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ABSTRACTS

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**A case of CANDLE syndrome**

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Introduction/Background: Chronic Atypical Neutrophilic Dermatosis with Lipodystrophy and Elevated Temperature (CANDLE) syndrome is a rare autoinflammatory disorder that can cause symptoms from infancy. Mutations in the genes encoding proteasome subunits (PSMB8, PSMB4, PSMA3 and PSMB9) have been identified as the cause of CANDLE syndrome. These mutations lead to malfunction of the proteasome, which results in buildup of cellular waste products. It is hypothesized that dysregulation in the interferon (IFN) signaling pathway in response to this waste is the driving mechanism of the inflammatory response, and may serve as a therapeutic target in these patients.
Objectives: To describe a case of suspected CANDLE syndrome successfully treated with tofacitinib.
Methods: Retrospective chart review was conducted with respect to diagnosis, treatment and response.
Results: A 16-month old Caucasian male was admitted to the hospital for evaluation of profound anemia. His medical history was significant for extreme prematurity (born at 22 weeks from premature labor), intraventricular hemorrhage grade IV that resulted in hydrocephalus needing ventriculoperitoneal shunting, and developmental delay. He was noted to have a hemoglobin of 6.2 g/dL during a neurosurgical evaluation for routine shunt revision. He developed hemodynamic decompensation and required hospital admission for packed red-blood cell transfusion. Review of systems was remarkable for intermittent pruritic macular rash, daily temperature fluctuations (fever to hypothermia), joint pain/swelling/stiffness of multiple sites, poor weight gain, irritability, irregular breathing, abdominal distention, and regression of gross motor milestones (no longer rolling over, sitting without support, or pulling up to stand). Workup excluded infections and lymphoproliferative malignancies, and he met clinical criteria for systemic juvenile idiopathic arthritis.
His initial laboratory studies showed systemic inflammation (WBC 14x 10^3/uL, Hgb 6.0 g/dL, platelets 558 x10^3/uUL, CRP 14.2 mg/dL, sedimentation rate 70 mm/h, ferritin 2370 ng/ml). Imaging studies revealed serositis with right-sided pleural and pericardial effusions. He also had myositis supported by imaging and elevation of muscle enzymes (AST 52 U/L, aldolase 30.6 U/L). The patient was started on pulse IV methylprednisolone 30mg/kg daily x3 days, followed by oral prednisolone 2mg/kg/day and anakinra 2mg/kg/day with partial improvement and he was discharged home. He was readmitted 3.5 weeks later due to concerns for macrophage activation syndrome (ferritin 12,362 ng/ml) in the setting of a gastrointestinal infection and anakinra was increased to 4.5mg/kg/day. However, he continued to have persistently elevated inflammatory markers and so the dose was increased again to 7mg/kg/day. Three months after initial presentation, he had an upper respiratory and ear infection and became ill with generalized rash, increased work of breathing, and poor perfusion. Anakinra was considered a treatment failure at that time. He required several doses of pulse steroids and initiation of tocilizumab 12mg/kg IV every 4weeks with improvement on systemic symptoms. Methotrexate 15mg/m² weekly was added soon after for persistent arthritis and inability to wean systemic steroids. He continued to have abnormal inflammatory indices, including ferritin (1,586 ng/mL) and IL-18 levels (35,588 pg/mL, normal 89-540). Proband only whole exome sequencing revealed a single heterozygous mutation in the PSMB4 gene (c.-9G>A), a published pathologic variant. Based on this finding, the patient was started on tofacitinib 2.5mg orally twice a day with a dramatic response. Laboratory markers of inflammation normalized, and he was able to walk within the first month of treatment. Further genetic testing to detect an additional proteasome subunit variant, as well as functional testing on a research basis to demonstrate an interferon signature are being pursued.
Conclusions: This case highlights the value of early genetic studies in patients with autoinflammation so that initiation of targeted therapy is not delayed in efforts to achieve control of symptoms and evade future complications. This case also illustrates the challenges in diagnosing monogenic autoinflammatory disorders in young patients that present with recurrent fevers, generalized rash, arthritis, and systemic inflammation that mimic systemic juvenile idiopathic arthritis. Our experience contributes to the understanding of janus kinase inhibition in type I interferonopathies.

**Submission ID#382424**

**A case of complete STAT1 loss of function**

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Introduction/Background: Complete signal transducer and activator of transcription 1 (STAT1) deficiency is a rare autosomal recessive condition that results in complete functional impairment of STAT1-dependent type 1 and type 2 interferon responses. Affected patients are predisposed to early and severe mycobacterial and viral infections, and usually succumb to infection in the first two years of life. Attempts at HSCT have been rare, and are usually unsuccessful due to either severe infection at the time of transplantation or profound GVHD in the post-transplant period.
Objectives: To characterize the clinical course of a patient with STAT1 loss of function.
Methods: A chart review was performed.
Results: The patient is a former full-term Caucasian male born to non-consanguineous parents who presented at 14 months of age with a history of two ear infections, RSV infection at 6 months of age requiring hospitalization, as well as vaccine-strain Measles and Varicella infections at 13 months of age. The diagnosis of VZV was made by positive DFA for VZV skin lesions, with viral culture that confirmed Vaccine-strain Varicella. Laboratory testing was notable for eosinophilia. Lymphocyte subsets were normal with the exception of decreased naïve CD8 T cells and increased activated T cells. Immunoglobulin levels were normal except for an elevated IgG of 1600 mg/dL. T cell proliferation was normal to candida but decreased to tetanus; however, tetanus antibody level was adequate. NK cell functional testing was severely decreased to absent. At 18 months of age the patient was again hospitalized with HHV6 meningoencephalitis and sepsis complicated by respiratory failure, hyponatremia, pancytopenia, and hypertension. He was also found to have disseminated MAC infection. Whole exome sequencing of primary immunodeficiency disease associated genes sent during this hospitalization, revealed compound heterozygous mutations (c.873G>T p.E625X; c.25C>T p.Q9X) in the STAT1 gene. Subsequent functional studies revealed absent STAT1 phosphorylation in response to IFN -gamma. The patient underwent hematopoietic stem cell transplant with a 9/10 MUD after reduced intensity conditioning with Busulfan, Fludarabine, Thiotepa and Anti-Thymocyte Globulin. His transplant course was complicated by hypertensive urgency, severe GVHD of the skin and gut, VOD, severe fluid overload requiring CRRT, elevated EBV/PTLD, a GI bleed, thrombotic microangiopathy, bacteremia, and disseminated candida infection. The patient ultimately succumbed to profound metabolic and respiratory acidosis, as well as severe electrolyte derangements resulting in cardiac arrest.
Conclusions: We report the outcome of a patient with complete functional absence of STAT1 who underwent HSCT from a 9/10 matched unrelated donor, but unfortunately did not survive. Of the two published reports of patients with complete STAT1 deficiency who had HSCT only one survived, but both had significant post-transplant complications like our patient. Moving forward these patients should be considered at high risk of complications during HSCT.

**(3) Submission ID#416907**

**A Case of Cutaneous T-cell Lymphoma in a Patient with Netherton Syndrome**

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Introduction/Background: Netherton syndrome syndrome is a rare autosomal recessive condition characterized by congenital ichthyosis, trichorrhexis invaginata (bamboo hair), and atopic diathesis.
Objectives: To describe a unique case of cutaneous T-cell lymphoma (CTCL) in a patient with Netherton syndrome.
Methods: A 35-year-old gentleman was evaluated in Allergy/Immunology clinic for possible immune deficiency syndrome. He described severe eczema in early childhood, asthma in elementary school, environmental allergy, and hair changes as a child. He also described a history of recurrent infections involving the skin and lungs as well as gastrointestinal symptoms of soft stools and gas. The patient was diagnosed one year prior to evaluation with CTCL and completed treatment with bexarotene. His IgE level was 4,347 kU/L (normal less than 214kU/L). The patient underwent genetic testing for Hyper-IgE syndromes and two pathogenic alterations in the SPINK5 gene were identified. Based on genetic testing results and clinical history he was diagnosed with Netherton syndrome.
Results: Netherton syndrome is a rare autosomal recessive condition characterized by congenital ichthyosis, trichorrhexis invaginata (bamboo hair), and atopic diathesis. Hypergammoglobulinemia E, high complement (C3/C4) and decreased numbers of natural killer cells make these patients more susceptible to infections and increase the incidence of angioedema, allergic rhinitis, and food allergy. There are several papers that describe the development of cutaneous neoplasia in patients with inherited ichthyoses, including Netherton syndrome, but after reviewing the literature we could find no reported cases of cutaneous T-cell lymphoma (CTCL) described in Netherton patients.
Conclusions: The present case raises the possibility of an association of CTCL and Netherton syndrome which may be linked by the presence of alterations in T-cell function.

**(4) Submission ID#421105**

**A case of Good syndrome with autoimmune complications and large granular lymphocytic leukemia**

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Introduction/Background: Good syndrome is a rare adult-onset primary immunodeficiency characterized by thymoma, hypogammaglobulinemia, and low or absent number of B and T cells. Males and females are equally affected and the syndrome carries a high association with autoimmunity. The incidence of malignancy is exceedingly rare.
Methods: A 63-year-old male presents with new onset fatigue, generalized weakness worsening in the afternoon, diplopia, non-bloody diarrhea, recurrent sinusitis and pneumonias, and anemia with thrombocytopenia. In concern for an underlying immunodeficiency and autoimmunity, the patient undergoes an extensive evaluation for primary immunodeficiency, myasthenia gravis, autoimmune cytopenia and enteropathy. Initial work-up is notable for positive Coombs test, which in the setting of thrombocytopenia, suggests Evans syndrome. Antibodies are negative to acetylcholine receptor, however clinically the patient is diagnosed and treated for myasthenia gravis. A CT chest is ordered to further evaluate the cause

of his recurrent infections and etiology of myasthenia gravis. Results of the CT chest are notable for a thymoma. Thymectomy with biopsy reveals benign pathology with a mixture of type A and B cells. He continues to have persistent fatigue, generalized weakness, diplopia, diarrhea and recurrent respiratory infections after thymectomy. Immunoglobulin and lymphocyte subset panels reveal hypogammaglobulinemia with absent B cells. Endoscopy reveals villous atrophy and blunting without evidence of celiac disease, inflammatory bowel disease or infection suggesting autoimmune enteropathy. The constellation of clinical and laboratory features are consistent with Good syndrome with Evans syndrome, seronegative myasthenia gravis and autoimmune enteropathy. The patient is started on immunoglobulin replacement therapy and pyridostigmine with resolution of recurrent infections and improvement of fatigue, generalized weakness and diplopia. Three years later his fatigue and Evans syndrome recur with new onset loss of appetite and a thirty pound weight loss. Repeat immunologic labs were notable for elevated CD3, borderline low CD4 and highly elevated CD8 cells with low absolute number and fraction naïve CD4 and CD8 cells suggesting worsening combined immunodeficiency with peripheral T cell expansion. A bone marrow biopsy reveals large granular lymphocytic (LGL) leukemia and he is started on methotrexate. Serum antibodies targeting IFN, and IL-12 are negative four years after removal of thymoma.
Conclusions: This case is consistent with a classic presentation of Good syndrome represented by thymoma, T and B cell-mediated immunodeficiency, increased susceptibility to infections and autoimmune manifestations of Evans syndrome, myasthenia gravis and autoimmune enteropathy. In this case the combination of Evans syndrome, autoimmune enteropathy and LGL leukemia as malignancy further worsen prognosis and is typically not seen together in Good syndrome. This case depicts well the crossroad of infection, autoimmunity and malignancy in late onset immunodeficiencies.

**(5) Submission ID#427200**

**A Case of Hyper-IgE Syndrome with Novel Variants in DOCK8 Gene**

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Introduction/Background: Dedicator of cytokinesis 8 (DOCK8) deficiency is a known cause of autosomal recessive hyper-IgE syndrome with a combined immunodeficiency. Most of the mutations in DOCK8 are loss-of-function homozygous or compound heterozygous point mutations or deletions. DOCK8 deficiency has been associated with low lymphocyte counts with impaired antibody responses, as well as eosinophilia, recurrent bacterial and cutaneous viral infections, malignancies, and severe atopy. We report the case of a 47 year old man with history of hyper-IgE syndrome, severe atopy, eosinophilia, and antibody deficiency, phenotypically atypical for DOCK8, who was noted to have two variants of unknown significance in the DOCK8 gene.
Objectives: We report the case of a 47 year old man with history of hyper-IgE syndrome, severe atopy, eosinophilia, and antibody deficiency, phenotypically atypical for DOCK8, who was noted to have two variants of unknown significance in the DOCK8 gene.
Methods: A 47 year-old man presented to us for evaluation of known hyper-IgE syndrome. He had a long history of elevated IgE, peripheral eosinophilia, severe atopic dermatitis, food allergies, asthma, severe eczema since early childhood which failed to respond to methotrexate, mycophenolate, cyclosporine, and omalizmuab, but ultimately responded to intravenous immunoglobulin (IVIG). His infectious history included MRSA skin infections and one episode of pneumonia, and he reported a history of fungal skin infections but the history was unclear. Initial immune workup revealed eosinophilia of 2898, IgE level 3540 (as high as 20,000), IgG level 603, IgM level 106, and IgA level 124. He had no random antibodies to streptococcus pneumonia 13 serotypes, but he had protective antibodies to diphtheria and tetanus. Lymphocyte subsets showed CD3 1874, CD4 1597, CD8 277, CD19 85. He had normal mitogen stimulation to PHA but decreased mitogen stimulation to candida. DNA testing for a STAT3 mutation was negative.
Results: We found two missense variants of uncertain significance in the DOCK8 gene (1.p.V194I, NM_203447.3:c.580G>A and 2.p.L1330V,NM_203447.3:c.3988C>G). The first variant had previously been reported in the ClinVar database as a variant of uncertain significance, and the second variant had not been previously reported in the literature to our knowledge. Our assay could not determine if the two DOCK8 variants were on the same allele or on different alleles. DOCK8 protein expression testing is currently pending.
Conclusions: Our patient presented with history of elevated IgE, eosinophilia, atopy, severe eczema, and cutaneous MRSA and fungal infections. He was noted to have variants of uncertain significance in the DOCK8 gene. Homozygous or compound heterozygous pathogenic variants in DOCK8 are associated with an autosomal recessive hyper-IgE syndrome and combined immunodeficiency with clinical features of recurrent bacterial infections, cutaneous viral infections, severe atopic disease, as well as susceptibility to malignancy. Our patient does not have all the typical features of DOCK8 deficiency and he seems to have a less severe phenotype. Notably, he does not have the cutaneous viral infections or malignancy often seen in DOCK8 mutation hyper-IgE cases. Our case demonstrates new missense mutations, which have not previously been described in the literature, possibly causing a milder phenotype of DOCK8 deficiency.

**(6) Submission ID#421797**

**A Case of IgM deficiency and Adult-onset Still's Disease**

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Introduction/Background: Selective IgM deficiency is a rare immunodeficiency disorder characterized by decreased serum levels of Immunoglobulin M (IgM). Those with IgM deficiency commonly present with recurrent infections and increased predisposition to allergic and autoimmune diseases. Adult-onset Stills disease (AOSD) is a rare auto-inflammatory disease characterized by high spiking fevers, characteristic evanescent salmon-colored rash, and multi-organ involvement. Association of Selective IgM deficiency with AOSD has not been previously reported. We present the first case of AOSD with known Selective IgM deficiency.
Objectives: To present the first known case of selective IgM deficiency and Adult-onset Stills Disease.
Methods: A comprehensive chart review of the patients disease process and hospital course was performed. Data was collected on her laboratory evaluation, diagnostic imaging and procedures results. A literature review was performed on selective IgM deficiency and AOSD.
Results: A 53-year-old African-American female with selective IgM deficiency and non-specific lymphadenopathy was admitted for evaluation of fevers without a source, bilateral arthralgias, and diffuse pruritic rash. Inpatient monitoring demonstrated a quotidian cycle of fevers along with the appearance of a rash during her fevers. She previously had extensive evaluations for fevers of unknown origin (FUO). With each admission, infectious evaluations were all

negative. Previous biopsy of her cervical and thoracic lymphadenopathy was unremarkable for malignancy.
During her hospitalization, serum immunoglobulins were performed, which demonstrated normal levels of IgG and IgA, with IgM level of <10 mg/dL (reference range 40-230 mg/dL), consistent with selective IgM deficiency. Liver function tests revealed an elevated Aspartate Aminotransferase (AST) of 177 U/L and an Alanine Aminotransferase (ALT) of 177 U/L with a total bilirubin of 1.7 mg/dL and an alkaline phosphatase of 146 U/L. Her ferritin was elevated at 349 g/L.
The patient fulfilled Yamaguchi criteria for AOSD with three major criteria of evanescent rash, intermittent fevers in a quotidian pattern, bilateral arthralgias in the hips, knees, and ankles. She also met two minor criteria of liver abnormalities and lymphadenopathy.
Conclusions: Selective IgM deficiency is an uncommon immunodeficiency disorder associated with increased risk for autoimmune disorders. The recognition of co-morbid autoimmune illnesses in an immunodeficient patient is often complicated by a paucity of examples in the literature and potential confounding of laboratory serology analysis. We report the first case of a patient with selective IgM deficiency and AOSD.

**(7) Submission ID#426367**

**A case of recurrent protamine hypersensitivity**

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Introduction/Background: Anaphylaxis to protamine is an uncommon but life-threatening complication of cardiac surgery and insulin therapy. Here we present a case of recurrent protamine hypersensitivity during vascular surgery.
Objectives
1. Recognize clinical signs of protamine hypersensitivity
2. Recognize recurrent hypersensitivity to protamine as a serious complication of anesthesia
Methods: A 63 year old man with a history of diabetes, previously on NPH insulin, hypertension, hyperlipidemia, chronic smoking, and peripheral artery disease with multiple vascular interventions was admitted to undergo a right lower extremity saphenous vein graft bypass. Three years earlier during a similar intervention, the patient had developed intraoperative hypotension after protamine sulfate administration. Protamine was subsequently held for additional surgeries, however the patient was able to tolerate protamine with slower infusion one year later. For the current vascular surgery, the patient was pretreated the day of surgery with diphenhydramine and dexamethasone, and a test dose of protamine was infused prior to full dosing. The patient initially appeared to tolerate the full protamine dose, but quickly developed facial erythema and angioedema. Due to concern for laryngeal edema he remained intubated and was transferred to the surgical intensive care unit, where he received additional diphenhydramine and dexamethasone. His symptoms resolved and he was successfully extubated the next morning.
Results: Anaphylaxis to protamine is an uncommon but life-threatening complication of cardiac surgery and insulin therapy. Protamine sulfate is a polypeptide used widely to neutralize heparin anticoagulation during cardiac and vascular surgeries, and in NPH insulin. Severe anaphylactic or anaphylactoid reactions caused by injection of protamine sulfate are well documented in literature, and the product contains a black box warning for such. The pathophysiologic mechanisms underlying these reactions are not clear, but IgE-mediated hypersensitivity appears to play a role in many reactions, and prior sensitization or cross-sensitization (eg, to fish) have been suggested. Type B adverse drug reactions are idiosyncratic drug reactions and are often unpredictable, as in our patient who previously tolerated protamine but subsequently developed an adverse reaction. Hypersensitivity reactions during anesthesia should be thoroughly studied to identify the responsible drug and minimize exposure in recurrent surgeries.
Conclusions: This case illustrates the potential for severe reactions even with newer protamine formulations, and highlights the unpredictable nature of type B adverse drug reactions. It is important for clinicians to exhibit awareness of the potential adverse effects of protamine sulfate in such situations.

**(8) Submission ID#427849**

**A case of X-linked lymphoproliferative syndrome type 2 with CD4 lymphocytopenia and B cell subset abnormalities in a 26 year old male with ulcerative colitis**

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Introduction/Background: X-Linked Lymphoproliferative Syndrome Type 2 (XLP-2) is a rare primary immune deficiency caused by loss of function in the X-linked inhibitor of apoptosis protein (XIAP). Common reported manifestations include recurrent hemophagocytic lymphohistiocytosis, splenomegaly, Crohns-like inflammatory bowel disease, and transient hypogammaglobulinemia without reductions in major T cell or B cell repertoires, with the exception of iNKT cells and MAIT cells. However, with only ~100 known cases worldwide, we are likely only beginning to understand the phenotypic spectrum of this disease.
Objectives: To describe additional manifestations of XLP-2 that expand our current understanding of its phenotype.
Methods: A 26 year-old male with adult-onset, treatment refractory ulcerative colitis was evaluated in the immunology clinic for a history of recurrent sinopulmonary infections, skin abscesses, and recurrent EBV and VZV infections. Extensive laboratory testing was performed in the course of his evaluation, including lymphocyte immunophenotyping, lymphocyte proliferation and cytotoxicity studies, quantification of total immunoglobulin levels and specific antibody function, HIV testing, and genetic testing.
Results: Laboratory testing was significant for persistent CD4 lymphocytopenia ranging from 380-459 cells/mcL (RR: 5031736 cells/mcL). Total B cell count was normal but B cell subsets showed an elevation in the percentage of naïve B cells (range: 85.2-85.4%, RR: 48.4-79.7%), low non-switched memory B cells (range: 4.7-6.0%, RR: 7.0-23.8%), and low to low-normal switched memory B cells (range: 7.2%-8.3%, RR: 8.3-27.8%), a pattern that has been seen in some autoimmune diseases. Genetic testing with a commercial immune deficiency panel (Invitae Corp) showed a pathogenic mutation in XIAP [Exon 2, c.664C>T (p.Arg222*)]. This mutation has previously been reported to cause a premature stop codon and reduced XIAP function. The patient was referred for hematopoietic stem cell transplant and is currently awaiting transplant with a matched unrelated donor.
Conclusions: XLP-2 is typically reported as having normal T cell, B cell, and NK cell counts, but the presence of persistent CD4 lymphocytopenia in this patient illustrates that this is not always the case. Our patient also had abnormalities in his B cell repertoire that have not been previously reported in XLP-2. Additionally, XLP-2 has been associated with Crohns disease and celiac-like bowel diseases, while our case indicates that the phenotype may also include ulcerative colitis.

**(9) Submission ID#420740**

**A case report: Enteroviral Encephalitis as a consequence of partial humoral immunodeficiency in a Chronic Lymphocytic Leukaemia patient treated with Rituximab**

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Introduction/Background: Enteroviral (EV) infections are prevalent and usually self limited or cause mild gastrointestinal manifestations. However , in the context of primary antibody deficiency , rare cases has been reported to develop meningoencephalitis and been linked to poor outcome with fatality or chronic course. EV Meningoencephalitis is even far rare reported in the era of rising secondary humoral immunodeficiency as a consequence of B cell depleting therapy e.g. Rituximab and Lymphoproliferative malignancies. Limited treatments for EV encephalitis are available to date, apart from Intravenous Immunoglobulin replacement which has variable efficiency.
Objectives: Studying such rare cases of EV meningoencephalitis as a consequence of antibody deficiencies would help to develop guidelines for Intravenous Immunogobulin replacement for treating these infections to improve outcome as well as predicting patients at higher risk who should be considered for prophylactic Immunoglobulin therapy.
Methods: Herein, we report a rare case of proven enteroviral Meningoencephalitis following Rituximab based therapy for B-cell Chronic Lymphocytic Leukaemia and an uneventful six months period of follow up. He was found to have persistent absent B cells six months after completing six cycles of Fludarabine, Cyclophosphamide and Rituximab therapy. Interestingly, he had partial Pneumococcal IgG serotypes deficiency, whilst his total IgG, IgM and IgA were all within normal limits throughout the course of the disease. The patient was treated empirically with Intravenous Immunoglobulin when his subtle confusion progressed to overt behavioural changes. Initially his level of consciousness continued to deteriorate and he was not communicating.
Results: Fortunately enough, the patient did have a remarkable improvement of GCS within couple of days and a slower recovery of higher mental functions e.g. memory and calculations in the next couple of months.
Conclusions: Early suspicion and detection of Entervorial Meningoencephalitis in patients at risk of secondary antibody deficiency is crucial for timely IVIg replacement and better outcome. Patients with haematological malignancies and those on B cell depleting immunotherapy should be screened for pneumococal IgG serotypes as part of secondary immunodeficiency workup. Further studies on enteroviral neurological meningo/encephalitis are required to optimise IVIg therapy and prognostication. Furthermore, such studies provide an important asset to reveal the underlying mechanisms for humoral/B-cell mediated protective response against EV compared to other T-cell mediated viral immunity, whilst highlighting the mechanisms of immunodeficiency in CLL and immunotherapy.

**(10) Submission ID#418733**

**A Comparison of Immune Reconstitution following Human Placenta-Derived Stem Cells (HPDSC) with Umbilical Cord Blood Transplantation (UCBT) vs. UCBT alone in Pediatric Recipients with Malignant and Non-malignant Diseases**

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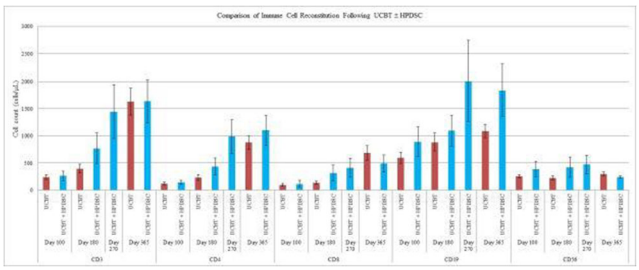
Introduction/Background: UCBT is a safe and effective treatment in children (Geyer/Cairo et. al BJH, 2011). However, due to a limited concentration of hematopoietic progenitor cells (CD34+) in UCB, UCBT has been associated with delayed hematopoietic reconstitution and a higher incidence of engraftment failure. HPDSCs contain a rich population of HPCs, are low in HLA Class I/II expression and T-cells, and have regenerative, anti-inflammatory, and immunosuppressive properties (Cairo et al BMT, 2015).
Objectives: To determine whether UCBT + HPDSC (vs. UCBT alone) is associated with enhanced hematopoietic and immune cell reconstitution in children with malignant and non-malignant diseases.
Methods: Immune cell reconstitution at days +100, 180, 270 and 365 was assessed in children who received UCBT with HPDSCs at NYMC (NCT01586455, IND#14949). Minimum TNC was 5 x 10^7/kg (4/6 HLA match) or 3.5 x 10^7/kg (5-6/6 HLA match). Immune cell subset counts at these time points were compared to those from a historical population of pediatric recipients of UCBT alone (Geyer/Cairo et. al BJH, 2011).
Results: Twenty four patients 18 years were enrolled. Mean age was 6 (range, 0.3-17) years. Malignant diseases =14, non-malignant diseases =10. Fourteen patients received myeloablative conditioning (MAC) and ten patients received reduced toxicity conditioning (RTC). There were no severe adverse events associated with HPDSC infusion. Two patients with non-malignant disease receiving RTC using alemtuzumab experienced primary graft failure. Probability of neutrophil engraftment was 91.6 %, median day 22 (13-53). Of evaluable patients at day 100, the probability of platelet engraftment in neutrophil engrafted patients was 100%, median day 43.5 (20-98). At days 30, 60, 100 and 180, mean percent donor chimerism in whole blood was 94, 98, 95, and 99%, respectively. Average percent of whole blood HPDSC chimerism was 1% at day 30 and <1% at beyond day 60. One patient with malignant disease relapsed. 12 month overall survival was 83.3%. There was no significant difference in CD3, CD4, CD8, CD19 and CD56 immune

cell reconstitution following UCBT + HPDSC vs. UCBT alone (Image 1).




Conclusions: These results suggest that UCBT ± HPDSC results in similar immune cell reconstitution. A larger cohort with extended follow-up would be required to confirm these preliminary findings.

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**(11) Submission ID#382302**

**A decade of disseminated abscesses due to Mycoplasma faucium in a patient with activated PI3K syndrome 2 (APDS2)**

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Introduction/Background: PIK3R1 monoallelic mutations are known to be responsible for APDS-like 2 syndrome, a rare form of primary immunodeficiency presenting as combined immunodeficiency or Hyper-IgM like phenotype. This study reports a patient carrying heterozygous PIK3R1 mutation with early onset and long-term disseminated abscesses due to Mycoplasma faucium in both peritoneal abscess and skin, with generalized involvement in neck and both upper extremities.

Objectives: We describe clinical management of retroperitoneal and skin abscesses before molecular diagnosis was available in a patient with a primary immunodeficiency. Identification by rDNA 16s in so-called sterile abscesses may confirm the clinical suspect of an oportunistic infection. Furthermore, this study offers insight on the PIK3R1 suspicion even in the absence of HIGM-like phenotype.

| Parameter/ Age | 16 yo Absolute number / % | 19 yo Absolute number / % | Normal Range Absolute number / % |
|---|---|---|---|
| Total lymphocytes | 382 | 704 | 1200 – 3000 |
| CD3+ | 313 / 83% | 614 / 87% | 850 - 2250 / 62 – 81% |
| CD4+ | 145 / 38% | 284 / 40% | 500 - 1450 / 32 – 59% |
| TCR CD3+ulfa/beta+ | | 549 / 78% | 1000 - 1350 / 60 – 80% |
| TCR CD3+gamma/delta+ | | 49 / 7% | 0 - 150 / 0 – 7% |
| Double negative T cells | N.D. | 1.8% | < 2.5% |
| CD4+CD45RA+CCR7+ (naive) | | 31% | 20-60% |
| CD4+CD45RA-CCR7+(central memory) | | 30% | 25-50% |
| CD4+CD45RA-CCR7-(efector memory) | | 38% | 5-30% |
| CD4+CD45RA+CCR7-(efector memory expresing CD45RA) | | 1% | 0-5% |
| CD8+ /uL | | 319 / 45% | 160 - 950 / 15 – 36% |
| CD8+CD45RA+CCR7+ (naive) | | 6% | 15-45% |
| CD8+CD45RA-CCR7+ (central memory) | | 4% | 5 – 25% |
| CD8+CD45RA+CCR+/- (effector memory) | | 75% | 25-55% |
| CD8+CD45RA+CCR7+/- (TEMRA) | | 15% | 5-20% |
| CD4+CD45RA+CD31+ | N.D. | 20% | 26 – 58% |
| CD19+ | | 42 / 6% | 100 – 500/ 8 - 20% |
| CD56+CD3- | | 42 / 6% | 60 – 450/ 4 – 22% |
| CD3+HLA-DR+ | | 106 / 15% | 0 - 250 / 0 – 10% |
| Serum immunoglobulins | | | |
| IgG (mg/dL) | 1170* | * | 700 - 1600 |
| IgA (mg/dL) | < 6.67 | | 70 - 400 |
| IgM (mg/dL) | 7 | | 40 - 230 |
| IgD (mg/dL) | 0 | | 1 - 5 |
| T-cell proliferation (cpm) | | | Response Index |
| PHA | 16 | 40 | >57 |
| PMA+Ionomycin | 33 | 27 | >65 |
| sCD25 (pg/mL) | | 3684 | 0-2000 |

- Under IgG replacement therapy

Methods: A 16-year-old girl with 2-year history of recurrent peritoneal effusion, which had been drained repeatedly was admitted in our Institution for a 10-year history of multiple supurative cutaneous and lymph node- abscesses (Fig1&2A-C). She had prior diagnosis of agammaglobulinemia under standard subcutaneous immunoglobulin replacement therapy and subcutaneous interferon-gamma treatment.

On physical examination at the age of 16 years, she was stunted (weight and height below the 3rd percentile), with facial, arm skin abscesses and right fistulized axillary lymphadenopaties, 5-6cm hepatomegaly and giant splenomegaly

Results: She had her first immunological work up at the age of 6 years during one isolated episode of knee arthritis and first episode of skin abscesses. Serum immunoglobulins revealed panhypogammaglobulinemia (IgG<7.3 mg/kg, IgA <5.8 mg/kg, IgM <4.3 mg/kg) with low B cell count. On her back, there was a 10x15cm, elastic, neither painful nor tender mass. After proper assessment by CT scan and MRI she had her retroperitoneal abscess drained percutaneously, and healed with sclerotherapy (percutaneous alcohol and polidocanol instilation) by the interventional radiologist (Fig 2B). Analysis of drained pus as well as pus of skin abscesses was made by 16S rDNA PCR, having coincidence of 99.9% with Mycoplasma faucium . Combination antibiotic therapy (doxycycline and ciprofloxacin) was started with favourable response. Unfortunately skin abscesses then relapsed.

T-cell phenotype only showed T-cell lymphopenia with senescent (TEM & TEMRA expansion) phenotype. Whole exome sequencing revealed a heterozygous mutation, previously reported (c.1425+1G>T)

Conclusions: In summary, this report emphasizes the suspicion of a combined immunodeficiency in the presence of multiple abscesses by Mycoplasma, the usefulness of rDNA 16s in order to achieve proper

diagnosis in disseminated Mycoplasma faucium infection and particularly APDS deficiency even with low IgM.

**(12) Submission ID#396277**

**A novel EP300 mutation associated with Rubinstein-Taybi syndrome presenting as combined immunodeficiency**

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Introduction/Background: The Rubinstein-Taybi syndrome (RSTS; OMIM #180849, #613684) is a rare developmental disorder characterized by craniofacial dysmorphisms, broad thumbs and toes, and mental and growth deficiency. It affects equally males and females, with prevalence of 1:100.000 to 1:125.000 liveborn infants. Mutations in the cAMP response element-binding protein (CREB)-binding protein (CREBBP) or in the E1A-associated protein p300 (EP300) have been demonstrated in 55% and up to 8% of the patients, respectively. Variable cellular and humoral defects have been rarely described1-4.

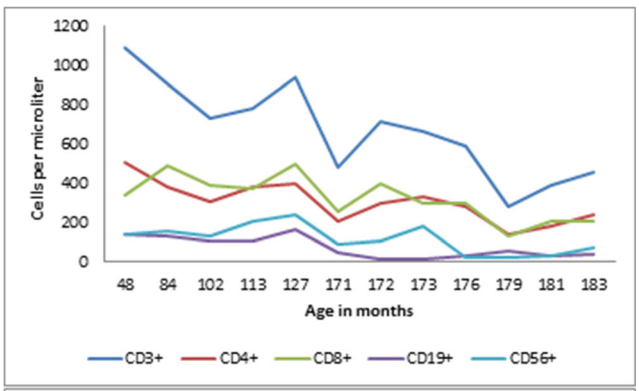
| | 4 years of age | 7 years of age | 14 years of age |
|---|---|---|---|
| WBC | 1970/mmc | 5040/mmc | 4820/mmc |
| Lymphocytes | 1370/mmc | 1220/mmc | 820/mmc |
| Neutrophils | 200/mmc | 3330/mmc | 3480/mmc |
| Platelets | 33000/mmc | 139000/mmc | 70000/mmc |
| CD3+ | 79.4% (56-78.9) | 74.6% (59.1-80.9) | 85.2% (58.1-80.1) |
| CD4+ | 37.0% (29.4-55.7) | 31.5% (24.9-51.1) | 32.5% (27.9-53.4) |
| HLA-DR+ | 6.5% (1.3-12.1) | | 19.2% (1.4-11.5) |
| Naïve CD45RA+CCR7+ | 50.3% (49.2-85.8) | | 10.7% (35.1-82.2) |
| RTE CD45RA+CCR7+CD31+ | n.a. | | 1.4% (26.2-67.1) |
| Central memory CD45RA-CCR7+ | 40.5% (9.6-31.9) | | 63.1% (10.7-44.3) |
| Effector Memory CD45RA-CCR7- | 7.3% (2.8-16.9) | | 24.7% (5.4-25.3) |
| Terminal differentiated CD45RA+CCR7- | 1.8% (0.7-4.8) | | 1.7% (0.6-6.5) |
| CD8+ | 24.6% (11.6-32.4) | 40% (13.8-31.2) | 46.9% (12.3-31.9) |
| HLA-DR+ | 11.0% (0.9-33.2) | | 44.8% (1.8-31.4) |
| Naïve CD45RA+CCR7+ | 47.2% (22.8-79.9) | | 9.5% (15.1-76.7) |
| Central memory CD45RA-CCR7+ | 7.1% (0.9-11.3) | | 4.4% (1.3-15.4) |
| Effector Memory CD45RA-CCR7- | 33.4% (4.7-31.3) | | 62.7% (6.1-40.1) |
| Terminal differentiated CD45RA+CCR7- | 12.3% (6.8-52.7) | | 23.5% (6.8-46.7) |
| CD19+ | 10.0% (10.7-34.9) | 10.7% (8.6-26.3) | 2.8% (6.3-25.4) |
| RBE CD38hiCD10+ | 13.3% (10.6-42.6) | | 7.7% (4.2-28.5) |
| Naïve IgD+CD21hiCD10-CD27- | 46.3% (34.2-65.5) | | 40.3% (39.9-74.1) |
| CD19hiCD21lo | 25.6% (1.5-9.8) | | 31% (1.0-15.8) |
| Switched memory IgD-CD27+CD21hi | 1.3% (1.5-14.2) | | 1.3% (2.7-16.5) |
| IgM Memory IgD+CD27+CD21hi | 9.9% (2.9-15.3) | | 11.1% (3.4-18.0) |
| Terminal differentiated CD38hiCD27hiCD20- | 2.9% (0.4-15.3) | | 4.7% (0.2-7.1) |
| PC CD38hiCD27hiCD20-CD138+ | n.a. | | 0.7% (0.1-2.4) |
| CD56+CD16+ | 9.9% (3.0-21.3) | 13% (3.3-22.8) | 10.7% (3.8-24.6) |
| CD3+CD4+CD8- a/b (total/CD3+ lymphocytes) | n.a. | 2.66%/3.57% (1.5-2.5) | 4.6% /5.7% (1.5-2.5) |
| Isohemoagglutinin | Anti-B absent, Anti-A 1:8 | | |
| Mitogen proliferation | Normal | Reduced to PHA and ConA | |
| TRECs | 33147 (3521+-17922) | | |
| IgG / A / M | 685 / 73 / 83 mg/dl | 274 / 17 / 185 mg/dl | 321 / 7 / 59 mg/dl |
| Anti-tetanus / Anti-diphtheria Ab | Absent / Absent | Absent / Absent | Absent / Absent |
| Spleen diameter | 11.6 cm | 14 cm | 17 cm |

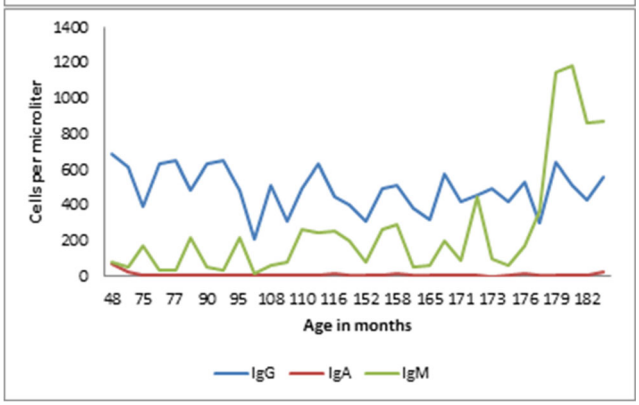
Objectives: We describe a 15-year-old male patient with novel heterozygous mutation of EP300 gene; his first manifestations were initially characterized by infections, cytopenia and hypogammaglobulinemia suggesting a Common Variable Immunodeficiency (CVID), but later on, persisting lymphopenia was suggestive of a combined immunodeficiency.
Methods: The patient was born to unrelated healthy Italian parents at 34 weeks gestation with adequate weight for gestational age. Shortly after birth, he underwent several surgical procedures due to interventricular defect, aortic coarctation, double outlet right ventricle, open Botallis duct, and gastroesophageal reflux. At the age of four, he came to our attention due to stomatitis. Clinical examination revealed dysmorphisms (microcephaly, wide forehead, sparse eyebrows, high nasal root, low-hanging columella, thick lips, micrognathia), splenomegaly (spleen diameter 11.6 cm at abdominal ultrasound), and severe developmental delay. In the course of the infectious episode, blood tests showed leukopenia associated with neutropenia (white blood cells 2.210/mm3; Neutrophils 20/mm3) and thrombocytopenia (platelets 1.000/mm3). Analysis of bone marrow aspirate revealed normal differentiation of both myeloid and erythroid lineages. Treatment with high doses immunoglobulin resulted in increase of platelet counts (up to 44000/mm3 after 1 month), while neutrophil counts spontaneously returned to normal when the infection resolved. However, thrombocytopenia relapsed (2000/mm3) after 2 months and intravenous high-doses of corticosteroids did not achieve normal platelets count. Despite oral corticosteroid treatment started at the age of six, two episodes of autoimmune hemolytic anemia occurred. During the following six years of follow-up, the patient experienced recurrent infections (stomatitis, upper respiratory tract infections, and skin abscesses), but none of the episodes has required hospitalization. But, at the age of ten, he was admitted to the hospital because of severe cultures negative diarrhea. Despite immunoglobulin replacement therapy was started at the age of fourteen, he was admitted twice due to bilateral pneumonia requiring continuous positive airway pressure and, a few months later, acute respiratory failure with evidence of Mycoplasma pneumoniae and Rhinovirus infections.
Immunological evaluation under chronic corticosteroid treatment at different time points showed persisting lymphopenia, with lymphocyte counts ranging from 350/mmc3 to 2100/mm3 , thrombocytopenia (platelets ranging from 0/mm3 to 308000/mm3), undetectable anti-diphteria and anti-tetanus toxoid antibodies, and splenomegaly. Interestingly, analysis of isohemoagglutinins, revealed low titers of anti-A (1:8 ) at 4 years of age, but normal immunoglobulins (IgG 685 mg/dl, IgA 73 mg/dl, and IgM 83 mg/dl). At the age of seven, reduced mitogen proliferation, hypogammaglobulinemia (IgG 274 mg/dl; IgA 17 mg/dl, IgM 185 mg/dl), increased CD3+TCR+CD4CD8 T-cell counts (2.7/3.6%) and impaired FAS mediated apoptosis as measured in two separate assays (Table 1 ). At the age of fourteen, evaluation of B-cell subsets showed increase of CD21loCD38lo cells and reduction of switched memory B-cells. Analysis of T-cell compartment unveiled a decreased proportion of CD31+CCR7+CD45RA+ recent thymic emigrants (RTE) cells and CCR7+CD45RA+ naive cells, with prevalence of effector memory T-cells (CCR7-CD45RA-) (Table 1). Interferon signature gene expression showed borderline levels of IFI27 (data not shown). Because of the decrease of IgG and the infectious episodes IVIG treatment was started at age of fourteen.
A molecular investigation performed by whole exome sequencing (WES) revealed a novel heterozygous missense mutation (NM_001429.3:c.4763T>C , p.Met1588Thr) in the exon 29 of the gene EP300 encoding the Histone Acetyltransferase (HAT) protein p300.
Results: Few immunological reports are available in RSTS patients1-4. In keeping with previous data1,3,4, our patient presented with progressive B- and T-cell lymphopenia, hypogammaglobulinemia with poor antibody response but also reduced naïve T cells, Evans syndrome, splenomegaly, and defective lymphocyte apoptosis with increased DNT. At the age of seven, the patient presented the features of CVID. Flow cytometry

revealed expansion of CD19hiCD21loCD38lo B cells, that is frequently associated with splenomegaly in CVID patients7, and reduced switched memory B-cell, previously reported in a RSTS patient with CREBBP mutation4 (Fig. S1). Lougaris et al. reported expansion of CD21loCD38lo B-cells in NF-kB1 haploinsufficiency 8, and this suggests in our opinion that alterations in the NF-kB pathway due to EP300 mutations may affect B-cell differentiation. Compared to healthy controls and CVID patients with predominant infectious complications, upregulation of interferon responsive genes in CVID subgroup with non-infectious complications (i.e hematologic autoimmunity, lymphoproliferation) and lymphopenia with reduced total B cells and switched-memory B cells has been demonstrated9. Borderline levels of IFI27 expression under corticosteroid treatment represent a novel finding in RSTS. Interferon signature may identify and better characterize subgroup of RSTS patients with autoimmune cytopenias and lymphopenia.

At the age of fourteen, analysis of lymphocyte subsets revealed decreased total CD3+, CD4+, CD8+, and of both naïve CD4+ and CD8+ cells. Elevated and persisting IgM levels were also observed (Fig. S2). According to our data, increased IgM levels may be related to high proportion of terminal differentiated IgM+ cells. These data (infections requiring hospitalization, immune dysregulation, lymphopenia, reduced naïve T cells, and reduced proliferation to mitogen) together with clinical history (Fisher Evans syndrome and lymphoproliferation) and exclusion of known syndromic immunodeficiencies, suggested a diagnosis of combined immunodeficiency10.

Conclusions: Our case underlines the value of WES in patients with difficult phenotype-genotype correlation. No RSTS typical traits were present and, prior to WES, several syndromes and immunodeficiencies were excluded. Our report expands the phenotypic spectrum of EP300 mutations, thus in syndromic patients with clinical and immunological overlap between CVID and CID ruling out EP300 mutation should be advisable. Furthermore, immunological work-up should be taken into consideration in RSTS patients, in order to early identify immunological abnormalities that may lead to severe immune-hematological complications.




**(13) Submission ID#415038**

**A Novel IFNGR1 Mutation Leading to Interferon-Gamma Receptor 1 Deficiency Treated Successfully with Umbilical Cord Blood Transplantation**

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Introduction/Background: Interferon gamma receptor (IFNGR1)-related disorders are rare variants of Mendelian susceptibility to mycobacterial diseases. Although hematopoietic stem cell transplantation (HSCT) is curative, it is complicated by high rates of delayed or failed engraftment thought to be due to high concentrations of interferon (IFN)-gamma. Umbilical cord blood transplantation additionally increases risk of graft failure.

Objectives: Describe a pediatric patient with non-functional IFNGR1 who successfully underwent umbilical cord blood transplantation.

Methods: Direct clinical care of described patient with additional electronic medical record chart review.

Results: The patient is a 19-month-old boy of Yemeni descent who initially presented with significant hepatosplenomegaly and extensive lymphadenopathy, including a large mediastinal mass. He then developed Salmonella enteritidis sepsis requiring numerous antimicrobials, vasopressor support, intubation and continuous renal replacement therapy. His evaluation showed a hyperinflammatory state with elevations in ferritin, IFN-gamma, sCD25, IL-10, IL-13, IL-17, IL-6 and IL-8 levels. Maximal ferritin and IFN-gamma levels reached 12065.4 ng/mL and 463 pg/mL (normal <5 pg/mL), respectively. Flow cytometry revealed normal expression of IFNGR1 and IL12R but absent IFN-gamma-stimulated STAT1 phosphorylation, suggesting defective IFNGR1 signaling. Genetic testing showed a previously unreported homozygous mutation in IFNGR1 (c.373+2T>C) which affects a donor splice site in intron 3 and is predicted to cause absent protein function. Dexamethasone and a single dose of alemtuzumab (0.2 mg/kg) were given to decrease inflammation. He then underwent allogeneic HSCT using a 5/6 human leukocyte antigen matched umbilical cord unit following a reduced-toxicity conditioning regimen of alemtuzumab (0.6 mg/kg), fludarabine (180 mg/m2) and busulfan (AUC 55 mg/L*h). Plasma IFN-gamma was undetectable prior to starting conditioning and on the day of transplant. Neutrophil engraftment occurred on day +14 with day +30 post-transplant chimerism analysis of peripheral blood myeloid cells showing the presence of donor cells only.

Conclusions: These early results suggest that umbilical cord blood transplantation may be feasible in patients with IFNGR1-related disorders provided adequate control of inflammation is gained prior to transplant.

**(14) Submission ID#426745**

**A Novel Mutation in the Inhibitor of Nuclear Factor Kappa-B Kinase Subunit Beta (IKKB) in an Adult Male WIth Anhydrotic Ectodermal Dysplasia with Immunodeficiency**

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Introduction/Background: BACKGROUND: Anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID) is caused by mutations leading to impairment of NF-KB activation. Hypomorphic mutation of the NF-KB-activating NEMO/IKKG/IKBKG and gain-of-function mutation of NF-KB-inhibiting IKBA/NFKBIA have been identified as genetic etiologies of EDA-ID. IKKB/IKBKB binds to NEMO in the NF-KB-activating IKK complex and directly phosphorylates inhibitors of KB (IKBA, IKBB, and IKBE) leading to their proteasomal degradation. Complete loss of IKKB is embryonic lethal in mice; however, homozygous deletion of IKBKB causes severe immunodeficiency in humans. Interestingly, no association with ectodermal dysplasia has been reported in human IKKB deficiency. In contrast to loss of function, somatic, activating mutations in IKBKB have been associated with numerous cancers but not immunodeficiency. Lys171Arg is a particularly potent mutation that results in constitutive phosphorylation and polyubiquitination of IKKB as well as induction of STAT3 activation. Lys171Arg has not previously been observed as an inherited variant. We report an adult patient with germ-line Lys171Arg in IKBKB and clinical features consistent with EDA-ID phenotype.
Objectives: OBJECTIVES: To characterize a novel form of EDA-ID in an adult male with the germline K171R mutation in IKBKB.
Methods: METHODS: A 28-year old male with clinical features of anhidrotic ectodermal dysplasia with immunodeficiency was initially identified in the Adult Allergy Immunology Clinic at National Jewish Health. The patient presented with lifelong anhidrosis, recurrent skin eruptions, dystrophic nails, history of abnormal conical teeth and premature dental loss (hypodontia), severe bronchiectasis, recurrent sinopulmonary infections with Streptococcus pneumonia, CMV viremia, chronic diarrhea and granulomatous uveitis. Lymphocyte phenotyping, quantitative immunoglobulins, and whole-exome sequence analysis were performed. Intracellular staining for IKBA in stimulated PBMC was also performed.
Results: RESULTS: The patient demonstrated mild leukopenia with WBC 3.5 (4-10.5 K/mcL), mild neutropenia 2.6 K/mcL (2.8-11.1 K/mcL), lymphopenia 0.3 K/mcL (0.4-2.5 K/mcL), and normal monocyte numbers. CD4, CD8, CD19, and CD16/56 cell counts were reduced (absolute counts: CD3 = 240 (78 to 2504), CD19 = 14 (96 to 515), CD4 = 30 (414 to 1679), CD8 155 (162 to 1038), CD16/56 = 11 (45-523). Quantitative immunoglobulins prior to replacement IgG therapy revealed total IgG 768 mg/dL, IgA 111 mg/dL and IgM 66 mg/dL with a relative reduction in IgG subclass 2 (15 mg/dl, range 117-746). HIV antibodies were not detected. Capillary sequencing of genomic DNA revealed no variant in IKBKG. Trio whole-exome sequencing identified a de novo heterozygous variant in IKBKB, c.512A>G, resulting in Lys171Arg. No variant was seen in NFKBIA. The variant was confirmed by capillary sequencing of whole blood and buccal swab. IKBA degradation by flow cytometry was measured in LPS-stimulated PBMCs demonstrating dramatic reductions in baseline and post-stimulation IKBA levels in both lymphocytes and monocytes (Figure 1). Despite baseline depletion, some IKBA degradation was observable.
Conclusions: CONCLUSIONS: We present a novel autosomal dominant cause of EDA-ID in a 28-year-old male with a de novo, germline, heterozygous variant in IKBKB. Lys171Arg causes a syndrome including lifelong recurrent infection, ectodermal dysplasia, and skin eruptions. At least in this instance, humoral immune development was mostly normal, and survival into adulthood was possible; however, at the time of evaluation, the patient had severe lymphopenia and systemic viral infection. Decreased IKBA was evident in lymphoid and myeloid compartments, presumably the result of chronic phosphorylation by IKKB. It is not clear if lymphopenia onset at birth or as an adult. Nonetheless, he suffered from lifelong recurrent infection, ectodermal dysplasia, skin inflammation, and progressive bronchiectasis, suggesting lifelong immune abnormality.
Supported by the Grifols and the Eugene F. and Easton M. Crawford Charitable Lead Unitrust.

**(15) Submission ID#421135**

**A Novel Mutation in the SH2 Domain of Brutons Tyrosine Kinase Leading to X-Linked Agammaglobulinemia.**

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Introduction/Background: Introduction: BTK is a cytoplasmic tyrosine kinase that activates phospholipase C2 (PLC2) via phosphorylation, which ultimately leads to the activation of NFK, which is essential for B cell development and survival. Mutations in BTK lead to X-linked agammaglobulinemia (XLA). In addition to the pleckstrin homology and tyrosine kinase domains, BTK contains two Src homology domains, SH2 and SH3, which are essential for BTK function. We describe a novel BTK mutation (c.1197A>T) resulting in V335A substitution in the SH2 domain that results in aberrant XLA function with nearly normal BTK protein expression.
Objectives: Case report/results: The male proband presented with recurrent otitis media, persistent fevers and neutropenia beginning in the first year of life with an IgG level of 46 mg/dl and a lack of B cells (0 cell/mm3), as demonstrated by flow cytometry. BTK protein expression in monocytes, also determined by flow cytometry, was equivalent to controls. Family history is significant for a maternal uncle with history of recurrent sinus infections and pneumonias with low IgA and IgM, low to normal IgE, and an absent vaccine response. Flow cytometry also showed an absence of B cells and essentially normal BTK protein expression in his monocytes compared to controls. Targeted high throughput sequencing of both proband and the uncle revealed a previously unreported missense mutation in exon 12, leading to the substitution of an aspartic acid residue for a valine (V335D.) The mutation is in the highly conserved SH2 domain of BTK (conservation PhyloP conservation score 2.8.) The probands mother and his sister were shown to be carriers of the same mutation and had normal serum immunoglobulin levels and normal numbers of B cells.
Methods: We hypothesized that if the BTK V335D mutant protein was non-functional, the female carriers of the mutation would only express wild type (wt) BTK in their B cells while their monocytes would express both wt and mutant BTK. To test this hypothesis CD19+ B cells and CD14+ monocytes were purified from PBMC by fluorescence activated cell sorting (FACS) from the sister, cDNAs were generated from the respective populations of cells, and the BTK cDNA was sequenced using high-throughput sequencing.
Results: At a read-depth greater than 16,000, CD19+ B cells demonstrated BTK expression only from the wt allele (~99% wt BTK) whereas both the wild type and mutant allele of BTK were expressed at approximately equal levels in monocytes.

Conclusions: Discussion: These results define a novel mutation in BTK that nominally affects protein expression, but alters function. The V335A substitution is found in the D structural element of the SH2 domain that is part of a hydrophobic phosphotyrosine binding pocket. A mutation in the adjacent residue, Y334S, has been shown to alter protein conformation and decrease binding affinity to PLC2 by roughly 4-fold resulting in XLA. Our study, using a carrier harboring the c.1197 A>T mutant BTK, demonstrated that in contrast to mononuclear cells, B cells only expressed the wt allele. This is consistent with the loss of function of V335A BTK protein, thereby causing XLA in both the proband and affected uncle.

**(16) Submission ID#420909**

**A novel NFkB1 (nuclear factor kappa B1) mutation (c.A2415G; p.Q805Q) associated with pyoderma gangrenosum and common variable immune deficiency**

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Introduction/Background: Pyoderma gangrenosum (PG) is often associated with systemic autoimmune diseases but it has rarely been reported with common variable immune deficiency (CVID). While genetic analysis has been increasing in both disease domains, there has been little investigation into the genetic components associated with the co-occurrence of these entities. Heterogeneous NFkB1 mutations have recently been identified in familial cases of CVID, though rarely have they been associated with PG.
Objectives: This case describes a novel NFkB1 mutation that may link both CVID and PG, and bolsters the recent identification of heterogeneous NFkB1 mutations in CVID.
Methods: A 24-year-old woman with a history of frequent skin infections in childhood presented with persistent, infected wounds following cholecystectomy. Upon admission, she was started on broad spectrum antibiotics but continued to have fevers and leukocytosis. Labs were also notable for elevated CRP (340; Normal (N):0.1-3) and ESR (120; N:0-20), with low C3 (60; N:81-145), C4 (<10; N:16-39), and CH50 (<10; N:>74). Wound cultures grew multi-drug resistant coagulase negative Staphylococcus. Despite broad spectrum antibiotics, the wounds failed to heal. Dermatology was consulted and punch biopsy revealed a dense neutrophilic infiltrate and no identifiable pathogens, which supports the diagnosis of PG. The patient was started on high dose steroids (1mg/kg/day) and had a rapid response with decreased skin inflammation and lesion expansion. Unfortunately, the patient developed posterior reversible encephalopathy syndrome (PRES) on steroids and therefore PG treatment was changed to infliximab as recommended by Dermatology. Further laboratory testing found that the patient also had low IgG (499; N: 700-1600) and IgA (34; N: 70-400), and a diagnosis of CVID was made given her clinical history of recurrent skin infections. Genetic testing was pursued to evaluate additional PG therapy options and this revealed a heterozygous mutation in NFkB1 (c.A2415G; p.Q805Q), located 2 base pairs upstream of the splice donor site for exon twenty-one. While this mutation has not been previously identified as a pathogenic variant, similar mutations in this gene have been linked to autosomal dominant CVID. The patients father also carried a similar mutation but without any evident clinical phenotype.
Results: NFkB1 plays a crucial role in both immune and inflammatory responses. This case highlights a novel mutation in NFkB1 that has not been previously described as a disease-causing change. Other mutations resulting in NFkB1 haploinsufficiency have been associated with CVID and rarely with concurrent PG, as in this case. Based on the location of the mutation, it is expected that the variant causes obliteration of the normal splice site and therefore results in defective mRNA that encodes p50/p105. Interestingly, studies on p50 knockout mice show decreased levels of IgG, IgA, and IgE but not IgM and our patient similarly had low levels of IgG and IgA but normal IgM.
Conclusions: Further studies are needed to determine if there are other links to this novel NFkB1 mutation in patients with CVID and PG.

**(17) Submission ID#412594**

**A Rapid Flow Cytometric Analysis Of DNA Repair Proteins Reveals A Radiosensitive Phenotype In BCL11B Deficiency Associated with Severe Combined Immunodeficiency (SCID).**

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Introduction/Background: BCL11B deficiency has been previously reported with a syndromic form of severe combined immunodeficiency (SCID). We describe here a male infant, identified by abnormal TREC, in newborn screening, who had pan-lymphopenia, global developmental delay, cortical vision loss, agenesis of the corpus callosum, ASD, dysmorphic features, microcephaly, cleft palate and focal epilepsy. WES revealed a de novo heterozygous variant of uncertain significance in the BCL11B gene, c. 2513A>C, p.Lys838Thr. The patient had hypogammaglobulinemia, abnormal TCR diversity, decreased naïve T cells, abnormal T cell proliferation to mitogens, with a partial antibody response to TT post-vaccination.
Methods: The patient was assessed for radiosensitivity using a novel flow cytometry assay measuring phosphorylated ATM, SMC1, and H2AX in T, B and NK cells before and after exposure to low-dose (2Gy) radiation.
Results: The patient had a subset of B cells with constitutive H2AX expression, even without induction of DNA damage via radiation. Also, NK cells demonstrated increased sensitivity to radiation at 24h compared to healthy control, with a significant loss of NK cells. T and B cell analysis post-irradiation demonstrate only a subset of cells have undergone SMC1 dephosphorylation at 24h post-radiation, as would be expected. The majority of these cells have persistent phosphorylated (p) SMC1, even at 24h. In the NK cell subset, only half the cells dephosphorylate ATM, SMC1 and H2AX at 24h.
Conclusions: These data, which are in contrast to healthy controls, suggests impaired DNA repair, which may be related to the underlying BCL11B gene variant. Therefore, BCLL1B deficiency may be a new radiosensitive SCID.

**(18) Submission ID#428316**

**A rare case of Familial HLH due to homozygous mutations in PRFI with isolated CNS involvement.**

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Introduction/Background: The patient is a 9-year-old boy of consanguineous Syrian origin. There was no significant family history of note. The family moved to Canada as refugees in 2016, and following his arrival he was referred for evaluation of presumed degenerative leucodystrophy. The patient first presented at 4 years of age in Syria with acute neurological symptoms, and loss of the ability to walk. He was diagnosed with ADEM and managed with IV and oral steroids and one dose of IVIG with some improvement. He required multiple courses of prednisone in the year following presentation, with relapse and regression of his neurological function with each trial of steroid cessation. At 5 years of age a 6-month period off steroids lead to significant deterioration with inability to stand or walk unsupported, dysphasia and seizures. The patient remained on oral prednisone 10mg PO daily for the subsequent 2 years with a plateau of neurological function. There was no history of fever.
Further trial of steroid cessation in November 2016 necessitated admission for neurological deterioration. At that time, he was found to have spastic tetraplegia, progressive dysphagia, dysphasia as well as recurrence of seizures, exotropia and neurogenic bladder. He was also noted to have splenomegaly, marked osteopenia as well as adrenal insufficiency following steroid withdrawal. Brain MRIs in 2016 and 2017 demonstrated cerebral atrophy with diffuse white matter abnormalities. CT in January 2017 demonstrated progressive cerebral atrophy with possible small areas of calcification. Extensive metabolic work up was normal. Chromosomal microarray, autoimmune work up, muscle biopsy, EMG were also normal.
Immunological work up was performed in 2017. Lymphocyte phenotype demonstrated: lymphocyte count (2400), CD3 85% (2040), CD4 48% (1152), CD8+++ 33% (792), reduced CD31+/CD45RA+/CD4+ 37%, CD19 7.2% (173), reduced CD27/CD19 memory B cells 3%, NK 5.6% (134). Activated CD8+ T cells were elevated at 55-64%. Ferritin was normal at 76ng/ml, triglycerides 1.93mmol/L, CRP 0.3mg/L, fibrinogen 1.3-2.0mg/L, full blood count and liver function normal. IgG 7.36g/L, IgA 0.28g/L and IgM 0.24g/L. Hair biopsy and telomere lengths were normal.
Whole exome sequencing was performed and confirmed homozygous mutations in PRF1 (c.673C>T). Subsequent perforin expression was found to be absent in both T and NK cells. The patient was therefore diagnosed with Familial HLH due to PRF1 mutations with isolated CNS involvement. Initial management was IT Methotrexate (x4 doses) with addition of IT Hydrocortisone and Ara-C (x3 doses), Alemtuzumab (total 2.5mg/kg) and IV Methylprednisone. Lumbar puncture prior to his first dose of IT therapy confirmed the presence of CSF lymphocytosis (93%) and activated CD8+ T cells of 83%, with no significant reduction following IT therapy. He required further Alemtuzumab for his first episode of systemic activation in the context of lymphocyte reconstitution and reactivation of CMV. He underwent a haploidentical HSCT on 01/11/2017 with reduced intensity conditioning with Alemtuzumab, targeted Busulfan and Fludarabine followed by cyclophosphamide post-transplant. To date his course has been complicated by severe mucositis, HSV infection and respiratory deterioration requiring a period of intubation.
This case highlights a rare presentation of Familial HLH due to homozygous mutations in PRF1 with isolated CNS involvement, and illustrates the complexities and need for awareness surrounding such a diagnosis.

**(19) Submission ID#417357**

**A Second Immunocompromised Patient with DNA Ligase I Deficiency**

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Introduction/Background: We present the second report in the literature of a patient with immunodeficiency, dysmorphic features, growth retardation, and a homozygous variant in the DNA Ligase I (LIG1) gene with associated absence of full-length LIG1 protein.
Results: This is a now 2 year old girl who was the fourth child of parents who are first cousins. She has one healthy older brother, a second older brother who died within 12 hours of birth of meconium aspiration, and an older sister who died at six months of age of an upper respiratory illness / pneumonia after a history of congenital anemia, poor weight gain, cardiomegaly and hepatomegaly. She was born at 37 weeks and spent the first five weeks of life in a neighboring hospital Neonatal Intensive Care Unit (NICU) for hepatomegaly, mild cardiomegaly (previously identified on fetal ultrasound) and congenital anemia (requiring transfusions). Her exam was and remains notable for weight, height, and head circumference below 3rd percentile, prominent forehead, hypotelorism with epicanthal folds, downslanting palpebral fissures, and low set, posteriorly rotated and prominent ears. After discharge to home from the NICU, her course was subsequently complicated by poor weight gain, chronic diarrhea beginning after her first rotavirus vaccine, and multiple deep vein thromboses. She then became critically ill at 6 months of age with respiratory failure, and was transferred to our institution for respiratory oscillator support. Absolute lymphocyte count on admission to our institution was 0.3 k/μl (total WBC 4.0 k/μl, ANC 2.7 k/μl) with agammaglobulinemia. She was diagnosed with and treated for Pneumocystis jirovecii pneumonia, gradually weaned from oscillator to room air, and was discharged home five weeks later on 0.4 mg/kg every other week IgG replacement. She has had no serious infections requiring hospitalization in the 18 months since. ALC has remained persistently below 1.0 k/μl with a corresponding uniform deficiency of T, B, and NK cells and no detectable TREC positive T-cells. Whole exome sequencing identified homozygosity for a c.914G>A coding region variant in the LIG1 gene not previously reported in the literature. A fibroblast cell line was

successfully established and western blot shows an absence of full-length LIG1 protein. Further molecular characterization is in progress.
Conclusions: This second reported case provides further evidence for DNA Ligase I deficiency as a distinct clinical entity comprising immunodeficiency, dysmorphic features, and growth retardation.

**(21) Submission ID#421430**

**A novel STAT1 gain-of-function mutation in an infant with mucocutaneous candidiasis, rectolabial fistula, and enteropathy**

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Introduction/Background: Chronic mucocutaneous candidiasis (CMC) is associated with a heterogeneous group of primary immunodeficiencies. Autosomal dominant STAT1 gain-of-function (GOF) mutations have been identified in up to 50% of patients with CMC. These mutations lead to impaired IL17A/F T cell immunity although the underlying mechanism is unclear. There seems to be no genotype-phenotype correlation. Recently, JAK inhibitor therapy has been reported to improve CMC and autoimmunity in patients with STAT1 GOF mutation.
Objectives: We describe an infant with CMC associated with a novel STAT1 GOF mutation.
Results: A 7-month-old girl was referred to our immunodeficiency clinic with chronic diaper rash since 2 weeks of life, failure-to-thrive, and history of labial abscess complicated by rectolabial fistula. She was subsequently diagnosed with food protein-induced enterocolitis syndrome triggered by cows milk-based formula. Laboratory evaluation revealed normal CBC with differential, lymphocyte subsets, mitogen response, immunoglobulin levels, antigen response to Candida, and neutrophil oxidative burst assay. Whole exome sequencing identified a de novo heterozygous variant in STAT1 (c.1627T >C, p.Cys543Arg). Further evaluation of this mutation revealed increased GAS (gamma activation sequence) reporter activity in response to IFNg stimulation suggesting that this is a gain-of-function mutation. The patient later developed significantly elevated liver enzymes while on Fluconazole treatment, Candida parapsilosis sepsis, granulomatous lesions in the liver, splenic lesions, intermittent thrombocytopenia and normocytic anemia. Sepsis and liver lesions resolved on Amphotericin treatment but other findings, including TPN dependency persisted. We are planning to initiate a JAK inhibitor therapy, Ruxolitinib.
Conclusions: This case is a possible genotypic and phenotypic expansion of CMC due to STAT1 GOF.

**(22) Submission ID#421112**

**Aberrant Innate-Like Lymphocytes Causing Atopy and Immune Dysregulation in a Patient With a Novel BCL11B Variant**

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Introduction/Background: B cell CLL/lymphoma 11B (BCL11B) is a zinc finger protein transcription factor with a multitude of regulatory functions in the integumentary, central nervous, cardiac, and immune systems. It is critical for T cell lineage commitment, development, differentiation, survival, and function. In addition, it also specifies the identity and function of innate-like lymphocytes, including T cells, innate lymphoid cells (ILCs), and invariant natural killer T cells (iNKT). However, little is known about its function in the human immune system, especially in the context of immune disorders.
Objectives: To understand the immunopathogenesis of a novel p.C826Y BCL11B variant.
Methods: Research study protocols were approved by our institutional Research Ethics Board. Two members of the family were enrolled (the index patient and her father). Written informed consent for genetic testing and participation was provided by the parents for the child. Genetic, bioinformatic, proteomic, and biochemical analyses were performed.
Results: We have identified the second described case of immune disease caused by a de novo heterozygous damaging variant of BCL11B (p.C826Y). This young girl presented with intellectual disability, microcephaly, severe atopy, eczema, alopecia totalis, and brittle nails. Extensive clinical immunophenotyping of patient blood showed initially unremarkable B and T cell populations. However, the patient possessed abnormal rare innate-like lymphocyte populations (iNKT, DN T cells). Using mass cytometry (CyTOF), a technique capable of concurrently analyzing 40 parameters in a single cell, we were able to examine various innate-like lymphocyte populations, including T cells, ILC1-3, and NK cells. We found that the patient possessed severely compromised numbers of T cells, thus potentially implicating the p.C826Y variant in T cell development and function.
Conclusions: The identification of decreased T cells in a patient with a p.C826Y variant of BCL11B suggests that BCL11B is important for human T cell development and provides novel insights into the roles of both BCL11B and T cells in regulating atopy and autoimmunity.

**(23) Submission ID#419273**

**ADA2 Deficiency: Case Report of a Rare Phenotype with ALPS and CVID-like presentation**

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Introduction/Background: Adenosine deaminase 2 deficiency caused by mutations in ADA2 gene is a newly recognized disorder. It is associated with a spectrum of vascular and inflammatory phenotypes, ranging from early onset recurrent stroke to systemic vasculopathy or vasculitis.
Objectives: We describe a 13 year old female patient with features of early onset immune thrombocytopenia (ITP), autoimmune hemolytic anemia (AIHA), chronic splenomegaly and variable abdominal lymphadenopathy. She was diagnosed with Evans-syndrome and treated with Rituximab at 19 and 27 month of age. From 3 years of age she developed recurrent infections, hypogammaglobulinaemia with specific antibody deficiency, progressively decreasing class-switched memory B cells, and increased CD3+CD4-CD8-//T cells (4%). Differential diagnosis included common variable immunodeficiency (CVID) or autoimmune lymphoproliferative syndrome and therefore a broad search for causative genetic defect was initiated. The parents are first cousins of Middle-Eastern origin suggesting an autosomal recessive inheritance. Patient was stable on long-term mycophenolate mofetil (MMF) and immunomodulatory dose (1g/kg/month) IVIG treatment.
Methods: Genomic DNA of the patient was sequenced with next generation sequencing technology. A panel of 250 genes linked to primary immunodeficiency was analyzed. The identified variant was confirmed by Sanger sequencing.
Results: Genetic testing revealed a homozygous pathogenic mutation in the ADA2 gene with one base pair duplication in exon 2 (c.144dup. p.Arg49Alafs*13) that creates a frame shift starting at codon Arg49. The new reading frame ends at a stop codon 13 positions downstream, likely resulting in a truncated protein. Plasma ADA2 activity of the patient was markedly reduced (0.2 mU/ml, normal 4.8-27.2) and confirmed the diagnosis of ADA2 deficiency. The parents of the patient are heterozygous carriers of the same mutation. Unlike most previously reported cases, this patient had an extended phenotype with no neurological evidence of vascular pathology, however brain MRI revealed two silent lacunar infarct or vasculitis related changes. We speculate whether the long-term MMF or IVIG therapy might be protective against vasculitis.
Conclusions: ADA2 deficiency may present with a wide spectrum of clinical phenotypes beyond classical vasculopathy. The diagnosis should be considered in patients with hematological autoimmune disease, splenomegaly and/or CVID like presentation. Better understanding of pathophysiology of ADA2 deficiency may help diagnosis and targeted treatment.

**(25) Submission ID#420511**

**Adenosine Deaminase (ADA)-Deficient Severe Combined Immune Deficiency (SCID): Analysis of Cases Enrolled in Protocols of the Primary Immune Deficiency Treatment Consortium (PIDTC).**

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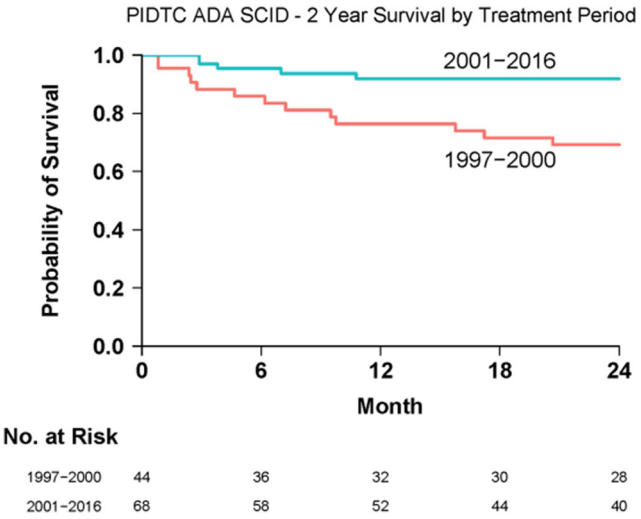
Introduction/Background: Adenosine deaminase (ADA) deficiency as a cause of severe combined immunodeficiency (SCID) is distinct from other forms of SCID in several ways. Historically, survival and clinical outcome of infants with ADA SCID have been inferior compared to infants with other SCID genotypes. There are multiple treatment modalities available for ADA-SCID, including enzyme replacement therapy (ERT), allogeneic hematopoietic cell transplant (HCT) and experimental autologous transplant of gene corrected cells (in recent years preceded by low dose busulfan), designated as gene therapy (GT). In addition, there is a growing body of evidence for effects of ADA deficiency on non-immunologic organ systems that may contribute to the historically poorer outcomes of these infants. Therefore, it is important to evaluate the cohort of patients with ADA SCID separately from other SCID cases.
Objectives: To capture incidence and treatment trends and to compare outcomes following available treatments for this rare inborn error of metabolism and other forms of SCID, the Primary Immune Deficiency Treatment Consortium (PIDTC), a network of 45 North American immunology and transplant centers, has collected standardized data for analysis.
Methods: 118 ADA SCID patients, first treated between 1977 through 2016, were enrolled from 26 centers (range 1-25 subjects/site). ADA accounted for 13% of the 926 total PIDTC SCID patients treated during that time. Patients were entered into either a retrospective protocol (PIDTC 6902, n=96) or a prospective protocol starting in 2010 (PIDTC 6901, n=22). ADA-SCID patients who received an HCT as first therapy entered either of two strata, as with other SCID patients in PIDTC studies, based on whether their initial presentation met definitions for typical (n=46) or leaky SCID (n=6); in contrast, patients initially treated with either ERT or GT were entered into a separate stratum (n=66).
Results: Sixty-four patients (54% of all ADA-SCID enrollees) had ERT as first therapy, but only 6 in this cohort received ERT as sole therapy; 58 went on to have subsequent HCT (n=30) or GT (n=28). There were various combinations of treatment cycles among these ADA-SCID patients. Most received HCT {+/- subsequent treatments} (44%), ERT followed by HCT {+/- subsequent treatments} (25%), or ERT followed by GT {+/- subsequent treatment} (24%); several patients received multiple successive treatment modalities, representing either failure of initial treatment or planned progression from ERT to cellular therapy. Two-year survival has improved over time from 69% in (1977-2000 to 92% 2001-2016 (p=0.007) (Figure 1). The survival for all other non-ADA SCID patients registered by PIDTC over these two eras were: 78% (1977-2000) and 81% (2001-2016). HCT (either as sole therapy or after ERT) accounted for >95% of cellular therapies between 1986 and 2007; in contrast, since 2007, GT was used as commonly as HCT (n=27 vs. n=29, respectively). There was a trend toward better two-year survival for patients receiving GT as first cellular therapy since 2001 (100%, n=28, all after initial ERT) compared to those receiving HCT over the same time period (87%, n=40, either as first therapy or after ERT), although this did not achieve statistical significance (p=0.073).




Conclusions: This study reveals the improved prognosis for patients with ADA SCID in recent years and the emergence of GT as a new treatment modality. Further analyses are investigating the impacts of prior infection and treatment modality, including effects of conditioning, on outcomes (survival, event free survival, clinical outcomes and completeness of immune reconstitution) for ADA-SCID in successive eras. This study may identify optimal treatment approaches for future ADA SCID patients.
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**(26) Submission ID#428054**

**Adherence to immunization as part of standard care of adult patients with diabetes mellitus**

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Introduction/Background: Patients with diabetes mellitus are immunologically vulnerable population to develop different types of microbial infections. Immunization has an important role in infection prophylaxis. In fact, Vaccines containing thymus-dependent antigens protect patients with diabetes as they produce massive and complex immune response and feature immunologic memory. The recommended vaccinations for patients with diabetes mellitus are influenza vaccination yearly and pneumococcal vaccination. In observational studies, influenza vaccine has been shown to be similarly effective in adults <65 years of age with diabetes as in older patients with or without diabetes [1]. Among immunocompetent elderly, vaccine efficacy of the 13-valent pneumococcal conjugate-vaccine (PCV13) was modified by DM with higher vaccine efficacy among subjects with DM [2]. The hepatitis B vaccination should be given to unvaccinated adults with diabetes mellitus who are ages 19 to 59 years. For older patients administration only after assessment of benefits and risks of acquiring hepatitis B virus (HBV). In fact, one review suggests that DM is associated with the progression of severe liver outcomes in adults with HBV [2]. On the other hand, tetanus and diphtheria vaccinations should be updated. In addition to, vaccinations such tick-borne encephalitis, meningococcal infections and other infections that put in risk diabetic patients travelling abroad. Accordingly, theres a variability of vaccines that can offer a preventive method to reduce morbidity, mortality, and medical expense. In our multicenter study among eastern province Saudi Arabia evaluated the degree of adherence of the physicians to the immunization recommendations for adult patients with diabetes mellitus Type 1 and type 2 to increased awareness of the immunization importance in diabetic patients.
Objectives: To increased awareness of the immunization importance in diabetic patients. In fact, Patients with diabetes mellitus are immunologically vulnerable population to develop different types of microbial infections. Immunization has an important role in infection prophylaxis.
Methods: This is a cross sectional study involving 500 adult patients with type 1 and type 2 diabetes mellitus using a questionnaire. Patients will be recruited from outpatient clinics including the primary care clinics and inpatient words of King Fahd Hospital- Al Khobar and other centers in the Eastern Province Saudi Arabia. After an informed consent, baseline data will be collected. Patients will be then asked if they received the recommended vaccines and who was the provider. Their knowledge regarding the needed immunization will be also tested. They will then be asked about frequency of upper respiratory tract infections and pneumonia they had over the last 2years.
Results: We are expecting to find low adherence to the recommended immunizations by the physician. We may also find that patients who received the vaccines has low incidence of related infections.
Conclusions: increased awareness of the immunization importance in diabetic patients and adherence to immunization as part of standard care of adult patients with diabetes mellitus that offer a preventive method to reduce hospitalizations, mortality, and medical expense.

**(27) Submission ID#428367**

**Analytic Validation of Oligonucleotide-Selective Sequencing Panels for Clinical Diagnostics of Primary Immunodeficiencies**

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Introduction/Background: INTRODUCTION: Genetics diagnostics of patients with suspicion of immunodeficiency has been affected by poorly validated genetic tests, lack of transparency, and testing solutions that are not optimized for maximal diagnostic yield.
Objectives: OBJECTIVE: Our goal was to develop a high quality next generation sequencing (NGS) platform based on oligonucleotide-selective sequencing, transparently and comprehensively validate its quality and performance, and report here our experiences with hundreds of patients suffering from retina disorders.
Methods: METHODS: We developed and validated a set of NGS tests (230 genes), with traceable sample sets, for detecting single-nucleotide variants (SNVs), insertions and deletions (INDELs) and deletions and duplications (DELDUPs). Our assay targeted the coding exons, the splice regions, and selected deep intronic variants. We also utilized this platform to diagnose hundreds of patients during 2016-17.
Results: RESULTS: We demonstrate, on average, 0.993 sensitivity, 0.999 specificity, 0.992 positive predictive value for detecting SNVs and 0.960,

0.884 and 0.667 sensitivity for detecting INDELs of 1-10, 11-20, and 21-30 bases. Repeatability and reproducibility of the assays were 0.994 and 0.998, respectively. 99.5% of the target regions were covered with over 15x sequencing depth. We showed that the assays had 0.748 sensitivity to detect single-exon DELDUPs and 1.000 sensitivity to detect copy number aberrations covering two or more exons. Using ACMG guidelines for variant classification a diagnosis was established in 20% patients that were sent for comprehensive panel analysis.
Conclusions: CONCLUSIONS: Our results demonstrate the analytic validity of the developed tests and show that the technology is well-suited for clinical diagnostics of inherited eye disorders. It also demonstrated a cost-effective diagnostic tool to simultaneously diagnose various types of mutations from SNVs to copy number variations.

**(28) Submission ID#428412**

**Assessment of calcium response in a non-phenotypic adult patient heterozygous for a purported pathogenic mutation in ORAI1**

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Introduction/Background: Loss of function (LOF) and null mutations in ORAI1 and STIM1 cause a rare autosomal recessive immunodeficiency by abolishing calcium release-activated calcium (CRAC) channel function and store-operated Ca2+ entry. The clinical presentation is characterized by SCID-like disease, dental enamel defects, muscular hypotonia and anhidrotic ectodermal dysplasia.
Objectives: Here we present the outcome of calcium assessments performed on lymphocytes from an adult patient with unusual infections and a purported novel single pathogenic variant in ORAI1.
Methods: The Ca2+ response in lymphocytes following activation by varying concentrations of non-cross-linked anti-CD3 was assessed by flow cytometry.
Results: The patient was a 47 year old female with a history of seasonal and medication allergies and recurrent sinus infections, who had recently developed an acute infection of her right first metatarsal joint. Cultures from the joint space grew Neisseria gonorrhea and atypical mycobacteria at two different time points. HIV screening was negative. Follow-up testing with the Mantoux test and QuantiFERON Gold suggested that she also had latent tuberculosis infection, for which she was started on rifampicin therapy. Immunologic evaluation revealed normal complete blood count and differential, normal T, B and NK cell counts, normal immunoglobulin G, A, and M levels, as well as normal responses to polysaccharide vaccines and normal T cell proliferative responses to mitogen and antigen stimulation. However, given the identification of atypical organisms from joint fluid cultures as well as latent tuberculosis (despite the lack of significant risk factors), genetic testing was recommended by her local physicians to rule out an underlying primary immunodeficiency. An Invitae Primary Immunodeficiency Panel identified a single novel pathogenic variant in ORAI1. She was then referred to our institution for further evaluation.
So far, individuals identified as heterozygous for LOF or null mutations in either ORAI1 or STIM1 have lacked any phenotype associated with CRAC channelopathy. However, there have been reports of abnormalities in calcium response in parents of a number of these patients, who are heterozygous for the disease-causing mutation. To examine the functional effect of the observed mutation in our patient, her freshly isolated PBMCs were loaded with indo-1 and the Ca2+ response of her lymphocytes were assessed by flow cytometry. This showed a dose-dependent decrease in the patients T cell response to non-cross-linked anti-CD3 in comparison to the normal control, i.e. there was a clear decrease in the patients Ca2+ response at 1 microgram/ml in comparison to the normal control, but the decrease was rectified upon stimulation with 5 microgram/ml or more of anti-CD3.
Conclusions: These findings provide functional support for the identification of a new pathogenic mutation in ORAI1. Nevertheless, it is not yet clear if the mutation has any mechanistic role in the patients recent clinical presentation.

**(29) Submission ID#428742**

**Association of IgA deficiency, encephalitis and epilepsy: a propos of 2 very similar cases**

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Introduction/Background: Patient 1: 10 years old boy, born to non-consanguineous parents, with hypothesis of autoimmune encephalitis (vasculitis), which was not characterized. CSF has already been routed to Barcelona 2 times and to Vienna 1 time. Refractory Epilepsy Remains (uses 5 drugs yet). He had continuous fever during the entire hospitalization. He had ADHD (took Ritalin for 2 years) and central auditory processing deficit.
One year ago he began to have fever for 3 days, improving later but evolving with bilateral otitis, predominantly on the left ear, accompanied by sinusitis. Soon after that he began presenting epilepsy that worsened severely. He was interned and took Acyclovir EV, associated with Hydantoinate (had Stevens-Johnson by the drug, suspending and improving quickly). In March had UTI + AMO, several infections requiring meropenem + Vanco, among others. Received Flebogamma 500 mg/day/5 days, twice; He also received weekly Rituximab for a few months. In use of carbamazepine, Clobazan, phenobarbital, Levotiracetam and Vigabatrin 3 times a day. Personal antecedents: Allergic rhinitis, bronchial asthma, recurrent otitis media, IgA deficiency.
Patient 2: Male, 9 years old, born to non-consanguineous parents, with history of repeated infections in the upper respiratory tract from one year of age with prolonged dry cough and sore throat in all episodes, treated with Dexamethasone without improvement, followed by antibiotics with resolution of the condition.
At age five, in February 2012, he had a new episode of sore throat and cough that lasted three months, improving spontaneously thereafter. In June 2012, new episode of sore throat with elevated fever and whitish plaques in the tonsils, being prescribed Benzetacil, without improvement in three days. He returned to the same emergency room, the antibiotic was replaced by Zinnat (axetil-cefuroxime), but within the hospital he began to convulsionate, entering into an epileptic crisis, being hospitalized for 33 days in this service and being transferred to another hospital specialized in pediatrics, intensely investigated and treated with partial improvement of the condition. He remained in coma, not walking and talking for some months, recovering slowly with physical therapy and speech therapy.
Of relevant exams have: reduced IgA, but before it was normal (probably induced by anticonvulsants); Full-body magnetic resonance imaging demonstrates generalized lymphadenomegaly and hepatosplenomegaly; PET-CT showing signs of hypoperfusion in temporal (right), occipital and cerebellum regions (suggestive of hypoperfusion - vasculitis?)

My first impression was of possible mevalonate kinase (MVK) or autoimmune lymphoproliferative syndrome (ALPS) deficiency. With these tests described above, I believe that the first diagnostic suspicion is that the epilepsy was triggered by hemophagocytosis in the central nervous system and consequent extremely severe epilepsy, triggered by EBV infection.
Objectives: To compare the clinical and genetic similarities and differences of both patients.
Methods: Both patients wer submitted to whole exome sequencing looking for the genetic alterations associated to the disease of these patients.
Results: WES of patient 1 showed an allelic variant in the gene of RAI1 (c.4625G.A; P.arg1542Gln) possibly pathogenic and that could be related to the clinical features of the patient.
WES of patient 2 showed an allelic variant in the gene of CD27 (c.281G>A; p.Arg94His) heterozygous and of uncertain significance. Another allelic variant was found in the gene of BTK (c.707G>A; p.Arg236Gln) classified as of uncertain significance and hemizygous (as BTK is in chromosome X). The expression of both proteins evaluated by flow cytometry is normal, decreasing but not abolishing the possibility of pathogenicity.
Conclusions: The similarity of the clinical presentations is striking, but the genetic alterations are totally different, leading to the presentation of this abstract.

**(30) Submission ID#427965**

**Autoantibodies to Interferon in a Patient with Treatment Resistant Mycobacterial Infection and Autoimmunity**

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Introduction/Background: Autoantibodies to Interferon-gamma (INF- ) have been associated with adult onset immunodeficiency in patients of Asian origin. Pathogens that cause infections in these patients include Mycobacterium avium-intracellularae (MAI), non-Typhoidal salmonella, Cytomegalovirus, Penicillium marneffei, and Varicella zoster virus.
Methods: Chart Review of One Patient
Results: We present a thirty-two-year-old Filipino female, with Sjogrens Syndrome, Penicillin and Vancomycin allergy, and shellfish allergy suffering from recurrent MAI spinal osteomyelitis.
After three months of conservative management of back pain, MRI showed an abscess at L4/L5 and L5/ S1 vertebrae, which was diagnosed as acid fast bacilli on biopsy. She was treated for Mycobacterium tuberculosis with Rifampin, Isoniazid, Pyrizinamide, and Ethambutal with subsequent change in antibiotic therapy after six weeks once cultures grew MAI. MRI showed spread of abscess to L3-S2 vertebrae. Three months into treatment, she was found to have a new abscess at a different spinal site, and antibiotics were again changed. Two months later, she had recurrence of disease with multiple large iliopsoas abscesses, cutaneous fistulas, insufficiency fractures of the sacrum bilaterally, and osteonecrosis of the L4 vertebra, requiring extensive surgical debridement. Medications were adjusted and she was referred to immunology eight months after initial presentation to Infectious Disease.
Laboratories were notable for elevated IgG (2580 mg/dL) and IgA (474 mg/dL) and decreased CD4 (425 cell/uL) and CD16/56 (49 cell/uL) cells. Serum electrophoresis showed low albumin, elevated gamma fraction, and polyclonal gammopathy. Specific antibody titers, lymphocyte proliferation assay, CH50, and immunofixation were within normal limits. Cytokine panel was significant for elevated IL-2 receptor CD25 (3860 pg/mL), IL-6(15 pg/mL), and IL-13 (12 pg/mL), and normal TNF, INF, IL-1, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12, and IL-17. Further serologic testing was positive for autoantibodies to INF-. Patient continues treatment with IV antibiotics and is awaiting enrollment in a Rituximab trial.
Conclusions: Autoantibodies to INF- should be considered in patients of Asian origin presenting with adult onset immunodeficiency, particularly those with severe or recurrent infection with MAI. A high level of suspicion is required to make this diagnosis: failure to consider this disease entity leads to delay in diagnosis with potentially significant consequences for the patient.

**(31) Submission ID#420431**

**Autoimmunity and Rheumatologic Complications in Patients with Common Variable Immunodeficiency (CVID)**

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Introduction/Background: Autoimmune and inflammatory conditions are common in CVID. These have been associated with increased morbidity and mortality.
Objectives: We sought to further understand and evaluate the prevalence of autoimmune and rheumatologic manifestations in patients with common variable immunodeficiency (CVID).
Methods: We performed a retrospective analysis of CVID patients with rheumatologic/autoimmune complications in the Partners HealthCare CVID cohort. We evaluated baseline patient characteristics as well as autoimmune and rheumatologic complications in this cohort of patients.
Results: In the Partners CVID cohort, 120/210 (57%) had autoimmune or rheumatologic disease. Autoimmune cytopenias were reported in 37/210 (18%) patients, including Coombs positive autoimmune hemolytic anemia (n=18), idiopathic thrombocytopenic purpura (n=30), and autoimmune neutropenia (n=10). Autoimmune thyroid disease was reported in 43/212 (20%) patients, including hypothyroidism (n=25) and Hashimotos thyroiditis (n=18). Inflammatory arthritis was present in 35/210 (17%), most commonly seronegative rheumatoid arthritis (RA) (n=18), followed by inflammatory arthritis (n=8), seropositive RA (+RF or +CCP antibody) (n =6), psoriatic arthritis (n=2), and juvenile idiopathic arthritis (n=1). Systemic autoantibody disease was diagnosed in 26 patients (12%), with diagnoses including vasculitis (n=7), systemic lupus erythematosus (n= 6), polymyalgia rheumatica (n=4), antiphospholipid syndrome (n=3), mixed connective tissue disease (n=2), CREST/ scleroderma (n=2), myositis (n=2), Sjogrens syndrome (n=2), and discoid lupus erythematosus (n=1). Inflammatory neuropathy was diagnosed in 21 patients, with small fiber polyneuropathy (n=10), uveitis (n=3), myasthenia gravis (n=2), Bells palsy (n=2), and multiple sclerosis (n=1). Autoimmune skin conditions were diagnosed in 24 patients with diagnoses including psoriasis (n=13), alopecia (n=8), and vitiligo (n=3). While the mean IgM was higher in the patients with autoimmune/rheumatologic manifestations than in other CVID patients (83 vs 57mg/dL), this difference did not reach statistical significance (p=0.15).
Conclusions: Autoimmune and rheumatologic complications are present in over half of patients with CVID. Increased vigilance for autoimmune and rheumatologic complications is important as survival outcome are worse in CVID patients with non-infectious complications as previously described. Further evaluation of these patients to understand the mechanism of immune dysregulation is essential, as this may promote targeted therapies and improve clinical outcomes.

**(32) Submission ID#419509**

**Biochemical characterization, pathogen safety and stability of Octanorm, a new 16.5 % subcutaneous immune globulin product**

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Introduction/Background: Immunoglobulin concentrates have been successfully used for decades to treat patients with primary or secondary immunodeficiency disorders. This treatment has substantially decreased the frequency of life-threatening infections in these patients. Octanorm is a newly developed maltose-formulated subcutaneous immune globulin (human) 16.5% liquid for the treatment of patients with primary immune deficiency (PID) and secondary immune deficiency (SID).
Objectives: Biochemical and physico-chemical properties were investigated.
Methods: Molecular size distribution of monomers, dimers, polymers and fragments were determined (according to European Pharmacopeia (EP) monograph 8.0) by size exclusion chromatography (SEC). IgG and IgG subclass concentrations were quantified by respective nephelometric methods. Functionality of the IgG was demonstrated by measurement of Fc function, opsonophagocytosis and Fc gamma receptor binding assays. Dynamic light scattering measurement and size exclusion chromatography were used to characterize the integrity of the IgG molecule. Measurement of potential procoagulant activity was done by NATEM and TGA (FXIa-like activity). The capacity of the octanorm manufacturing process to robustly inactivate/remove pathogens was investigated in spiking experiments with prions and viruses.
Results: Octanorm contains more than 96 % of human IgG and is characterized by an especially low content of polymers and aggregates, low viscosity, low isoagglutinin titres, low IgA and IgM contents with a broad spectrum of antibodies against infectious agents. It has a distribution of immunoglobulin G subclasses closely proportional to that in native human plasma. In the final product, potential procoagulant activity is not detectable. Functionality and physico-chemical properties of the IgG molecules were demonstrated by state-of-the-art methods. Virus safety of octanorm is obtained via a combination of three validated orthogonal methods as part of the manufacturing process: cold-ethanol fractionation, Solvent/Detergent (S/D) and pH 4 treatment. A substantial depletion of prions during the manufacturing process was demonstrated.
Conclusions: Octanorm is a state-of-the-art subcutaneous immunoglobulin. Based on the excellent stability the intended shelf life of octanorm is 24 months stored at +2°C to +8°C protected from light. Within its total shelf life the product can be stored at room temperature up to +25°C for up to six months. Efficacy and very good tolerability of this new subcutaneous normal immune globulin 16.5% were shown in a clinical phase III study performed in 18 centers in North America and Europe.

**(33) Submission ID#428663**

**Carriage of Streptococcus pneumoniae and Haemophilus influenzae Italian CVID population: immunological and clinical correlates**

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Introduction/Background: Primary antibody deficiencies (PAD) are characterized by defective Ig production resulting in high susceptibility to bacterial infections, especially caused by S. pneumoniae and H. influenzae. There is a limited evidence on the rate of microbial airway epithelial colonization and on the role of bacterial carriage on the development of recurrent respiratory tracts infections in such populations.
Objectives: The aim of this study was to investigate the prevalence of S. pneumoniae and Haemophilus influenzae colonization in PAD adults in Italy and its clinical and immunological correlates.
Methods: Nasopharyngeal and oropharyngeal swabs were obtained from 93 CVID and 16 patients with idiopathic primary Hypogammaglobulinemia (IPH) over 18 years of age and under Ig replacement treatment during the period October 2016-April 2017. Presence of S. pneumoniae and H. influenzae was investigated using conventional cultural methods and RT PCR. S. pneumoniae isolates were serotyped by the Quellung reaction; capsular type of H. influenzae isolates was determined by PCR. The pattern of associations between the two species and potential risk factors were investigated. Respiratory infections rate was recorded over 12 months of follow up.
Results: Among CVID prevalence of carriage assessed by traditional culture was 11% and 27% for S. pneumoniae and H. influenzae, respectively. RT PCR allowed to identify a higher rate of carriage of S. pneumoniae and H. influenzae compared to standard culture. CVID and IPH had not different rate of pneumococcal colonization, whereas CVID had higher rate of H. influenzae carriage identified by a culture methods and RT PCR.
No synergistic association between S. pneumoniae and H. influenzae colonization was observed. Among CVID, S pneumonia and H. influenzae carriage were associated to low IgA and IgM levels. CVID under antibiotic prophylaxis did not have an increased prevalence of carriage. No association was found between carrier status detected by culture and having chronic lung disease, bronchiectasis or the rate of infections during the follow up. RT PCR identified merely the association between IgM levels and H. influenzae carriage. Antibiotic resistance from isolated stains was also assessed.
Conclusions: This is the first study assessing the prevalence of S. pneumonia and H. influenzae carriage in CVID.

**(34) Submission ID#421125**

**Cartilage hair hypoplasia detected on newborn screening**

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Introduction/Background: A 4-month old female was born to unrelated parents of Eastern European origin with undetectable TRECs on newborn screening. Skeletal dysmorphism was noted during pregnancy.
Objectives: To discuss clinical challenges in a subject with congenital hair hypoplasia detected on newborn screening with preserved T-cell mitogen responses but declining naive T-cell counts. Parents are Jehovah's witnesses, which adds complexity to clinical decision-making.
Methods: Immune evaluation included complete blood count, lymphocyte subsets, flow cytometry to define T-cell subsets, immune globulins, repeat TREC assay from peripheral blood, human immunodeficiency virus (HIV) and cytomegalovirus (CMV) DNA PCR, screen for maternal engraftment, chromosome microarray analysis, lymphocyte proliferation to mitogens, T-cell proliferation to interleukins, T-cell receptor V-beta diversity analysis by spectratyping, and thymic ultrasound.
Results: Repeat TREC assay from peripheral blood on day 17 confirmed undetectable TRECs. CD4+ thymic emigrants were low at 147 (Reference 733-3181 cells/uL). Naïve CD4+ and CD8+ T cell compartments declined as did naïve CD4+ T cells from 48 to 17 cells/uL and naïve CD8+ T cells from 33 to 5 cells/uL.
Lymphocyte proliferation to mitogens was preserved except for the CD19+ response to pokeweed mitogen. Interleukin proliferation of CD45+ lymphocytes was slightly decreased after stimulation with anti-CD28 (14%, normal >38%). However, T-cell proliferation with other interleukins (IL-2, anti-CD3+Il-2, antiCD28 as %CD3) was preserved. The T cell receptor repertoire had intermediate diversity.
Antibody replacement therapy was prescribed for declining IgG with no history of severe infections except for rhinovirus prior to discharge from the NICU at 8 weeks, during which she required continuous positive airway pressure (CPAP) therapy. The viral load for CMV and HIV DNA were undetectable. Breastfeeding was discontinued early because the mothers CMV serology revealed positive CMV IgG. The child has had intermittent anemia, which is common in patients with CHH.[2] Tests for autoimmune hemolytic anemia were not performed because the patient required IVIG from an early age. The parents of the child are Jehovahs witnesses, complicating requests for blood transfusions.
Conclusions: This case highlights challenges in clinical decision making for a newborn with CHH identified on NBS. T-cell function is preserved, but declining naïve T cell counts and absent TRECs lead us to consider hematopoietic stem cell transplant (HSCT). Indication and timing of elective HSCT is unclear and may depend on the natural progression of disease, including infections and anemia.
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**(35) Submission ID#417998**

## Cartilage hair hypoplasia: Heterogeneity in clinical features and management among siblings

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Introduction/Background: Cartilage-hair hypoplasia (CHH), caused by mutations in the ribonuclease mitochondrial RNA-processing (RMRP) gene, is associated with diverse immune abnormalities including combined immune deficiency (CID). Most patients with CHH are managed with supportive measurements, while few have received allogeneic hematopoietic stem cell transplantations (HSCT). The progression of the immune abnormalities and the impact of HSCT in patients with CHH and CID have not been well characterized.
Objectives: To characterize the progression of the immune abnormalities and the impact of HSCT in patients with CHH and CID
Methods: The clinical and laboratory findings of 2 siblings diagnosed in infancy with CHH and CID due to the common 70 A>G mutation in RMRP, including the effects of HSCT performed in 1 of them, were compared.
Results: Both patients suffered from recurrent respiratory infections at early age with reduced T cells numbers and responses. Patient #1 immune function continued to deteriorate leading to HSCT from an HLA-matched sibling at 4.5 years of age. The patient suffered acute and chronic graft versus host disease of the skin with residual mild joint contractures and scleroderma-like skin changes. Seven years after HSCT patient #1 has normal immune function. Immune evaluations of patient #2 in the first years of life indicated mild improvement. The patient did not have a suitable related HSCT donor and the family elected to continue with supportive care. At 7 years of age, patient #2 is clinically well and thriving with persistent T cell abnormalities.

| | Shortly after birth | 2y | 3y | 7y | 11y |
|---|---|---|---|---|---|
| Patient 1 | | | | | |
| CD3 cells/ul | NA | **867 (>900)** | **652 (>900)** | 2301 (>700) | 1405 (>800) |
| CD4 cells/ul | NA | **430 (>500)** | **356 (>500)** | 1439(>300) | 754(>300) |
| CD8 cells/ul | NA | **204 (>300)** | **154 (>300)** | 772(>300) | 452(>300) |
| PHA SI* | NA | 50% | **6%** | NA | 91% |
| CD3-16+56+ cells/ul | NA | 182 (>100) | 175 (>100) | **979 (<900)** | 508 (>70) |
| IgM/G/A gram/l | NA | 0.9/8.3/0.6 | 1.1/10.7/1.1 | 0.4/7.8/0.5 | 0.5/8.3/1.0 |
| Height c"m | NA | 72 | 76 | 88 | 100 |
| Patient 2 | | | | | |
| CD3 cells/ul | **647 (>2300)** | **749 (>900)** | 1235 (>900) | 890 (>700) | NA |
| CD4 cells/ul | **530 (<1700)** | **490 (>500)** | 799 (>500) | 531 (>300) | NA |
| CD8 cells/ul | **60 (<400)** | **132 (>300)** | **135 (>300)** | **167 (>300)** | NA |
| PHA SI | **6%** | **25%** | **23%** | **30%** | NA |
| CD3-16+56+ cells/ul | **1451 (<1400)** | 534 (<1000) | 482 (<1000) | 461 (90-900) | NA |
| IgM/G/A gram/l | 1.2/4.0/0.3 | 2.1/1.3/10.7 | 1.4/10.1/1.4 | 1.2/11.4/1.2 | NA |
| Height c"m | 49 | 67 | 75 | 95 | NA |

Conclusions: Close monitoring of immune function in early life for patients with CHH and CID as well as the availability of suitable donors assists in determining management, including HSCT

**(36) Submission ID#418390**

**Case report of Leukocyte Adhesion Deficiency in a pediatric patient in Lima-Peru**

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Introduction/Background: Leukocyte Adhesion Deficiency (LAD) represents a group of distinct inherited disorders, which inhibit the normal extravasation of neutrophils and their recruitment to sites of infection or inflammation.
Objectives
PRESENTATION OF CASE: The patient is a girl of 8 years old with no history of Primary Inmunological Disease (PID) in family members, including a healthy brother (currently 12 years old). She received all the immunization schedule until 1 year old (according to Peruvian schedule). She had several admission to the hospital since newborn; the 1st hospital admission was at 15 days of life with diagnosis of: sepsis, pneumony, onphalitis (Leukocytes count: 70 000), she had 3 more hospital admission and the leukocytes count were always above 25000. In summary, she presented other infections besides the ones admitted at hospital like: 2 episodes of sepsis, 1 episode of pneumony, 5 episodes of cellulitis (left eye, left elbow, right thigh and vaginal (2), 27 episodes of otitis, 7 episodes of tonsillitis, 6 episodes of diarrhea, 5 episodes of rhinoadenoiditis, 5 episodes of sinusitis, 3 episodes of gingivitis and 1 episode of whooping cough. She received different antibiotics for treatment, even broad-spectrum as Vancomycin, Meropenem and Cefepime.
The diagnosis of Leukocyte Adhesion Deficiency (LAD) was made at 1 year 9 months by clinical features like delaying in separation of umbilical cord, recurrent infections and persistent leukocytosis>25000.
Flow citometry was taken at 3 years old, resulting CD11b/CD18: 0,21%.
Results: DISCUSSIONF: or the severe phenotype, in which leukocytes express <1% of normal levels of CD18, death occurs at an early age because of severe infection unless patients receive bone marrow transplant. However, it does not happened with her so we suspected in a possible reversion in LAD1 so we took a second flow citometry at 5 years old and the results were: Total leukocyte: 27870; Linfocyte: 6777 CD11b+/CD18+: 0,04%; Granulocyte:17438 CD11b+/CD18+: 1,41% and the conclusion was C3 receptor (CD11b/CD18) absent in linfocyte, monocyte and granulocyte. Molecular study was taken at 7 years old, resulting a homozygous substitution c.562C>T identified in exon 5, causing a nonsense mutation: p.Arg188, confirming LAD1 diagnosis.
She is, currently, receiving profilactic antibiotic and antimicotic which has reduced considerably recurrent infections. It seems that our patient may have a mixed phenotype due to a clinical expression, which may not require Hematopoietic cell transplantation (HCT) despite features of the severe type.
Conclusions
CONCLUSION: We conclude that the clinical evolution of this patient is unsual because she has severe LAD1, she has not transplanted yet, profilaxis treatment has improved to decrease frecuency of infections, she exceeded life expectancy and last flow citometry confirmed that LAD1 was not reverted.

**(37) Submission ID#406485**

**CD40 ligand deficiency causes functional defects of peripheral neutrophils that are improved by exogenous IFN-**

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Introduction/Background: Patients with X-linked Hyper-IgM syndrome (X-HIGM) due to CD40 ligand (CD40L) deficiency often present with low blood neutrophil counts. However, even when not neutropenic and despite immunoglobulin (Ig) replacement therapy, CD40L-deficient patients are susceptible to life-threatening infections by opportunistic pathogens, suggesting impaired function of phagocytes, and requiring novel therapeutic approaches.
Objectives: To analyze whether peripheral neutrophils from CD40L-deficient patients display functional defects and to explore the in vitro effects of recombinant human interferon (rhIFN)- on such cells.
Methods: We investigated the microbicidal activity, respiratory burst and transcriptome profile of neutrophils from CD40L-deficient patients. In addition, we evaluated whether the lack of CD40L in mice also affects neutrophil responses.
Results: Neutrophils from CD40L-deficient patients exhibited defective respiratory burst and microbicidal activity which were significantly improved in vitro by rhIFN-. Similar to humans with CD40L deficiency, CD40L-deficient mice were found to have defective neutrophil responses. Moreover, neutrophils from CD40L-deficient patients showed reduced CD16 protein expression and a dysregulated transcriptome profile suggestive of impaired differentiation.
Conclusions: Our data suggest a non-redundant role of CD40L-CD40 interaction in neutrophil development and function that could be improved in vitro by rhIFN-, indicating a potential novel therapeutic application for this cytokine.

**(38) Submission ID#426424**

**CD96 correlates to human NK cell exhaustion and predicts the prognosis of HCC patients**

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Introduction/Background: To date, blocking CD96 has not been demonstrated to be of substantial benefit in patients with cancer.
Objectives: Here, we demonstrated that human CD96 works as a checkpoint in hepatocellular carcinoma (HCC) patients.

Methods: In this study, through the use of 23 healthy livers, paired peritumoural tissues (PT) and intratumoural tissues (IT) from 236 HCC patients.
Results: Increased expression of CD96 on NK cells was observed in intratumoral but not peritumoral regions, along with increased expression of its ligand CD155 and a poor prognosis. Human CD96+ NK cells exhibited functional exhaustion, showing decreased IFN- and TNF- productions, impaired cytolysis in response to in vitro stimulation, and high gene expression of IL-10 and TGF-1 with low expression of T-bet, IL-15, Perforin and Granzyme B by global transcriptomic analysis of sorted CD96+ and CD96- NK cells. Blocking TGF-1 specifically inhibited CD96 expression and reversed the dysfunction of NK cells. In addition, we compared other two receptors, CD226 and TIGIT, which share common ligand CD155 with CD96, and found CD96 plays a more important role in NK exhaustion.
Conclusions: These findings indicate that human CD96+ NK cells have features of functional exhaustion, suggesting that CD96-CD155 blockade has the potential to restore immunity against liver tumors by reversing NK cell exhaustion.

**(39) Submission ID#428151**

**Characterization of Patients and Carriers of P47phox Chronic Granulomatous Disease by Flow Cytometric Analysis of P47phox Expression and Droplet Digital PCR Analysis of NCF1**

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Introduction/Background: Mutations in NCF1 (encoding protein p47phox of the NADPH oxidase) result in an autosomal recessive form of chronic granulomatous disease (CGD), a rare genetic disease with impaired phagocyte production of reactive oxygen species and recurrent infections. Diagnosis of p47phox CGD is based on abnormal dihydrorhodamine assay and absence of p47phox protein by immunoblotting; however, these assays fail to diagnose carriers of NCF1 mutations. Instead, carrier status is inferred after the birth of a child with p47phox CGD. Furthermore, identification of the specific genetic defect in patients with p47phox CGD is complicated by two highly conserved (>98%) pseudogenes. The NCF1 gene has a GTGT at the start of exon 2, while the pseudogenes (NCF1B and NCF1C) delete one GT (GT). In p47phox CGD, the most common mutation in NCF1 is GT causing c.75_76delGT; p.Tyr26fsX26. Sequence homology between the wild type gene and pseudogenes precludes using standard Sanger sequencing to identify specific mutations in NCF1.
Objectives: To identify phenotypic and genotypic differences that facilitate the diagnosis of patients and carriers with p47phox CGD.
Methods: Expression of p47phox in neutrophils is determined by fixing and permeabilizing whole blood with IntraPrep, and then incubating with either anti-p47phox antibody or its corresponding isotype. AlexaFluor 488-conjugated secondary antibody is used to detect the target antigen. Expression of p47phox is based on the mean fluorescence intensity of cells within the neutrophil population gated using forward and side light scatter. Differential expression of p47phox by flow cytometry is validated using quantitative immunoblotting. To screen for the GT mutation, a droplet digital polymerase chain reaction (ddPCR) with two distinct probes recognizing either the wild-type GTGT sequence or the GT sequence was used to quantitate the ratio of GTGT vs. GT copies. A second ddPCR reaction established copy number by comparing one probe for an invariant region of NCF1/NCF1B/NCF1C and a second probe for the single-copy telomerase reverse transcriptase gene, TERT. The results of these two assays were combined to determine the total number of GTGT-containing and GT-containing NCF1 copies.
Results: Analysis of p47phox expression in permeabilized neutrophils determined that neutrophils from p47phox CGD patients had negligible p47phox expression; neutrophils from p47phox CGD carriers exhibited ~60% of p47phox expression compared to healthy volunteers independent of the mutation in NCF1. Of all p47phox CGD patients tested by ddPCR, 83.2% (109/131) exhibited 0 copies of GTGT, 6.9% (9/131) exhibited 1 copy of GTGT (compound heterozygotes with 1 non-GT mutation), and 9.9% (13/131) exhibited 2 copies of GTGT (two non-GT mutations). Moreover, ddPCR can identify the carriers among kindreds within the GT p47phox CGD families. Unexpectedly, among normal subjects tested, only 78.8% exhibited the expected 2 copies of GTGT per 6 total NCF1/NCF1B/NCF1C copies, designated 2/6; a significant number exhibited more than two copies of GTGT (14.6% with 3/6, 1.9% with 4/6); others exhibited NCF1/NCF1B/NCF1C copy number variation (2.8% with 2/7 and 1.9% with 2/5).
Conclusions: Flow cytometric analysis of neutrophil intracellular p47phox staining provides a quick method to identify patients and carriers of p47phox CGD.
Droplet digital PCR can be used to identify patients and carriers of p47phox GT, the most common mutation in p47phox CGD
Copy number variation is observed at the NCF1 locus among normal subjects tested.
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**(40) Submission ID#418435**

**Chronic EBV Infection as the Sole Manifestation of a Novel Homozygous Splice Site Mutation in the CD3 Gene**

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Introduction/Background: Defects in the CD3 subunits of the TCR/CD3 complex account for a small percentage of the SCID presentations. CD3 deficiencies are characterized by profound and T-cells lymphocytopenia, and normal numbers of B- and natural killer (NK) cells in the peripheral blood (T-B+NK+ SCID). Thymocyte development from double negative stage to double positive stage results arrested. Affected individuals typically present with severe and opportunistic infections in the early infancy.
Objectives: To evaluate possible underlying immunodeficiency disorder in the setting of chronic EBV infection.

Methods: Here we report our experience of a patient with an atypical presentation of CD3 deficiency.
Results: A 7-year-old girl previously healthy, first generation American-born of Gambia immigrants was referred to our clinic for further evaluation of chronic EBV infection. One year before presentation, she developed bilateral parotid enlargement, cervical, axillar, and hilar lymphadenopathy, bronchiectasis, and pulmonary nodules. Relevant previous studies included: EBV viremia (PCR quant 63,000 copies), EBV IgG and EA positive whereas IGM and EBNA were negative. Peripheral blood phenotyping was not suggestive for malignant process and cervical lymph node biopsy was consistent with a reactive process without evidence of a clonal lymphoproliferative disorder and BAL studies positive for EBV only. HIV and TB both negative. She was otherwise thriving with no history of recurrent infections and family history was negative for consanguinity or immunodeficiency. Our evaluation revealed: normal blood counts, EBV PCR quant 206,677 copies, normal immunoglobulin levels and vaccine titers. She had a normal Lymphocyte subsets, but skewed CD45 RA/RO consistent with low thymic output (very low CD4 naïve T cells (5.8%), low CD31+ naïve T cells (36.7%), and elevated TEMRA (42.6%)), poor mitogen, and antigen responses. All these findings were suggestive of a SCID like phenotype; therefore, SCID Next Generation Sequencing Panel was pursued, and showed a novel homozygous splice site mutation (C.55 +1 G>T) in the CD3 gene.
Conclusions: A homozygous mutation in the CD3 gene might not necessarily imply profound T-cell lymphopenia. Though this patient did not present with classic clinical course, and immune findings; her inability to clear EBV with persistent significant viremia does support a T-cell immune deficiency. She remains at risk for EBV-associated lymphoproliferative disorder, infections, and autoimmunity. BMT will be a curative treatment option. However, it is difficult to predict evolution of clinical phenotype given the atypical presentation. This case illustrates the importance of contextual interpretation of clinical findings, laboratory data, and genetic analysis for treatment approach.

**(41) Submission ID#421263**

**Clinical challenges of patient with POLE-1 deficiency and progressive immunological decline**

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Introduction/Background: Pol is multi-subunit polymerase that includes both POLE1 with catalytic activity and additional POLE2, 3 and 4. This holoenzyme plays a key role in proofreading damaged DNA and is required for proper DNA replication in proliferating cells, such as lymphocytes. Germline mutations are linked to rare cause of primary immunodeficiencies whereas somatic mutations are described in colon cancer.
Primary immunodeficiencies are reported POLE-1 deficiency in members of a large consanguineous French kindred and a Palestinian female with FILS (facial dysmorphism, immunodeficiency, livedo, and short stature). All reported cases with POLE1 deficiency have homozygous intronic splice site variant (c.4444+3A>G) that result in a deletion of exon 34 which lead to subsequent frame shift (from p.S1483V onwards) and a premature stop codon at position 1561; this transcript results in a degraded product. The proportion of the POLE1 transcript in T lymphoblasts is significantly lower (10%) in patients then carriers or healthy individuals.
Objectives: Hereby we describe the clinical progression and treatment challeneges of the Palestinian female with POLE-1 deficiency secondary to homozygous g.G4444+3 A>G substitution.
Results: Initially patient presented with viral and recurrent ear infections and CMV viremia. With age, patient had less episodes of infections even with intermittent pause in immunoglobulin replacement therapy (IgRT), however had multiple admissions for fever of unknown origin with negative cultures but increased ferritin level (3300 ng/mL) and low platelet count (46,000 count/mL). Autoimmune and inflammatory complications were not reported among the French kindred. Her skin also worsened with poor wound healing and scarring throughout that hinders IgRT via subcutaneous route. Furthermore, IgRT with intravenous administration has resulted in symptoms of aseptic meningitis likely related to the underlying inflammatory state.
In addition, patient shows decline in immune dysfunction. Initially, she had normal immunoglobulin G (IgG) however by 5 years of age, she developed hypogammaglobulinemia with low IgM unlike in the French family with patients. Also, pneumococcal, diptheria, tetanus titers were non-protective off of immunoglobulin replacement therapy (IgRT). Beyond low switched memory B cells, patient also developed low B cell by 5 yo age.
T cell dysfunction continued to decline from decreased lymphocyte proliferation to antigens at 2 yo age to fully absent lymphocyte proliferation to antigens and mitogens. Naïve CD4 and CD8 compartments continue to be preserved.
Currently management challenges include treatment strategies for thrombocytopenia, inflammation and progressive skin disease that complicates the proper selection of route for optimal IgRT.
Conclusions: Beyond progression of immunological decline, our patient developed inflammatory phenotype with age. The progression of her immunological decline may be related to further decrease in the proportion of the wild type POLE1 transcript and yet to be examined.
Overall, clinical follow up is essential in patients with POLE-1 deficiency as phenotype can change with age and may pose new challenges. Longitudinal follow up studies are needed to uncover the potential role of germline POLE1 and POLE2 pathogenic variants in cancer susceptibility.

**(42) Submission ID#419991**

**Clinical Characteristics and Genetic Profiles of Severe Combined Immunodeficiency: a Single Center Experience in China from 2004 to 2016**

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Introduction/Background: Severe combined immunodeficiency (SCID) is a heterogeneous group of rare inherited primary immunodeficiency diseases (PIDs). To date, its clinical manifestations and gene mutation analysis is poorly characterized in Chinese population.
Objectives: We retrospectively reviewed patients with SCID referred to our hospital from 2004 to 2016, and summarized their clinical manifestations and genetic features.
Methods: Distribution of lymphocyte subsets from peripheral blood were examined by flow cytometry. Targeted gene capture combined with next-generation sequencing technology and Sanger sequencing were used to find out related gene mutation.
Results: Among 725 patients with PID, 95 (13.1%) were diagnosed as SCID. The mean time of delay in the diagnosis was 2.76 months (range, 0.4-22 months). Fifty-six of the 95 patients (58.95%) died by the end of 2016 with the mean age being 8.55 months. Eight patients received

HSCT, one survived, who was transplanted twice. BCG complications occurred in 30 of the 66 patients who received BCG vaccination. Transfusion-induced GVHD occurred in 8 patients. Total 30 mutations in IL-2RG were identified in 42 patients, including 18 novel mutations.
Conclusions: In China, diagnosis of SCID has improved over the last decade, although a much higher number of cases had been expected. Complications of BCG vaccine are an important warning sign for the presence of SCID and account for significant morbidity during disease progression. Establishing more diagnostic centers dedicated to the care of PID will facilitate early, correct diagnosis and better care of SCID in China.

**(43) Submission ID#419997**

**Clinical Features and Genetic Analysis of 47 Chinese Patients with X-Linked Hyper-IgM Syndrome**

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Introduction/Background: X-linked hyper-IgM syndrome (XHIGM) is one type of primary immunodeficiency diseases, resulting from defects in the CD40 ligand/CD40 signaling pathways.
Objectives: Here, we retrospectively reviewed clinical, laboratory and genetic characteristic of XHIGM in Chinese population, thus further improving diagnosis and treatment for XHIGM.
Methods: We collected and analyzed 47 Chinese patients, who were diagnosed and followed up in hospitals affiliated to Shanghai Jiao Tong University School of Medicine from 1999 to 2016. Targeted gene capture combined with next-generation sequencing technology and Sanger sequencing were used to find out related gene mutation.
Results: The median onset age of these patients was 7 months (range: 20 days66 months). Thirty-six percent of them had positive family histories, with a shorter diagnosis lag. The most common symptoms were recurrent sinopulmonary infections (40 patients, 85%), neutropenia (22 patients, 47%), protracted diarrhea (21 patients, 45%), and oral ulcer (19 patients, 40%). Ten patients had BCGitis. Six patients received hematopoietic stem cell transplantations and four of them had immune reconstructions and clinical remissions. Twenty-seven unique mutations in CD40L gene were identified in these 47 patients, with 18 novel mutations.
Conclusions: To our knowledge, this report provides the largest cohort of patients with XHIGM in China. Mutation analysis is an important tool for XHIGM diagnosis.

**(44) Submission ID#427355**

**Concomitant OPV and BCG Vaccine Derived Complications in Two Infants with Severe Combined Immunodeficiency**

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Introduction/Background: Infants with severe combined immunodeficiency (SCID) are at risk of developing severe life threatening infections if given attenuated live vaccines. Vaccines that are contraindicated for use in SCID patients during the first years of life include the rotavirus, oral polio, BCG and MMRV vaccines. Infants with SCID are asymptomatic at birth and unless prompt diagnosis of the disease is made they may horrifically be vaccinated. Simultaneous appearance of two live vaccine associated infections in one person is rarely reported.
Objectives: In this study we present two infants with SCID, who received BCG and oral polio vaccines early in life before the diagnosis of immune deficiency was made. Both patients developed localized and disseminated infections originating from the BCG vaccine (BCGitis and BCGiosis, respectively) and in addition were diagnosed with chronic fecal secretion of vaccine-derived polio virus (VDPV); alarmingly, in both cases, the VDPV underwent reverse mutation of attenuated sites to the neurovirulent genotype. The rarity of concomitant infection from two live vaccines in one recipient, together with the multiple complexities originating from these infections in immunodeficient infants, led us to report these cases and to inquire the pathogenesis that underlies this unique condition.
Methods: Immunological evaluation:: Cell surface markers of peripheral blood mononuclear cells (PBMCs) were measured by immunofluorescent staining and flow cytometry Serum concentrations of immunoglobulins were measured using nephelometry. Quantitative analysis of the TCR V repertoire was performed by means of flow cytometry. Quantification of T cell receptor excision circles (TRECs) was determined by real-time quantitative (RQ)-PCR.
Genetic analysis: Genetic diagnosis of SCID was made for patient 1 by direct Sanger sequencing of candidate genes and retrieval of mutation in RAG2 , and for patient 2 by WES (whole exome sequencing), followed by validation of the DNA cross-link repair 1C (DCLRE1C) mutation using Sanger sequencing.
Poliovirus detection and characterization:
Stool samples were collected monthly and transported to the National Poliovirus Laboratory located at Israel Central Virology Laboratory (ICVL) for Polio detection and characterization.
Results: In both patients, immunological workup revealed undetectable serum IgA and IgM levels with normal IgG levels (Table 1), Lymphocyte immune-phenotyping using flow cytometry revealed complete absence of T and B cells with presence of NK cells (Table 2). TRECs, a DNA marker of naive T cells and thymic output, were absent in both patients. The diagnosis of SCID was made. We initiated prophylactic antibiotic (Trimethoprim-Sulfamethoxazole) and anti-fungal (Fluconazole) treatment, as well as monthly intravenous immunoglobulin (IVIG) infusions.
Genetic workup: The T- B- NK+ SCID phenotype in patient 1 led us to search for a mutation in the RAG complex genes. Indeed, a Sanger sequencing of the RAG2 gene, revealed a G104T homozygous mutation which predicts an amino acid substitution from Glycine to Valine in position 35 (Fig.1). For patient 2, who had a similar T- B- NK+ SCID phenotype, we identified a homozygous mutation in the DCLRE1C gene (del.4bp, c.817-CTTT) (Fig. 2), using WES. The genetic evaluation confirmed the diagnosis of SCID due to RAG2 deficiency in patient 1 and ARTEMIS deficiency in patient 2.
Clinical course: During hospitalization patient 1 developed disseminated BCG related disease with skeletal lesions involving the phalanx, tibia and maxillary bones, as well as involvement of the spleen, liver and pancreas. Anti-tubercular therapy with Isoniazid, Rifampicin, Ethambutol and Ciprofloxacin was initiated. Due to lack of response, empirical trial of G-CSF (Granulocyte colony-stimulating factor), in order to enhance macrophage activity . The patient showed good response to this combination therapy. Patient 2 developed a palpable rigid mass on her left shoulder with surrounded redness at the site of the BCG vaccine at the age of 5 months. The clinical diagnosis of BCGitis was established and triple anti-tubercular therapy with Isoniazid, Rifampicin and Ethambutol was initiated with good response. Throughout their hospitalization, both infants suffered from intermittent diarrhea. PCR for enterovirus was performed and detected the presence of type 3 and type 2 vaccine-derived poliovirus (VDPV) in patient 1 and 2, respectively. During the follow-up, stool samples collection revealed accumulation of several polio virus mutations and some of the neuro-virulence attenuation sites were reverted to the

neuro-virulent genotype .Fortunately, both patients did not show any signs of flaccid paralysis. Precautionary measures of isolation were taken to prevent spread of the VDPV.
Eventually, both patients underwent allogeneic bone marrow transplantation (BMT): Patient 1 had BMT without pre-conditioning, from a matched sibling donor. Due to engraftment failure, a second BMT was repeated, this time successfully. On follow up examination her T cell repertoire showed a normal TCR V polyclonality and TREC was detected, indicating the emergence of new T cells. Due to ongoing low Immunoglobulin levels this patient is still on regular IVIG-infusions and prophylactic antibiotic treatment, as well as Isoniazid. Currently, she is well, her BCGitis is not active and her stool specimens are negative for polio. Patient 2 underwent an urgent haplo-identical BMT with alpha-beta T cell depletion without pre-conditioning, due to her unstable medical condition. Flow cytometry analysis six months post BMT revealed lymphopenia of 7.7% (1334/mm³) with low mature T cells (CD3 32%) and absent B cells. TRECs were barely detected. Microsatellite analysis as a marker for engraftment revealed a stable donor chimerism of 10%. The patient is still on immunosuppressive therapy doing well, her BCGitis is not active and her stool specimens are negative for polio.
Conclusions: These cases highlight the importance of early recognition of SCID by neonatal screening or thorough family anamnesis, and the need to further defer the timing of administration of live vaccines.

**(45) Submission ID#398909**

**Correlation between percentage of B cells/B cell subsets and pneumococcal IgG, IgA and IgM concentrations**

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Introduction/Background: Measurement of B cells, B cell subsets and specific antibodies produced in response to vaccination are key tests used to investigate immune system function. Specific antibody production may indicate B cell functionality.
Objectives: In this study the correlation between the different B cell subsets and antibody responses to Pneumovax® in an immunocompromised population was investigated.
Methods: B cell subsets were assessed by flow cytometry and pneumococcal responses measured using the VaccZyme Pneumococcal Capsular Polysaccharide (PCP) IgG, IgA and IgM ELISAs (The Binding Site Group, Birmingham, UK) in 39 primary immunodeficiency patients (PID) vaccinated with Pneumovax®. Lower limits of normal were defined as follows: B cells: 6.6%; naive B cells: 65.6%; non-switched memory B cells: 7.4%; switched memory B cells: 7.2%; PCP IgG: 50 mg/L; PCP IgA: 125 U/mL; and PCP IgM: 140 U/mL.
Results: The correlation coefficients between percentage of B cells/B cell subsets and IgG, IgA or IgM Pneumovax® responses ranged from -0.61 to 0.39. The percentage of the cohort achieving a normal B cell and normal IgG, IgA or IgM response to Pneumovax® was 45%, 29% and 40% respectively. B-cell responses were measureable in the remaining patients but they did not produce normal concentrations of PCP IgG, IgA or IgM. Further stratification of patients who achieved normal percentage of switched or un-switched B cells but who failed to achieve normal IgG, IgA or IgM responses to Pneumovax® were 32%, 55% and 50%, respectively, for un-switched and 22%, 47% and 33%, respectively, for switched B cells.
Conclusions: The combined measurement of B cells and response to vaccination are required to provide a detailed insight into these disorders.

**(46) Submission ID#427937**

**CTLA-4 Haploinsufficiency Presenting in a Child with Very Early-Onset Colitis, Viraine Weearsooriya[3]**

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Introduction/Background: This is a 2-year-old boy of African American heritage with multiple congenital malformations who was diagnosed with very early-onset inflammatory bowel disease, which proved to be resistant to treatment. Subsequent testing revealed a heterozygous mutation in exon 1 of CTLA4. This variant has not been previously reported in the literature in individuals with CTLA4-related disease. Heterozygous mutations in CTLA4 cause a disease of immune dysregulation. Clinical presentation is variable and may be characterized by enteropathy, hypogammaglobulinemia, granulomatous lymphocytic interstitial lung disease, lymphocytic organ infiltration in non-lymphoid organs, autoimmune cytopenias, and recurrent infections. Early onset colitis has been reported with CTLA4-related disease and is unique to our patient's initial clinical presentation.
Objectives: This is a 2-year-old boy with cloacal exstrophy of the urinary bladder, omphalocele, imperforate anus, polydactyly, and sacral agenesis who was diagnosed with very early-onset inflammatory bowel disease at six months of age. He underwent cloacal exstrophy closure, omphalocele repair, and colostomy placement in the first week of life. At six months of age, he presented with dark tarry stools. Upper endoscopy and colonoscopy revealed polyps, ileitis, and colitis. He was p-ANCA positive and started on sulfasalazine. Unfortunately, he continued to have symptoms suggestive of active colitis, prompting a change to prednisone and azathioprine. Despite therapy, his colitis persisted leading to chronic bloody diarrhea and growth failure. Initial immune evaluation consisted of a normal complete blood count, serum immunoglobulin, lymphocyte subsets, and neutrophil oxidase burst assay. FOXP3 analysis by flow cytometry showed a moderately elevated percentage of FOXP3+CD25+ cells in the CD4+ T cell population, but the regulatory T cell immunophenotype was normal. Because suspicion was high for a monogenic immunologic disease to explain his symptoms, genetic sequencing was performed. A candidate gene panel was sequenced by Next Generation Sequencing, and a heterozygous mutation in exon 1 of CTLA4 (c.23G>A; p.Arg8Gln) was found. This variant has not been previously reported but is predicted to be pathogenic in Exac and PolyPhen databases.
Results: The patients diagnosis of CTLA-4 haploinsufficiency associated with very early-onset inflammatory bowel disease has provided opportunity for targeted treatment of his specific molecular defect. Given his poor response to treatment thus far, the patient will be started on abatacept. Abatacept is FDA approved for the treatment of rheumatoid arthritis but has been used successfully for the treatment of disease-related manifestations of CTLA-4 haploinsufficiency. Abatacept is a CTLA-4 fusion protein formed by the IgG1 Fc region linked with the extracellular domain of CTLA-4; it replaces the defective protein in CTLA-4 haploinsufficiency. In addition, given other manifestations of CTLA-4 haploinsufficiency including lymphoproliferative disease in non-

lymphoid organs, particularly the brain and lung, we have initiated further evaluation of these organs to evaluate for disease-specific manifestations.
Conclusions: The protein cytotoxic T lymphocyte antigen-4 (CTLA-4) is an essential negative regulator of T cells. Heterozygous mutations in CTLA4 cause a disease of immune dysregulation. Clinical presentation is variable and may be characterized by enteropathy, hypogammaglobulinemia, granulomatous lymphocytic interstitial lung disease, lymphocytic organ infiltration in non-lymphoid organs, autoimmune cytopenias, and recurrent infections. Inflammatory bowel disease may be associated with certain variants in CTLA4, but the literature remains limited, both by number of papers published as well as by ethnic subsets studied. Clinicians who are presented with children who have early-onset colitis, and particularly inflammatory bowel disease that is difficult to treat, should consider possible genetic abnormalities, such as CTLA-4 haploinsufficiency, as these can impact therapeutic decision-making and outcomes.

**(47) Submission ID#427616**

**Dapsone in the Management of Recurrent Skin Abscesses in Child with Cytokinesis 8 (DOCK8) Mutation.**

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Introduction/Background: DOCK8 deficiency is an autosomal recessive combined immunodeficiency syndrome associated with recurrent infections, eczema and other atopic diseases. The infections are usually viral, bacterial and fungal resulting in predominantly cutaneous and sinopulmonary manifestations. Homozygous or compound heterozygous deletions or mutations in the DOCK8 gene (9p24) lead to abnormal cytoskeletal organization and impaired function of dendritic cells and lymphocytes. An aniline derivative belonging to the group of synthetic sulfones, Dapsone has been employed in the treatment of chronic skin diseases characterized by an accumulation of neutrophils and eosinophils.
Methods: Chart Review of One Patient
Results: We present a four-year-old male with severe eczema, persistent asthma, allergic rhinitis, as well as peanut and egg allergy suffering from recurrent skin abscesses and prurigo nodularis. Abscesses began at age 18 months and required prolonged courses of antibiotics, eight in total prior to presentation. Other infectious history included otitis media and lymphadenitis. There was no history of pneumonia or other severe infections. Skin abscesses responded to oral antibiotics, but recurred shortly after completing extended courses of treatment. Laboratory results including quantitative immunoglobulins, specific antibody titers, myeloperoxidase staining, neutrophil oxidative burst and complement were within normal limits. Laboratories were notable for elevated IgE (10080 IU/mL) and eosinophilia (1200 eosinophils/microL). Lymphocyte immunophenotype was significant for mild elevations in CD3 and CD4. DOCK8 genetic sequencing by GeneDx revealed a heterozygous missense mutation in exon 17 (c.1979 C>A, amino acid change p.Ala660Asp).
Abscess cultures grew Methicillin Sensitive Staphylococcus aureus (MSSA) and Enterococcus faecalis. MSSA was sensitive to Ampicillin/Sulbactam, Cefazolin, Gentamycin, Moxifloxacin, Oxacillin, Rifampin, Tetracycline, and Vancomycin. Isolate was resistant to Ciprofloxacin, Clindamycin, Erythromycin, Levofloxacin, Penicillin, and Trimethoprim/Sulfamethoxazole. The patient was initially treated with emollients, mupirocin washes, topical steroids, anti-histamines, bleach baths, and Cephalexin three times a day. He improved clinically but was unable to tolerate Cephalexin for more than ten days secondary to abdominal pain. Cephalexin, with addition of probiotic, was attempted several months later and again had to be discontinued because of abdominal pain and vomiting. Due to limited antibiotic options, Dapsone was started after ruling out Glucose-6-Phosphate Dehydrogenase Deficiency. Dapsone was initiated at a dose of 1.5mg/kg, and the patient was monitored weekly for hemolytic anemia. After two weeks of treatment, anemia was noted and the dose was decreased to 1mg/kg. He has continued on Dapsone 1mg/kg once a day, with significant improvement in abscess number and severity. He has not required other systemic antimicrobials since starting Dapsone.
Conclusions: Dapsone may be considered as a treatment option for children with heterozygous DOCK8 mutation and recurrent abscesses, particularly those requiring prophylaxis, long term treatment and lacking antibiotic options.

**(48) Submission ID#420002**

**Defective B Cell Proliferation and Activation in Response to TLR7/9 Agonist Stimulation in B Cells from AD-HIES Patients**

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Introduction/Background: Autosomal dominant hyper-IgE syndrome (AD-HIES) is a rare complicated primary immunodeficiency disease (PID). Signal transducer and activator of transcription 3 (STAT3) gene mutation is found to cause AD-HIES. TLR7/9 signaling plays multiple roles in B cell proliferation, activation, class-switch recombination, and cytokine and antibody production. However, little is known about B cell response to TLR7/9 agonist in patients with AD-HIES.
Objectives: Here, we aim to study the response of B cells from AD-HIES patients to the TLR7/9 agonist.
Methods: PBMCs were isolated from peripheral blood of 5 AD-HIES patients and 10 age matched healthy controls. PBMCs were stimulated with TLR7 and TLR9 agonist (R848 and CpG ODN 2006, respectively), then B cells were analyzed for proliferation, the expression of certain surface markers (CD40, CD80 and CD86), intracellular immunoglobulin levels (IgM and IgG) and intracellular cytokine levels (IL-6 and IL-10) by flow cytometry.
Results: In response to TLR7/9 agonist, proliferative capabilities of B cells were reduced in AD-HIES patients compared with those in age-matched healthy controls. Besides, defective costimulatory molecule CD80 expression was observed in B cells from AD-HIES patients. Furthermore, significantly lower IgM and IgG levels, and IL-10 production was detected in B cells from AD-HIES patients. However, there was no significant difference in B cell apoptosis between AD-HIES patients and healthy controls.
Conclusions: These data demonstrated that STAT3 gene mutations in AD-HIES patients contributed to impaired B cell TLR7/9 signaling, and further affected B cell proliferation, activation, cytokine secretion and antibody production.

**(50) Submission ID#421129**

**Diagnosis of Primary Immunodeficiency Disease using Pneumococcal Avidity Assay: Interpretation and Clinical Outcomes**

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Introduction/Background: Antibody function is most commonly measured by a rise in antibody titers in response to antigen introduced by vaccination or natural infection. Specific antibody deficiency is defined as normal serum levels of immunoglobulins with reduced or absent antibody response to antigens, often after administration of the pneumococcal vaccine polyvalent (Pneumovax® 23). A paucity of information exists about measurement of antigen-antibody binding, or avidity, as a measure of antibody function, including persons with recurrent sinopulmonary infections who have normal response to immunization with pneumococcal vaccine polyvalent.
Objectives: The aims of this study are to identify and evaluate children with recurrent sinopulmonary infections who had appropriate rise in pneumococcal antibody titers following immunization with pneumococcal vaccine polyvalent but low response by avidity, and to assess response with IgG replacement therapy in these patients.
Methods: A retrospective chart review involved eight children with recurrent sinopulmonary infections with discordant pneumococcal antibody and avidity results following vaccination with pneumococcal vaccine polyvalent. These eight children subsequently received IgG replacement therapy.
Results: The mean age of subjects was 9.75 (range 2 - 15) years. The mean number of serotypes with a normal antibody response (>1.3 ug/ml) among 8 children following immunization with pneumococcal vaccine polyvalent was 18.1 (range 12-22) of 23 serotypes while the mean number of serotypes with a normal avidity response (1.0) was 4.4 (range 2-7) of 23 serotypes. IgG replacement was administered subcutaneously in 8 children. The mean IgG level was 720 mg/dL. Local reactions were all mild and observed in 4/8 (50%) children. No serious adverse events were reported. All 8 children experienced a marked reduction in respiratory illnesses while on IgG replacement therapy.
Conclusions: Discordance between pneumococcal antibody titers and pneumococcal avidity titers was identified in eight children with recurrent respiratory illnesses. In children with recurrent sinopulmonary infections despite normal antibody response to pneumococcal vaccine polyvalent, measurement of pneumococcal avidity may identify patients with poor pneumococcal antibody function. IgG replacement in these children was well tolerated and associated with a decrease number of respiratory infections.

**(51) Submission ID#382130**

**Diagnostic Utility of Exome Sequencing for Disorders of the Immune System**

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Introduction/Background: Exome sequencing (ES) is a powerful genomic tool that can be used to identify novel molecular causes of disorders with multiple etiologies. Immunologic disorders are clinically and genetically heterogeneous, and therefore present unique diagnostic challenges both in the clinic and in the laboratory.
Objectives: The objective of this study was to assess the utility of ES for determining the genetic etiology of immunological disorders and describe diagnostic yield and outcomes of exome sequencing (ES) for patients with immunologic disorders and immunologic phenotypes.
Methods: A retrospective review was performed of 315 individuals referred for clinical ES for primary abnormalities of the immune system and individuals with additional phenotypes where multiple immunologic features were reported as part of the clinical picture, as determined during internal curation. Analysis of clinical ES data was performed by board-certified clinical geneticists and all variants reported were confirmed by a secondary methodology. Positive ES outcomes required a pathogenic or likely pathogenic variant in a gene with autosomal dominant or X-linked inheritance, or compound heterozygous or homozygous pathogenic or likely pathogenic variants in a gene with recessive inheritance.
Results: The most common clinical indications for ES in this cohort were hypogammaglobulinemia (16%), neutropenia (14%), immune dysregulation (10%), lymphopenia (9%), and combined immunodeficiency (8%). The gender distribution was 59% male (n=185) and 41% female (n=130); 76% of cases were pediatric (<18 years, n=240) and 24% were adult (>=18 years, n=75). Positive results were reported in 83 cases (26%), comparable to the overall diagnostic yield of ES at our laboratory (Retterer et al., 2016). This included 20/90 (22%) of cases submitted as proband-only, 47/178 (26%) submitted as a trio, and 16/47 (34%) submitted as duo, quad or alternative family structure. Diagnostic results spanned 70 different genes and recurrently reported genes with identified pathogenic variants included FLG (n=10), RAG1 (n=5), SBDS (n=4), LRBA (n=3), STAT1 (n=3), and PIK3CD (n=3). Variants possibly associated with the phenotype, but not considered diagnostic, were reported in 58% of cases (n=183), while 13% of cases (n=41) had reportable findings in a candidate gene.
Conclusions: These results support that exome sequencing for individuals with immunologic-based phenotypes has similar diagnostic utility as the overall rate for clinical ES. Immunologic ES cases with trio family structures have a higher diagnostic yield than proband-only cases, as inheritance information improves confidence in classification of variants as pathogenic or likely pathogenic. Genetic heterogeneity, as demonstrated by the large number of distinct genes represented in this cohort of diagnostic ES cases and rapid candidate gene discovery make ES a valuable tool for genetic diagnosis in patients with immunological disorders.

**(52) Submission ID#414883**

**Diagnostic Value of an Enzyme-linked Immunosorbent Spot (ELISPOT) Assay in the Detection of Specific Pneumococcal Vaccine Response in Common Variable Immunodeficiency**

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Introduction/Background: Vaccination response to the 23-valent polysaccharide vaccine (PPSV23) is often used in the diagnosis of common variable immunodeficiency (CVID). Unfortunately, PPSV23 titers are often difficult to interpret and many CVID patients are started on IgG replacement therapy (IGRT) before adequate evaluation. Unlike PPSV23 titers, the enzyme-linked immunosorbent spot assay (ELISPOT) is independent of IGRT and can provide an ex vivo functional measurement of specific antibody production on the B cell level.
Objectives: Develop and test an ELISPOT assay to better determine vaccination response to PPSV23 compared to PPSV23 titers in CVID patients on IGRT, healthy controls, and IGRT patients without immunodeficiency.

Methods: An ELISPOT assay was successfully optimized and used to evaluate the PPSV23-specific B cell response in 8 healthy adult controls. ELISPOTs were performed on day 1, and day 7 (when plasmablasts are best evaluated). PPSV23 titers were measured on day 1, day 7, and day 30. For IGRT patients, flow cytometry for B cell subpopulations will be performed to further validate the assay.
Results: Normal controls demonstrated a significant increase in PPSV23 antibody spot forming units (SFU) between day 1 and 7 after PPSV23 vaccination. PPSV23 titers showed generally robust initial titers at day 1, and no significant change at day 7, with day 30 results pending.
Conclusions: Here we optimized an ELISPOT assay that functionally measures the specific antibody response to PPSV23 in normal controls with PPSV23 titer results pending. We are actively recruiting patients on IGRT (both with CVID and without immunodeficiency) for comparison. We hope to validate our assay as a useful alternative to PPSV23 titers that may be particularly useful when patients are on IGRT.

**(53) Submission ID#428683**

**Diagnostic Yield of Bronchoalveolar Lavage in Patients with Primary Immunodeficiency with Suspected Pulmonary Infection: a Single Center Experience.**

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Introduction/Background: Pneumonia and lower respiratory tract infections are the most frequent serious infectious manifestations of primary immunodeficiency diseases (PIDD). Identification of pathogens is important to guide treatment, given that patients have frequent prior antibiotic exposure and that their infections may be due to a wide range of typical and opportunistic organisms. Invasive diagnostic testing, such as bronchoscopy with bronchoalveolar lavage (BAL), fine needle aspiration (FNA), or open lung biopsy are used in PIDD patients to identify an etiologic organism in recurrent or recalcitrant pulmonary infections. Previous studies have demonstrated BAL may be a sensitive diagnostic method for treatment failures of clinically diagnosed pneumonias, even if performed under treatment with empiric antibiotics, and can lead to a culture-directed change in antimicrobial therapy in the majority of cases. However, it has also been reported that at least in one PIDDchronic granulomatous disease (CGD)the diagnostic yield of BAL was inferior to that of other diagnostic methods (Marciano et al., 2015). Further information on the diagnostic yield of BAL and other invasive procedures to obtain a specific organism diagnosis in PIDD patients with suspected pulmonary infection is needed.
Objectives: To characterize the yield of diagnostic procedures used in PIDD patients with pneumonia or other suspected pulmonary infections at the UCSF Benioff Childrens Hospital.
Methods: We screened our database of PIDD patients (encompassing patients seen from September 1, 1998 to September 1, 2017) cared for by the Pediatric Immunology Service at the University of California, San Francisco to identify patients with history of at least 1 BAL or other invasive diagnostic procedure (FNA or open lung biopsy) for etiologic diagnosis of suspected pulmonary infection. If multiple BALs were performed during a single episode of illness, only the first was used for this analysis.
Results: We identified 22 PIDD patients with history of at least one BAL or other invasive diagnostic procedure, for a total of 55 events. Most procedures (n=53) were performed at our institution, with 2 documented in outside hospital records. Patient diagnoses included CGD (11), NEMO deficiency (4), CVID (3), STAT3-deficient AD-HIES (2), DOCK8 deficiency (1), and MHC Class II deficiency (1). Of 49 BALs, 24/49 (49%) grew a predominant organism, but only 18/49 (37%) were positive for an organism believed to be causative by providers and/or for which the overall result of the bronchoscopy affected antimicrobial treatment. BAL yield was highest in patients with a clinical and/or radiologic diagnosis of pneumonia (13/27, 48%). Yield was poor (3/16, 19%) in minimally or chronically symptomatic patients referred for BAL for interval changes on chest CT (i.e. suspected fungal infection). Our NEMO deficiency cohort had the highest rate of positive organism isolation by BAL (6/11, 66%) for diagnosis of pulmonary infection. In our CGD cohort, 11/26 (42.3%) BALs grew a causative organism. Lung biopsy yielded positive organism isolation in 2/3 cases (2/2 in CGD, Burkholderia cepacia and Nocardia cyriacigeorgica; 0/1 in CVID) and FNA in 1/2 cases of CGD (Aspergillus fumigatus). FNA or biopsy was done concurrently or after BAL in 5 cases; in 4/5 (80%), FNA or biopsy, but not BAL, was positive for a causative organism.
Conclusions: At our institution, BAL overall had a 37% rate of causative organism isolation in PIDD patients, but had up to a 50% rate of organism isolation in those with clinical and/or features of pneumonia. Our rate of causative organism isolation was slightly higher than in previous reports. However, in specific instances, biopsy was still required to make a definitive diagnosis. BAL may have limitations in certain populations of PIDD patients, such as in CGD, but it may be a reasonable starting point in the diagnosis of pneumonia or worsening pulmonary disease in PIDD. Prospective research is needed to evaluate whether FNA or lung biopsy, though more invasive, could result in overall shorter time to institution of appropriate directed therapy and shorter hospitalizations for specific PIDD patients.

**(54) Submission ID#402818**

**Disseminated BCG Presenting as an Orbital Mass in an Infant with Undiagnosed SCID**

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Introduction/Background: Prior to the introduction of newborn screening, cases of severe combined immunodeficiency often presented with severe or disseminated infections. Herein, we report an infant from India who presented for evaluation and treatment of a periorbital mass, presumed to be malignant. However, he was found to have disseminated Bacille Calmette Guerin (BCG), as well as multiple other infections, and was eventually diagnosed with X-linked SCID.
Results: A 4-month old boy was born to unrelated parents in India. He initially presented with growing periorbital mass and fever for two weeks. PET scan showed hypermetabolic areas in the bone marrow, spleen, mesenteric lymph nodes, and left shoulder, along with the periorbital mass. Biopsy of the mass revealed numerous B lymphocytes without malignant transformation. There was no evidence for malignancy on bone marrow biopsy, though numerous granulomas and a significant decrease in T lymphocytes were seen. Peripheral blood flow cytometry showed a complete lack of T cells. The bone marrow biopsy was reexamined, and innumerous acid-fast bacilli were found. Cultures grew Mycobacterium bovis, and he was diagnosed with disseminated BCG disease. Genotyping revealed a novel splice site variant in IL2RG, consistent with X-linked SCID. Further evaluation revealed multiple other infections. These included extended-spectrum beta lactamase-produce E. coli

bacteremia, human metapneumovirus, cytomegalovirus, and Pneumocystic jiroveci. He was also tested for vaccine-associated poliovirus because he had received the oral polio vaccine, but this was negative.
Four-drug therapy was started for the BCG, and the periorbital lesion completely resolved within several weeks. Additionally, he was treated with intravenous ribavirin for the human metapneumovirus, and sulfamethoxazole-trimethoprim for the P. jiroveci. Although the CMV was initially treated with ganciclovir, the virus eventually developed resistance and required treatment with foscarnet.
Due to his many infections, he underwent haploidentical stem cell transplant plus donor lymphocyte infusion from mom. This transplant failed to engraft, but a matched unrelated donor was eventually found, and our patient received a second bone marrow transplant. He currently is showing signs of engraftment, and is continuing to be treated for his multiple infections.
Conclusions: Disseminated mycobacterial disease due to the Bacille Calmette-Guérin (BCG) vaccination has been noted in cases of X-linked SCID previously, often consisting of lymphatic, skin, and pulmonary manifestations. However, there is a paucity of published cases presenting with multiple other co-infections. Nor are there reports of disseminated BCG presenting as a localized mass. This case highlights the unique considerations when evaluating a patient with immunodeficiency from another country, where vaccination practices and epidemiology differ. Specifically, unusual presentations of infections or masses may warrant investigation for severe immunodeficiency.

**(55) Submission ID#419520**

**Distinct Clinical and Immunologic Course in Two IL7Ra Deficient Patients Harboring the Same Mutation, Identified in the Israeli Newborn Screening Program for SCID**

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Introduction/Background: Severe combined immunodeficiency (SCID) is a fatal primary immunodeficiency (PID) caused by mutations in genes involved in the development of T, B and natural killer (NK) cells. The prototypic T-B+NK+ immune phenotype is caused by mutations in the IIL7Ra gene. The IL7 signaling has an important role during T-cell development in the thymus, contributing to cell proliferation and survival. In addition, in mouse models, the rearrangement of the T Cell Receptor genes, specifically the gamma locus (TRG), has been shown to be regulated by IL7 signaling. Similar to other SCID phenotypes, patients with IL7Ra deficiency are predisposed to acquire opportunistic infections early in life and to display poor outcome and death, unless their immune system is restored by mean of hematopoietic stem cell transplantation (HSCT). While most patients with IL7Ra mutations have full IL7Ra deficiency resulted in a severe T cell depletion, some have a partial deficiency with residual cells or leaky phenotype, or even present symptoms later in life.
Objectives: Here we report two non-related infants detected by the Israeli national newborn screening program for SCID. Despite having similar IL7Ra missense mutation (F40L) they displayed distinct clinical and immunological course, resulted in a completely different treatment approaches; observation in one patient with an unusual recovery, and HSCT in the other patient
Methods: Patient lymphocytes were examined for subset counts, Thymic output (via excision circles), T cell receptor repertoire diversity (TCRB) and IL7Ra expression and function. The pathogenic IL7Ra mutation was found by whole exome sequencing (WES). High Throughput immune-sequencing was performed to characterize the TRG repertoire.
Results: We established patients' diagnosis by validating the pathogenic IL7Ra mutation, and showing profoundly impaired T cell immune work up and abnormal IL7Ra expression and function, determined by STAT5 phosphorylation assay. All these measurements improved over time for the patient with less severe clinical presentation, while remained low in the patient with the severe phenotype. Characterization of their TRG immune repertoire using High Throughput immune-sequencing revealed restriction of T cell receptor repertoires of both patients upon their initial diagnosis, compared to healthy controls. However, skewed usage of variable (V) gene segments and abnormalities of the CDR3 length distribution were more prominent in the patient with the severe phenotype.
Conclusions: These studies illustrate the gap that exists in our understanding of other non-genetic parameters that may influence disease course and severity in patients harboring a similar genetic defect. Furthermore, the results reinforce the role of IL-7 signaling not only in cell proliferation but also in TRG rearrangements

**(56) Submission ID#420685**

**Dock8 Immunodeficiency Syndrome Presented SLE in 16 Month-Year-Old Boy**

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Introduction/Background: DOCK8 immunodeficiency syndrome is primary immunodeficiency disease caused by loos of function mutations in the DOCK8 gene, which was known to play a critical role in the survival, proliferation and function of several types of immune system, especially lymphocyte. DOCK8 immunodeficiency syndrome is the most common cause of autosomal recessive hyper-immunoglobulin E syndromes (HIES) and mainly expressed as recurrent infections and severe allergic disease affecting the skin. In addition, autoimmune features including systemic lupus erythematosus, hemolytic anemia or idiopathic thrombocytopenic purpura may be presented in DOCK8 immunodeficiency syndrome.
Objectives: We report a case of 16-month-old boy diagnosed with DOCK8 immunodeficiency syndrome, which was initially expressed as SLE without recurrent skin infections.
Methods: A child with atopic dermatitis was admitted to another hospital because of fever lasting more than 3 days accompanied by swelling of the hands and foot. He developed whole body edema, perioral purpura and oliguria. Complete blood count was normal and blood urea nitrogen , creatinine and albumin levels were normal on his laboratory findings. However, C-reactive protein level was high at 9.85 mg/dL, coagulation parameters were abnormal (prothrombin time 14.8 sec; activated partial thromboplastin time 67.0 sec, D-dimer 5.82 ug/mL). So he was transferred to our hospital for further examination and treatment on the 5th day of fever. Additionally, ulceration of the tonsil and maculopapular rashes on the abdomen and both legs were observed in physical examination. We suspected a meningococcal infection and administered antibiotics. However, no bacterial isolates were identified in the blood and CSF culture test. Fever persisted despite the administration of antibiotics. We checked immunoglobulin level, complement level and autoantibodies based on fever of unknown origin. Immunoglobulin G, M and A were normal, complement fractions C3, C4, and CH50 were low at 80.0 mg/dL, 6.7 mg/dL and 13.3 U/mL, respectively. Antinuclear antibodies were positive at 1:128 with homogenous fluorescence. Anti-ds DNA antibody was positive at 84.4. The tests for anti-SSA, anti-SSB, anti-ribonucleoprotein, anti-scleroderma 70, and anti Jo1 antibodies were all negative. He developed leukopenia and thrombocytopenia over time.

Results: He was treated with steroid satisfied diagnostic criteria for systemic lupus erythematosus (SLE) and fever subsided. He was confirmed lupus nephritis by renal biopsy later. Because of onset of SLE at the young age, we performed diagnostic whole exome sequencing and multiplex ligation-dependent probe amplification assays.
Conclusions: He was confirmed DOCK8 gene deletion (a deletion on one allele and point mutation on the other allele). He is preparing for hematopoietic stem cell transplantation due to autoimmunity and non-reversible parenchymal organ damage form infections although he has not yet experienced a life-threatening infection.

**(57) Submission ID#382728**

**Does the Measurement of IgG Subclasses have a Utility in CVID Patients?**

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Introduction/Background: During immunological investigation, it is important to distinguish those individuals who may have hypogammaglobulinemia (HYPO) without fulfilling the criteria for the severe antibody deficiency common variable immunodeficiency (CVID) e.g. unspecified hypogammaglobulinemia from those with CVID.
Objectives: Since low IgG3 concentrations may support a diagnosis of CVID, we sought to investigate whether the measurement of additional IgG subclass antibodies (IgGSc) may provide further discrimination between patients with CVID and those with HYPO.
Methods: IgGSc concentrations were measured in serum samples from CVID patients (n=15, 1:1.3 M:F, median age 41.5 years, range 20-78) and HYPO patients (n=19-21, 1:1.3 M:F, median age 41.5 years, range 20-78).
Results: CVID patients had lower median IgGSc concentrations for all IgGSc: IgG1 290mg/dL (range 46-478) vs 365mg/dL (range 174-601); IgG2 - 88mg/dL (range 8-190) vs 116mg/dL (range 13-546); IgG3 - 15mg/dL (range 7-58) vs 30mg/dL (range 15-62); IgG4 6mg/dL (range 0.2-25) vs 11mg/dL (range 0.31-108). This was significantly lower for IgG1 and IgG4 (P=0.04 and 0.01 respectively).
A higher percentage of CVID patients had IgGSc below the lower limit of the normal range compared to HYPO patients: IgG1 (80 vs 57.9%); IgG2 (100 vs 90.5%); IgG3 (60 vs 28.6%); IgG4 (26.7 vs 10.5%). 100% of CVID patients had low concentrations of 2 or more IgGSc vs only 68.5% of HYPO patients (P=0.02). 5.2% and 26.3% of HYPO patients had low levels of 0 or 1 IgGSc, respectively.
Conclusions: IgG subclass measurements may have some utility in distinguishing CVID patients from hypogammaglobulinemia patients.

**(58) Submission ID#419343**

**Does Thymectomy During Cardiac Surgery Affect TREC levels and Infection Risk?**

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Introduction/Background: The thymus is often removed during cardiac surgery for repair of congenital heart disease, but the extent of tissue removed varies between procedures and surgeons. Previous studies have shown decreased T cell counts after thymectomy but there is limited data on the effect of thymectomy on T Cell Receptor Excision Circle (TREC) levels and infection risk.
Objectives: To determine the effect of partial and complete thymectomy during cardiac repair surgery on TREC levels and infection risk.
Methods: A retrospective study of electronic medical records was performed on children who received cardiac surgery before age one at New York Presbyterian/Morgan Stanley Childrens Hospital between 7/1/2013 and 3/31/2017. Patients with heart transplant or primary immunodeficiency were excluded. Data was recorded on TREC levels (abnormal TREC < 200 copies/μl on New York State Newborn Screen), number of positive cultures, viral PCR panels, and infiltrates on chest x-ray. Patients were followed for a minimum of six months after cardiac surgery. Study was IRB approved.
Results: Cardiac surgery was performed on 256 patients and data was available for 133 patients. Of patients included, 65 had a partial and 68 had a complete thymectomy. TREC levels after surgery were recorded for 79 patients. Only 4% of patients had an abnormal TREC level on newborn screen. There was no difference between partial and complete thymectomy on risk of abnormal TREC ($p = 0.58$) and mean total number of infections at 6 months ($p = 0.42$).
Conclusions: Thymectomy rarely causes low TREC levels in children undergoing cardiac surgery. Complete thymectomy does not significantly increase infection rates in these children compared to partial thymectomy. These findings are possibly due to presence of ectopic thymic tissue or thymic regeneration and are reassuring for children undergoing complex cardiac surgeries. However, long-term follow-up of these children will be necessary to determine residual function of the thymus and clinical response.

**(59) Submission ID#421382**

**Dried Blood Spots, an Affordable Tool to Collect, Ship and Sequence gDNA From Patients with an XLA Phenotype Residing in a Developing Country**

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Introduction/Background: New sequencing techniques have revolutionized the identification of the molecular basis of primary immunodeficiency disorders (PID), not only by establishing a gene-based diagnosis, but also by facilitating defect-specific treatment strategies, improving quality of life and survival, and allowing factual genetic counseling. Because these techniques are generally not available for physicians and their patients residing in developing countries, collaboration with overseas laboratories has been explored as a possible, albeit cumbersome, strategy.
Objectives: We sought to determine whether blood collected by Guthrie cards could be shipped across continents by regular airmail to a CLIA-approved laboratory for confirmatory testing.

Methods: Blood was collected and blotted onto the filter paper of Guthrie cards by completely filling three circles. We enrolled 20 male patients with presumptive X-linked Agammaglobulinemia (XLA) cared for at the Vietnam National Children's Hospital, their mothers and several sisters for carrier analysis. DBS were stored at room temperature until ready to be shipped together, using an appropriately sized envelope, to a CLIA-certified laboratory in the US for Sanger sequencing. The protocol for Sanger sequencing was modified to account for the reduced quantity of gDNA extracted from DBS.
Results: High-quality gDNA could be extracted from every specimen. BTK mutations were identified in 17 of 20 patients studied, confirming the diagnosis of XLA in 85% of the study cohort. Type and location of the mutations were similar to those reported in previous reviews. The mean age when XLA was suspected clinically was 4.6 years, similar to that reported by Western countries. Two of 15 mothers, each with an affected boy, had a normal BTK sequence, suggesting gonadal mosaicism.
Conclusions: DBS collected on Guthrie cards can be shipped inexpensively by airmail across continents, providing sufficient high-quality gDNA for Sanger sequencing overseas. Using this method of collecting gDNA we were able to confirm the diagnosis of XLA in 17 of 20 Vietnamese patients with the clinical diagnosis of agammaglobulinemia.

**(60) Submission ID#421938**

**Dual Cancer in a Patient with ZNF341 Deficiency**

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Introduction/Background: ZNF341 is a positive regulator of STAT3 expression. It has recently been described that nonsense mutations in ZNF341 account for the STAT3-like phenotype in four autosomal-recessive kindred. Patients presented with reduced STAT3 expression and diminished Th17 cell numbers, in absence of STAT3 mutations.
Objectives: Here, we decribed a Turkish case having nonsense mutation in ZNF341 developed dual malignancy in 2 years.
Results: A 26-year-old female patient presented with severe eczema, recurrent cold skin abscesses, herpetic skin lesions, sinopulmonary infection, otitis media and hearing loss. The parents are cousins. Two younger sisters of the index case had eczema and recurrent skin infections since their infancy period. Physical examination of the patient revealed severe eczema, high palate, micrognathia, maxillary hypoplasia and hearing loss. Laboratory findings showed reversed CD4/CD8 ratio, high serum IgE level (13.916 U / L) and low IgG2 level (186 mg / dl). The patient was diagnosed as hyper IgE syndrome and IVIG therapy (400 mg / kg, every 3 weeks) was initiated because of IgG subgroup deficiency and recurrent sinopulmonary infections. At the age of 32, a polypoid mass filling the left nasal cavity was detected in her examination. Paranasal sinus CT revealed a mass obliterating the left nasal cavity, left ethmoid sinus and frontal sinus. Immunohistochemical stains showed a small round cell malignant tumor in the nasal cavity. She was treated with chemoradiotherapy successfully. A homozygous nonsense mutation has been detected at exon 8 in the ZNF341 gene (c.1156C> T) (kindly provided by Grimbacher's lab) very recently. She developed papillary thyroid carcinoma two years after completing the cancer therapy.
Conclusions: The relationship between ZNF341 defect and cancer development is unknown. The development of a second malignancy in this patient for a short time of completing the therapy might imply us a tendency for malignancy in ZNF 341 patients. Additionally, the likelihood of increased radiosensitivity in these patients should be taken into consideration.

**(61) Submission ID#422298**

**Efficacy, Safety, and Tolerability of Prometics Immune Globulin Intravenous (Human) 10% (Prometic 10% IGIV) in Adult and Pediatric Subjects with Primary Immunodeficiency Diseases (PIDD)**

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Introduction/Background: ProMetic 10% IGIV contains purified IgG, 95% as monomer; with a distribution of IgG subclasses proportional to that in native human plasma. We report the interim results from a phase 3 trial in the USA of Prometic 10% IGIV in adults and children with PIDD.
Objectives: This was a phase 3, single-arm, open-label, multicenter trial to evaluate the safety, tolerability, and efficacy of Prometic 10% IGIV in adults and children with PIDD.
Methods: Adults and pediatric subjects with PIDD on a stable dose of IgG replacement therapy (200-900 mg/kg) for at least 3 months with serum IgG trough levels > 500 mg/dL were included. Subjects received Prometic 10% IGIV every 3 to 4 weeks for approximately 1 year at the same dose and schedule as their previous IgG replacement therapy.
Results: An interim analysis was conducted when data were available on 50 adult subjects who received at least one dose of Prometic 10% IGIV (total of 461 infusions), with 15 subjects receiving at least 6 months of treatment (exposure = 27.72 subject years). At this time, pediatric exposure was only 4.29 subject years. There were no serious bacterial infections (SBIs) reported, and rate/yr of infections other than SBIs was 2.49 which was comparable to rate while on commercial product (2.80) All subjects achieved an IgG trough level > 500 mg/dL. There were no deaths, and no subject had a study drug-related serious adverse event or an adverse event that resulted in permanent discontinuation of study drug. A total of 145 adverse reactions (AR) (0.315/infusion) occurred in 32 subjects (64.0%), with 92 infusions (20.0%) associated with an AR. Most infusions (97.0%) were completed without a rate reduction. Most ARs were mild or moderate in severity, with 6 severe ARs (0.013/infusion) occurring in 3 subjects (6.0%). The most frequent ARs were headache (20.0% subjects or 0.056/infusion) and fatigue (14.0% subjects or 0.017/infusion).
Conclusions: In adults treated with Prometic 10% IGIV, there were no SBIs and infusions were well tolerated.

**(62) Submission ID#428751**

**Epigenetic Changes in Immune Cells Following Successful Desensitization with Multi-Food Allergen Oral Immunotherapy**

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Introduction/Background: Desensitization to food allergies is being studied in clinical trials using oral immunotherapy (OIT). There are limited data regarding the immune changes associated with successful OIT. Epigenetics involves heritable changes in gene function without modification of the underlying DNA sequence. This is mediated by methylation, histone modification, or changes in microRNA.
Objectives: To study methylation changes in the loci of four key genes of immune cells involved in allergy, interleukin 4 (IL-4), interferon gamma (IFN-g), forkhead box protein 3 (FoxP3), and interleukin 10 (IL-10), comparing baseline to post-OIT.
Methods: We completed a phase 2, randomized, placebo-controlled, multi-food OIT trial using omalizumab, an anti-IgE biologic, to facilitate desensitization for 48 multi-food allergic individuals. Double-blind, placebo-controlled food challenges (DBPCFCs) to multiple foods were conducted at entry and after 36 weeks of treatment, the primary endpoint. Omalizumab (n=36) or placebo (n=12) was administered for 16 weeks, with OIT for 2-5 foods starting 8 weeks after the beginning of omalizumab or placebo. After 36 weeks (28 weeks of OIT), participants underwent DBPCFCs to their offending foods. Treatment failures (n=11) were offered open-label omalizumab. Pyrosequencing of bisulfite treated genomic DNA purified from PBMCs from each participant at baseline and post-OIT was undertaken to investigate changes in methylation.
Results: Forty-four participants achieved successful desensitization, defined as passing DBPCFCs to 2 or more foods following OIT. We found that the -48 CpG site in the IL-4 promoter region is hypermethylated over time during successful multi-food OIT (FDR-adjusted $P < 0.001$ by Wilcoxon signed-rank test). The median % of methylation at baseline was 81.6 (interquartile range 2.1%) and was 84.5 post OIT (interquartile range 2.05%). There were no statistically significant (with a significance level of FDR adjusted P value of 0.001) changes in the IL-10, FoxP3, or IFN-g loci in the CpG sites we studied.
Conclusions: These preliminary results suggest that one immune mechanism involved in successful desensitization may involve suppression of Th2 function by hypermethylation of IL-4 in immune cells in the peripheral blood.

**(63) Submission ID#425322**

**Esculetin From Fraxinus Rhynchophylla Attenuates Dermatophagoides Farinae Extract /2,4-Dinitrochlorobenzeneinduced Atopic Skin Inflammation by Inhibiting Expression of Inflammatory Cytokines**

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Introduction/Background: Atopic dermatitis (AD) is a common chronic inflammatory skin disorder afflicting from infancy to adults with itching, scratching, and lichenification.
Objectives: We investigated the effects of esculetin from Fraxinus rhynchophylla on atopic skin inflammation.
Methods: For induction of atopic skin inflammation, we exposed the ears of female BALB/c mice with house dust mite (Dermatophagoides farinae extract, DFE) and 2,4-dinitrochlorobenzene (DNCB) during 4 weeks.
Results: Oral administration of esculetin reduced DFE/DNCB-induced atopic skin inflammation symptoms based on ears swelling and scratch numbers. The immunoglobulin (Ig) E, IgG2a, and histamine levels in serum were decreased and inflammatory cell infiltration in skin tissue was reduced by the esculetin. It suppressed Th1, Th2 and Th17 responses by inhibiting the production of inflammatory cytokines such as tumor necrosis factor (TNF)-, interferon (IFN)-, interleukin (IL)-4, IL-13, IL-31 and IL-17 in the ear tissue. Further, we investigated the effects of escueltin on activated keratinocytes, one of the most representative cells for studying pathogenesis of acute and chronic atopic skin inflammation. As results, esculetin suppressed gene expression of Th1, Th2 and Th17 cytokines and activation of nuclear factor-B and signal transducer and activator of transcription 1 in TNF-/IFN–stimulated keratinocytes.
Conclusions: Taken together, the results imply that esculetin attenuated atopic skin inflammation, suggesting that esculetin might be a potential therapeutic candidate for the treatment of AD.

**(64) Submission ID#420907**

**Evaluation Frequency and Methodology of Common Variable Immunodeficiency in Patients with Immune Thrombocytopenic Purpura at a Large Academic Medical Center**

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Introduction/Background: Autoimmunity is often seen in common variable immune deficiency (CVID) with immune thrombocytopenic purpura (ITP) being the most frequent manifestation at a prevalence of 8-14% in CVID patients. In such patients, ITP is often recognized and treated long before CVID, the implications of which are unknown. Primary ITP is a clinicopathologic diagnosis which includes an evaluation of other conditions that may mimic it including CVID. Currently, it is unknown how frequently CVID is evaluated during the diagnostic workup of ITP and what percentage of those patient actually have CVID.
Objectives: The two main objectives of this study were to determine the number of ITP patients that had an IgG level checked during their clinical course and if the globulin fraction can be used as a marker for hypogammaglobulinemia in ITP patients at the time of diagnosis.
Methods: A retrospective chart review was undertaken at a large academic medical center of patients with a new diagnosis of ITP between January 2009 and January 2017. IgG levels were collected and globulin fractions were calculated as the difference between serum total protein and albumin within 30 days of the initial ITP diagnosis.
Results: Six hundred and twenty-three patients were found to have a new diagnosis of ITP in the given timeframe. Of these, only 61 (9.79%) had IgG levels checked at any point during their clinical course. Twelve of the 61 (19.7%) had hypogammaglobulinemia with only 3 of the 12 (25%) having a formal immunologic follow-up evaluation. Two were diagnosed with a primary immunodeficiency (1 CVID and 1 CTLA4 deficiency). Globulin fractions were calculated on 52 patients at the time of ITP diagnosis. Mean calculated globulin fraction in hypogammaglobulinemic patients was 2.64 (range 2.0 3.7), versus 3.92 (range 2.4 7.5) in patients without hypogammaglobulinemia (p=0.0004).
Conclusions: The diagnosis of CVID is often delayed from the onset of symptoms which can include autoimmune conditions such as ITP. Our data indicate that clinicians do not routinely check IgG levels at the time of ITP diagnosis which should be considered standard of care based on the current guidelines. Our data suggest that although calculated globulin fractions were significantly lower in hypogammaglobulinemic patients, the variability was substantial and hypogammaglobulinemic patients would be missed using this as an indicator of low immunoglobulins. Future directives include a prospective study using IgG levels checked at the time of ITP diagnosis, with formal evaluation for CVID in any hypogammaglobulinemic patients to evaluate the true prevalence of CVID among ITP patients.

**(65) Submission ID#422171**

**Evaluation of CD4-CD8-TCR+ T Limphocytes in a Pediatric Age Patient Diagnosed as Evans-Fisher Syndrome.**

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Introduction/Background: Autoimmune Lymphoproliferative Syndrome (ALPS) is a disorder characterized by immune dysregulation due to the rupture of lymphocyte homeostasis, which occurs as a result of mutations in the apoptotic pathway mediated by Fas. This disease is sometimes misdiagnosed due to its variable phenotypic expression and the overlapping of symptoms with many other hematological and immunological disorders. (1)

A patient diagnosed with Evans-Fisher syndrome (SEF) was referred to the laboratory for immunity studies. This is a patient who from infancy had multiple admissions for severe sepsis, with severe anemia and severe thrombocytopenia.

In the evaluation of the history of the disease by the work team, a series of clinical data was observed that suggested the possibility of the patient presenting an ALPS, so it was decided to incorporate as part of the study, the quantification of the cells T CD3 + CD4-CD8- (double negative T cells or DNT) that express TCR +.

The differentiation pathways of DNT TCR + and the role of Fas in this process are not clear, some authors hypothesize that they may be represented by direct descendants of chronically activated positive simple T cells, with deregulated co-receptors, in a state of differentiation in which they were destined to perish by Fas-mediated apoptosis. (1-4)

The population of TCR + DNT cells required for diagnosis must be derived from the particular study of each laboratory in their populations, but several working groups agree that the pathological limit values are 1,5% in total lymphocytes or 2,5% in CD3 + lymphocytes. (4)

Evidence shows that DNT ALPS cells are not simply accumulated in senescent withdrawal; they and their precursors remain active and proliferate under the influence of activation signals. (5) Although numerous genetic deficiencies lead to lymphoproliferation of T cells, only that caused by a defective Fas pathway is dominated by negative double T cells. (2)

In clinical immunology, the distinction between CD45RA + and CD45RO + cells is particularly useful for determining the state of the "naive" cell compartment in relation to its thymic origin. Primary immunodeficiency disorders are characterized by a decrease / absence of thymus performance, and often involve a decrease in CD45RA + T lymphocytes.

Scientific evidence suggests the important role of the "naive" subpopulation in the origin and maintenance of self-reactive effectors in the periphery. (5)

Regulatory T cells are able to effectively control autoreactive T cells, especially when negative thymic selection is defective. Its differentiation and function is controlled by Foxp3. The decrease or absence of regulatory T cells leads to autoimmune diseases, specifically those mediated by CD4 + T cells, and to lymphoproliferation characterized by multiorgan inflammation and other autoimmune disorders. (4, 5)

Objectives

General: Establish the diagnosis of a pediatric patient with suspected primary immunodeficiency due to dysregulation, an Autoimmune Lymphoproliferative Syndrome (ALPS).

Specific: Evaluate the TCR + DNT population, relevant in the diagnosis of ALPS.

Quantify cell populations that exhibit markers of activation, differentiation and regulation of the relevant T cells in the study of this disease.

Methods: Monoclonal antibodies (mAb) CD4FITC / CD8PE / CD3PC5 triple labeled (Beckman Coulter), TCRAPC, CD4PE, CD45RAPerCP, CD45ROFITC, CD8FITC, CD45ROPE, CD4APC, CD25F, CD56PE, CD19PerCP, CD3PE, CD5PE, CD45PerCP, CD4FITC, CD38FITC were used , HLA DR and the CD4FITC / CD25PE / Foxp3APC regulatory T cell kit, all from Miltenyi Biotec.

The trial included the use of a healthy control. Both samples were processed in unison to ensure reproducibility. The samples were acquired in a Beckman Coulter Gallios cytometer, with the use of the Kaluza program, version 1.2.

The analysis strategy included the formation of overlapping "gate" windows for the quantification of DNT TCR + and regulatory T cells.

Results: The patient studied showed 11.60% of TCR + negative double T cells, in relation to 1.45% of the healthy control. The presence of this population of T cells in patients diagnosed with Evans syndrome, even in the absence of lymphoproliferation, is consistent with ALPS. (5) (Graph 1)

The presence of increased TCR + DNT and lymphoproliferation - expressed as lymphadenopathies and splenomegaly of noninfectious or malignant cause, of at least 6 months of evolution; plus the typical immunohistological findings found in the patient's lymph node biopsy (paracortical hyperplasia), the presence of autoimmune cytopenias (hemolytic anemia and thrombocytopenia) and hypergammaglubulinemia, led to the "probable diagnosis" of ALPS in the patient studied.

The accumulation of DNT in the lymph nodes and other peripheral organs is accompanied by qualitative changes in the composition of the T cell repertoire, so that the immunophenotype performed included markers of activation, differentiation and regulation.

The evaluation of the activation on T lymphocytes and NK cells showed that there was a slight decrease in the expression of the receptor for IL-2, CD 25.

The CD19 + CD25 + population was expressed in greater percent - although discretely- in the patient than in the healthy control, which was directly related to the presence of hypergammaglobulinemia, elevated IgA and the presence of autoantibodies. It was also highlighted by the high expression of the CD19 + CD5 + autoreactive population in the patient studied (11.73% vs 0.39%).

In order to know the impact on cell differentiation, the subpopulations of effector and memory cells for CD4 + and CD8 + T lymphocytes were evaluated by combining the CD45RA and CD45RO isoforms.

CD45RA is expressed in naive T lymphocytes. Particularly, the CD4 + CD45RA + population has an essential function as a suppression inducer, and it is diminished in the patient studied, in relation to the control. (29.11% vs. 62.75%, respectively) The same behavior was shown by the CD4 + CD45RO + memory and effector cells, 53.38% vs73.37%.

In the CD4 + population itself, it was possible to confirm the presence of a clone that expressed both receptors (CD45RA and CD45RO), probably a TEMRA population (terminally differentiated effector memory cells). When comparing the results, it was unexpectedly found that there was a slight decrease between patient and control (2.70% vs 4.0%).

As the characteristic phenotype of this cell population could not be corroborated, conclusive assessments could not be made.

The combination of CD45 isoforms was also used to study CD8 + T lymphocytes, but similar behavior between patient and healthy control was observed. (The lab results are shown in Table 1)

The analysis of the regulation of the immune response showed a decrease in the CD4 + CD25 + population and the expression of the Foxp3 transcription factor in the patient with respect to the control. (11.16% vs. 6.30%, 0.00% vs. 1.05%, respectively) (Graph 2)

Conclusions
1. The markedly high quantification of CD4-CD8-TCR + T lymphocytes allowed to define the "probable diagnosis" of Autoimmune Lymphoproliferative Syndrome in the patient under study.
2. The increased activation of CD19 + cells and the presence of the CD19 + CD5 + self-reactive population, largely responsible for autoimmunity and lymphoproliferation in the ALPS, could be confirmed.
3. The decrease in the CD4 + CD45RA + T-cell suppressor-inducing population evidenced corroborated its involvement in this disorder by primary immunodeficiency, in relation to the thymus dysfunction that originates and maintains self-reactive effectors in the periphery.
4. The decrease in the expression of the Foxp3 transcription factor observed, points towards a low regulation of the response that leads to autoimmunity and to lymphoproliferation, specifically mediated by CD4 + T cells.

**(66) Submission ID#409240**

**Evaluation of Pneumococcal Serotype-Specific Antibody Titers in Pediatric Patients with Recurrent Sinopulmonary Infections: A Pre and Post Booster Vaccination Assessment and its Clinical Correlation**

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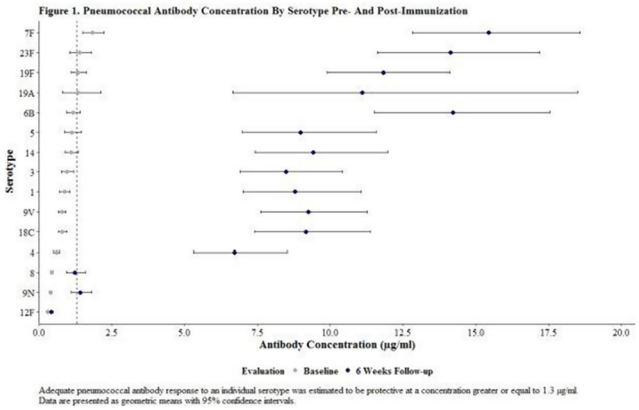
Introduction/Background: Specific antibody deficiency syndrome is characterized by a weak antibody response to bacterial polysaccharide antigens when no other immune system abnormalities can be found. Low titers to pneumococcal vaccine have become one of the most frequently recognized immune abnormalities in pediatric patients with recurrent sinopulmonary infections. Nonetheless, insufficient data and lack of consistent testing of the response to pneumococcal polysaccharides continues to affect the optimal diagnosis and management of this specific antibody deficiency.
Objectives: To characterize the pre- and post-immunization IgG antibody trend for each specific serotype included in the pneumococcal 13-valent conjugate vaccine (PCV13), as well as others that are routinely tested, in a cohort of pediatric patients with recurrent sinopulmonary infections. Secondarily, to understand differences in the immune response to the vaccine booster between age groups.
Methods: This retrospective review identified 182 patients with recurrent sinopulmonary infections. In this cohort, 131 required an immune workup, and 99 were found to have low pneumococcal titers needing a PCV13 vaccine booster. Baseline pneumococcal serotype-specific antibody titers at initial visit and 6 weeks after the vaccine booster were obtained. Patients were categorized by age: 2 years, 3-5, 6-10, and 11-18. An adequate response to the pneumococcal conjugate vaccine was deemed to be a 4-fold increase over baseline and/or a post-immunization titer of 1.3 μg/ml or greater.
Results: Overall, PCV13 booster provided a significant improvement in the number of protective titers, increasing from 3.6 (95% CI: 3.2-3.9) serotypes at baseline to 11.1 (95% CI: 10.7-11.5) serotypes at 6 weeks ($p < 0.001$). This increase correlated with improved clinical outcomes81% showed no signs of recurrent infection after the first booster and 94% after a second dose. All those who did not improve clinically suffered from co-morbidities (genetic abnormalities and rheumatologic diseases). Post-immunization antibody concentrations were significantly higher than at baseline for all serotypes ($p < 0.05$) and only 8, 9N, and 12F did not exhibit a greater than 4-fold increase ($p > 0.05$) 6 weeks following the booster. Across age groups, only 1, 7F, and 9V showed pre-immunization differences in titers. There were no differences between ages in post-immunization titer levels for all serotypes. Similarly, all age groups had a comparable number of baseline titers ($p = 0.63$) and at follow-up ($p = 0.10$).




Conclusions: In pediatric patients with recurrent sinopulmonary infections, an additional pneumococcal booster proved to be effective in the protection of these children from further infections. The PCV13 booster substantially increased titer levels and concentrations, and significantly improved clinical outcomes, independent of age. This investigation has provided us with a better understanding of the response after booster vaccination, and its role in the protection of patients from recurrent sinopulmonary infections. Further studies are needed to elucidate whether a fifth dose of PCV13 should be optional as part of the vaccination schedule of patients with recurrent sinopulmonary infections.

**(67) Submission ID#421463**

**Expanded Genetic Testing for Primary Immunodeficiencies: Findings From a 207-Gene Next-Generation Sequencing Panel**

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Introduction/Background: Many primary immunodeficiencies (PIDs) share overlapping presentations, complicating clinical diagnosis. Due to the ability to include many genes in one assay and the rapid turnaround time that these panels allow, expanded next-generation sequencing (NGS) panels are valuable in facilitating the diagnosis of patients with PIDs.
Objectives: We aimed to determine the clinical utility of an expanded NGS panel for the genetic diagnosis of patients with suspected PIDs.
Methods: We performed a retrospective analysis of the clinical utility of a 207-gene PID NGS panel used in a clinical diagnostic laboratory. From April to October 2017, 260 panels were ordered for patients with suspected or known PIDs.
Results: Seventy-four pathogenic or likely pathogenic (P/LP) variants were identified in 63 (24.2%) patients. Eight (10.8%) of the P/LP variants were copy number variations (6 deletions, 2 duplications). Fifty-two patients (20%) had 1 P/LP variant, and 11 patients (4.2%) had 2 P/LP variants. Of positive patients, 31.7% (n = 20) were heterozygous carriers for autosomal

recessive conditions where a second variant was not identified. Twenty-two patients had P/LP heterozygous variants in genes with autosomal recessive and autosomal dominant inheritance patterns, in which the positive findings may or may not explain the patient's phenotype. For example, 13 variants in this category were heterozygous variants in TNFRSF13B (TACI). Genetic diagnoses were established or likely in 30% of patients with P/LP variants (7.3% of all patients). Five patients were heterozygous for a single P/LP variant and a variant of unknown significance in the same autosomal recessive gene. Four patients in whom a genetic diagnosis was determined were also heterozygous carriers for a second, unrelated condition. One patient was found to have two distinct genetic diagnoses. Variants of uncertain significance (VUS) were identified in most (94.2%) patients. The average turnaround time from test requisition to return of results was 18 days. In total, 63% percent of genetic diagnoses were for conditions that are treatable with hematopoietic cell transplantation.
Conclusions: These results illustrate the utility of broad NGS panels for the diagnosis of patients with PIDs.

**(68) Submission ID#420840**

**Experiences of Parents of Patients with Severe Combined Immunodeficiency Disease (SCID) Identified by Newborn Screening: a Qualitative Study**

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Introduction/Background: Severe combined immunodeficiency (SCID) is a life-threatening immune deficiency manifest by extreme susceptibility to infection. Early diagnosis and definitive treatment with either hematopoietic cell transplant (HCT) or, in select cases, gene therapy (GT) has been shown to significantly improve survival. Newborn screening for SCID has allowed for the opportunity to promptly identify these patients before significant infections occur. At UCSF, newly diagnosed infants with SCID are admitted to the hospital for management and remain in isolation for definitive treatment and until adequate immune reconstitution occurs. Previous work by our group demonstrated up to 60% of these parents experience psychosocial trauma manifested by depression and post-traumatic stress disorder (PTSD). The psychosocial challenges that contribute to depression and PTSD in these parents have not been qualitatively described.
Objectives: To understand the range of experiences and feelings of parents/caregivers of infants with SCID diagnosed by newborn screening throughout their prolonged hospitalization and isolation in order to better support patients, parents, and their families.
Methods: Voluntary participation was elicited from parents of children with SCID who were status post HCT/GT for one year or longer. Semi-structured, in-person interviews lasting approximately 45-60 minutes were conducted with 11 parents; interviews were recorded and transcribed. Parents were asked to discuss their experiences from first notification of an abnormal screening result through discharge after HCT or GT. Emerging themes were identified from the transcribed interviews.
Results: We interviewed 6 mothers and 5 fathers of 7 infants with SCID. Six infants received HCT, while one underwent GT for ADA-SCID. All children were alive and well at the times when interviews were conducted. Overall, once admitted, parents reported feeling well supported by the medical team and support staff. However parents identified a number of stressful events. Uniformly reported key stressors included: receiving the first phone call regarding their childs abnormal newborn screening results, preparing for HCT, coping with prolonged isolation, and transitioning from hospital isolation to care with ongoing isolation at home. Other challenges described reflected the additional stressors of caring for a newborn, including coping with postpartum depression. Overall, we identified three major themes encompassing the challenges faced by parents of hospitalized SCID patients: (i) loss of normalcy and control over multiple aspects of life; (ii) prolonged waiting periods (especially the wait between diagnosis and HCT and between HCT and evidence of T cell engraftment); and (iii) perceived lack of guidance on realistic expectations during the hospital stay . Parents sought and relied on peer support from other SCID parents to learn about coping with the nuances of daily life as a parent of an infant with SCID.
Conclusions: We identified multiple psychosocial stressors and challenges uniquely faced by parents and caregivers of infants diagnosed with SCID by NBS. Parents described barriers to caring for their own physical and mental health, which can be especially harmful to parents experiencing postpartum depression. Recognizing these challenges allowed for the identification of opportunities to improve both healthcare delivery to their children and institutional support for future families affected by SCID (Table I). Emphasis should be placed on providing parents with SCID-specific resources as early as the time of diagnosis, connecting parents with SCID support networks, and facilitating access to psychosocial and mental health services for caregivers

**(69) Submission ID#414066**

**First Report of a Papulopustular Dermatitis as the Presenting Feature of Chronic Granulomatous Disease in Two Infants at 8 months and 14 months**

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Introduction/Background: Chronic granulomatous disease (CGD) is caused by a genetic defect that impairs phagocyte function. This disease results in recurrent infections and granuloma formation. Rarely do patients develop cutaneous symptoms, unless associated with autoimmune disorders such as systemic erythematous lupus. Previously described cutaneous findings include granulomas, abscesses, photosensitivity, malar rash, discoid lupus, vasculitis, and rarely vesicular rashes. Here we describe two term infants diagnosed with X-linked CGD who present, in addition to frequent infection, with a unique papulopustular skin rash initially diagnosed as non-classic appearing eczema refractory to usual eczema treatment and antibiotics.
Objectives: To characterize cutaneous findings in X-linked CGD and emphasize the importance of considering further work in patients who present with similar rashes in conjunction with concerning features for primary immunodeficiency.

Methods: Each infant was diagnosed with CGD based on abnormal DHR testing and/or genetic evaluation. After obtaining consent from both families, we have documented photographs of the development of rash in two newly diagnosed infants with CGD. One infant underwent cutaneous biopsy with resulting pathologic evaluation.
Results: Our first patient presented at 8 months of age with episodic fever with chronic leukocytosis, iron deficiency anemia, thrombocytosis, elevated inflammatory markers, proctocolitis with elevated calprotectin, and rash. EGD with flexible sigmoidoscopy showed no signs of inflammatory bowel disease, the left colon had macroscopically raised erythematous lesions but was microscopically normal with no signs of active colitis. He was initially diagnosed with FPIES and eczema in the setting of diagnosis of various infections (otitis, upper respiratory illness). The skin rash was described as non-pruritic, generalized pink-purple papulopustular lesions with a pink base, most prominent on upper and lower extremities, mostly sparing the trunk. Skin biopsy histopathology revealed an essentially unremarkable appearing epidermis and within the dermis, there was a superficial perivascular lymphohistiocytic infiltrate. Eosinophils and plasma cells were not abundant. The histologic changes were thought to be non-specific, but could be seen in a drug reaction or urticaria. A viral exanthem could also demonstrate these changes. Giemsa stain and a stain for mast cell tryptase revealed normal numbers of mast cells in the biopsy specimen. He was not on any oral medications at the time and did not respond to treatment with topical moisturizers, oral antihistamines, topical steroids, or any standard for eczema care. He had a maternal uncle who passed away in infancy from infection. Heightened clinical suspicion for primary immunodeficiency led to obtaining a neutrophil respiratory burst assay which was consistent with CGD and genetic testing was positive for X-linked CGD. Pathogenic mutation nucleic acid change was c. 469c>T; Hemizygous; Amino acid alteration was p.157Arg>Stp within the CYBB gene locus. He had no other features of auto-immune disease including negative ANA obtained later in his clinical course and his colitis diagnosed as CGD-associated colitis. His rash did not respond to systemic treatments for his colitis, oral antibiotics, and during HSCT.
Our second patient presented at 14 months of age with persistent fevers, inguinal lymphadenopathy, leukocytosis, elevated inflammatory markers, non-bloody diarrhea and rash. There was a family history of autoimmune GI disease. He presented to the hospital with fever of unknown origin with concern for infection, atypical Kawasakis, and drug rash. His rash was described as a blotchy, pink, papular rash most prominent on the upper arms but also present on lower arms, legs, and chest, faint on face. There is some induration and thickness to the rash in some areas (confluent on the arms with more erythema and induration) and more faint pink papules (scattered on legs) elsewhere. A biopsy of his inguinal lymph node showed granulomatous lymphadenitis with neutrophilic abscess formation, and culture was positive for Serratia marcescens. Neutrophil respiratory burst assay was consistent with CGD and genetic testing was positive for X-linked CGD, mutation with the CYBB gene. Pathogenic nucleic acid change was c. 141+5G>A; Hemizygous; Amino acid alteration was p. deletion of Exon 2. The rash remained unchanged with treatment for infection, initiation of antifungal and bacterial prophylaxis, along with topical steroid therapy.
Conclusions: In patients who present with frequent infections and who have unusual cutaneous findings that do not fit with common infant rashes, consideration of work up for CGD should be pursued. Specifically, generalized papulopustular rash with a negative skin biopsy can be misdiagnosed as atopic dermatitis, and in the right clinical context CGD should be considered.

**(70) Submission ID#420138**

**Fractional Exhaled Nitric Oxide Measurements in Common Variable Immunodeficiency Patients with Chronic Lung Disease**

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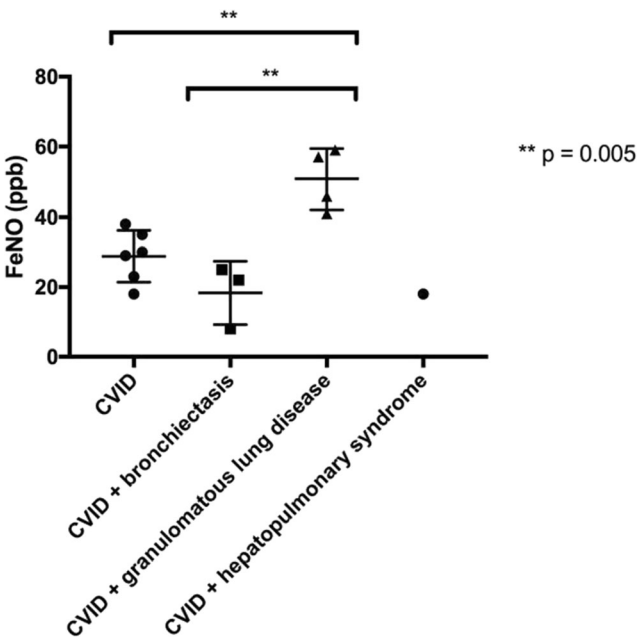
Introduction/Background: Chronic lung disease in common variable immunodeficiency (CVID) is heterogeneous, and it is a leading cause of morbidity and mortality in this population. Currently, the diagnosis and monitoring of chronic lung disease in CVID rely on radiographic findings, biopsy and/or pulmonary function tests. Fractional exhaled nitric oxide (FeNO) is a noninvasive biomarker of airway inflammation, and it has been widely utilized to aid the in-office diagnosis, characterization, and management of inflammatory airway disease, such as asthma. However, exhaled nitric oxide in CVID patients with chronic pulmonary complications has not been examined.
Objectives: We aimed to determine fractional exhaled nitric oxide levels in CVID patients.
Methods: We measured exhaled nitric oxide in CVID patients with or without chronic lung disease.
Results: FeNO measurements were obtained in 13 CVID patients (mean age: 53, range 34-68). Five patients had no known lung disease; 8 patients had chronic lung disease (bronchiectasis, n=3; lung granulomas, n=4; hepatopulmonary syndrome, n=1). Four patients were on inhaled steroid (bronchiectasis, n=3; lung granulomas, n=1), 2 patients were on systemic steroid (lung granulomas, n=1; hepatopulmonary syndrome, n=1), and 1 patient was on oral budesonide (no known lung disease). FeNO was elevated (> 25 parts per billion [ppb]) in 9 of 13 patients, 6 of whom had known chronic lung disease. Patients with granulomatous lung disease had higher FeNO (mean 50.8 ppb, range 41-59 ppb) compared to patients without known lung disease (mean 31 ppb, range 23-38 ppb, p=0.005), patients with bronchiectasis (mean 18.3 ppb, range 8-25, p=0.005), and the patient with hepatopulmonary syndrome (18 ppb). FeNO levels remained elevated in granulomatous lung disease in patients with inhaled or systemic steroid use.




Conclusions: This is the first report of FeNO measurements in CVID patients. FeNO is elevated in a subgroup of CVID patients, and it may differ according to the underlying lung pathology. Further investigation is warranted to determine the utility of FeNO in the diagnosis and management of chronic lung disease in CVID.

**(71) Submission ID#422038**

**Functional Evaluation of a Novel Homozygous Variant in Caspase Recruitment Domain Family Member 11 (CARD11)**

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Introduction/Background: Whole exome sequencing (WES) has revolutionized the discovery, diagnosis, and treatment of primary immune deficiency diseases (PIDs), a group of disorders with high genetic and phenotypic heterogeneity. Current bioinformatic analysis approaches for WES rely heavily on population databases to exclude variants present in the populations represented by these databases. This approach may confound the ability to detect true disease causing variants, and conversely, flag variants that are absent from population databases only by virtue of originating from minority populations. Ultimately, the pathogenicity of novel variants can only be mechanistically established through rigorous biochemical and functional validation.
Objectives: To evaluate the pathogenicity of a novel homozygous variant in caspase recruitment domain family member 11 (CARD11) (c.1798G>T, p.C600Y), in a patient with features of combined immunodeficiency (CID).
Methods: Research study protocols were approved by our institutional review board. Six members of one family (the affected child, healthy siblings and their parents) were enrolled. Written informed consent for genetic testing and participation was provided by the parents for their children. Genetic, bioinformatic, biochemical and immunological investigations were performed.
Results: Targeted Sanger sequencing confirmed the presence of the homozygous CARD11 variant c.1798G>T, p.C600Y in the index patient (and subsequently in one apparently healthy sister). Each parent was found to be a heterozygous carrier of the variant. NFKB activation in vitro was found to be normal for both the index patient and sister, and was indistinguishable from unaffected family members.
Conclusions: Although multiple in silico tools predicted the c.1798G>T, p.C600Y CARD11 variant to be pathogenic, the patients B and T cells did not have aberrant NFKB activation in vitro, as would be predicted given the central role of CARD11 in NFKB activation. This practical experience highlights how imperative it is to functionally characterize novel variants found by WES, even when variants are predicted to be damaging.

**(72) Submission ID#417394**

**GATA2 Deficiency with Mutation c.1061C>T p.(Thr354Met) in a Brazilian Patient.**

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Introduction/Background: GATA2 is a zinc finger transcription factor essential for embryonic and definitive hematopoiesis. Heterozygous mutations leading to GATA2 deficiency were first described in 2011. The age of clinical presentation ranges from early childhood to late adulthood, with most of them in adolescence to early adulthood. Patients present clinical findings as monocytopenia, nontuberculous mycobacterial infections, myelodisplasia, viral (mainly HPV and EBV), fungal and bacterial infections. Patiens may arise with many phenopytes, showing how complex is the effect of this transcription factor.
Objectives: We report a patient with GATA2 mutation.
Methods: Report: A 34 year old female patient was referred due to a Mycobacterial non-tuberculosis pneumonia. She was a healthy child until puberty. At the age of 15 she presented genital herpes and recalcitrant vulvo-vaginal warts (HPV). At 18, she complained of lower back pain with no diagnosis for months, medication was unnefective, and she developed ischemic stroke. Hemiparesia was mild and temporary. Endocarditis with no agent identified was also diagnosed. Therapy with acetilsalicilic acid was mantained until the age of 28. Five years later, vaginal HPV spread through vulve and anal region evolving to neoplasia. She was submitted to surgery, radio and chemotherapy until October 2016. One year later, profound cytopenias led to hospitalization. During that time, she had cough and fatigue and Pulmonary Tuberculosis was diagnosed. Despite therapy with Rifampin, Isoniazid and Pyrazinamid, there was no improvement. Bronchoalveolar lavage was positive for Mycobacterium avium. Main immunological evaluation showed: Monocytes 162 (6%), T cells /mm3; CD4+: 28 (1,5%), CD8+: 74 (4%); B cells 2/mm3 (0,1%); NK: 9/mm3 (0,5%); Normal Immunoglobulin levels; incomplete response to Pneumococcus serotypes; IgM and IgG positive for CMV; negative serologies for HIV, EBV and HTLV1/2. Molecular analysis identified an heterozygous mutation c.1061C>Tp.(Thr354Met) in gene GATA2.
Results: We report a patient with GATA2 mutation. The same GATA2 mutation was previously described in National Institute of Health NIH-USA (n=5) and in Australia (n=3).
Conclusions: It took almost 20 years for diagnosis suspicion. Gynecologists should be warned about this diagnosis in order to improve patients prognosis. Early diagnosis is crucial with adequate prophylaxis, prompt treatment of infection, surveillance of malignancy, and, moreover, family screening and genetic counseling. This is the first report of this mutation in Latin America.

**(73) Submission ID#427139**

**Gene Therapy for Adenosine Deaminase-Deficient Severe Combined Immunodeficiency (ADA SCID) with a Lentiviral Vector.**

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Introduction/Background: Severe combined immunodeficiency (SCID) due to adenosine deaminase (ADA) deficiency was the first human monogenic disease to be approached with gene therapy, and ongoing research advances over 25 years led to the approval by the European Medicines Agency of a stem cell gene therapy product for its treatment using a gammaretroviral vector (GV), Strimvelis®. Despite the high success rate using GVs for ADA gene transfer without vector-related complications,

the development of leukoproliferative complications from the use of GVs in gene therapy of other disorders led us to develop lentiviral vectors (LVs) to deliver the corrective ADA sequence/cDNA (Corrigan-Curay et al, Mol Ther 2012; Modlich et al, Mol Ther 2009.). LVs, typically derived from HIV-1 and devoid of all viral genes, can be produced in a self-inactivating (SIN) configuration, in which the viral long-terminal repeat enhancers are absent, eliminating the major identified cause of insertional oncogenesis due to GVs. We developed a LV (EFS-ADA) that carries a normal human ADA cDNA (codon-optimized) and demonstrated in pre-clinical studies its efficacy to transfer and express the ADA protein, with evidence of significantly decreased potential for insertional mutagenesis compared to gammaretroviral vectors using the immortalization (IVIM) assay and in murine bone marrow transplant models (Carbonaro et al, Mol Ther, 2014). This new LV was evaluated for safety and efficacy in parallel clinical trials of gene therapy for ADA SCID performed at sites in the U.S (University of California, Los Angeles and the National Institutes of Health {NIH} Clinical Center) and U.K. (University College London/Great Ormond Street Hospital) enrolling subjects between 2012 and 2016. We report here results from the 20 patients treated in the U.S. between 2013-2016.
Objectives: This is a prospective, non-randomized Phase I/II clinical study to assess the safety and efficacy of EFS-ADA lentiviral vector stem cell gene therapy in ADA-SCID subjects older than 1 month.
Methods: 20 subjects with ADA-SCID were enrolled, screened to document eligibility and underwent bone marrow harvest (15-20 cc/kg). Bone marrow was processed to isolate CD34+ cells, which were pre-stimulated by overnight culture in serum-free medium containing c-kit ligand, Flt-3 ligand, and TPO followed by culture with the EFS-ADA lentiviral vector overnight. Subjects received a single dose of busulfan (4 mg/kg) IV for reduced intensity conditioning. Cells were removed from culture, washed, formulated and administered by IV infusion at least 24 hours after busulfan administration. Enzyme replacement therapy (ERT) with pegylated bovine ADA (PEG-ADA) was continued for one month post-transplant and then stopped. Subjects were followed over 24 months to assess safety and efficacy end-points.
Results: With 17-54 months follow-up, overall survival is 100%. Event-free survival has also been 100%, as all subjects are alive, remain off PEG-ADA ERT, and none have required a second transplant. Successful engraftment of gene-corrected cells was observed in all subjects at 6 months, and persisted over the 24 months of observation, based on vector gene marking in granulocytes and peripheral blood mononuclear cells (PBMCs), changes from baseline in RBC ADA enzyme activity, and levels of metabolic detoxification of deoxyadenosine nucleotides. Immune reconstitution was observed in all subjects and was sustained over the two years of observation, based on improvement of peripheral blood absolute lymphocyte counts and lymphocyte subsets (T, B and NK cells, and naïve CD4+ T cells). Eighteen of 20 patients have been able to stop receiving immunoglobulin replacement therapy. Subjects who had routine infections all recovered with standard of care treatment, and there were no severe or opportunistic infections.
Conclusions: Conclusions: Gene therapy using the EFS-ADA LV has a favorable safety profile and was efficacious in this trial. A current follow-on trial at UCLA is using a cryopreserved formulation of the cell product and pharmacokinetic-adjusted busulfan dosing, sponsored by the California Institute for Regenerative Medicine (CLIN2-09339) and Orchard Therapeutics.
Acknowledgements: This study was supported by research grants from the National Institutes of Health (U01 AI100801; 2P01 HL073104-01; NHLBI GTRP RSAs #1101 and 1129) and the California Institute for Regenerative Medicine (CL1-00505; FA1-00613); support from the UCLA David Geffen School of Medicine Human Gene and Cell Therapy Program and the UCLA Eli & Edythe Broad Center of Regenerative Medicine and Stem Cell Research; and funding from the NHGRI intramural program. DBK has potential financial conflict of interest as a member of the Scientific Advisory Board for Orchard Therapeutics and as an inventor on intellectual property which UCLA has licensed to Orchard Therapeutics related to gene therapy for ADA SCID.

**(74) Submission ID#421809**

**Genome Editing of Long-Term Human Hematopoietic Stem Cells for X-Linked Severe Combined Immunodeficiency**

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Introduction/Background: X-linked severe combined immune deficiency (SCID-Xl) is a rare monogenic primary immunodeficiency disorder (PID) where male infants are born without an adaptive and innate immune system. It is a life-threatening disease due to patients inability to fight viral and bacterial infections. SCID-Xl is driven by any of the two hundred known pathogenic mutations in the interleukin 2 gamma receptor (IL2RG) gene, which function is required for proper development of T, B and NK cells. The most effective treatment for SCID-Xl, if performed in the first few months of life, is allogeneic hematopoietic stem cell transplantation (allo-HSCT). This treatment is limited by absence of match donors, incomplete immune reconstitution, graft versus host disease and the need for long-term immunosuppression. An alternative curative treatment for SCID-Xl would be genome editing-based gene therapy by ex vivo genome correction of the patients long-term hematopoietic stem cells (LT-HSCs) prior to autologous stem cell transplantation (auto-SCT).
Objectives: Here we report a proof-of-concept genome editing-based approach for correcting SCID-Xl disease.
Methods: Using a CRISPR/Cas9-rAAV6 platform, we deliver a full-length codon optimized IL2RG complementary DNA (cDNA) at the endogenous start site in CD34+ hematopoietic stem and progenitor cells (HSPCs).
Results: Using an optimized genome editing protocol we achieved >80% genome editing as early as 48h and a median of 45% genome targeting while retaining >80% viability and promoting greater than 70% ex vivo expansion of CD34+ HSPCs from healthy donors. We demonstrate that our approach retains proper IL2RG signaling function in T-cells derived from healthy male donors and rescues the

lymphopoietic defect from a patients derived mobilized CD34+ HSPCs both in vitro and in vivo. We further show robust in vivo primary human engraftment potential and multi-lineage hematopoietic reconstitution of IL2RG gene targeted HSPCs: a median of 26% (bone marrow), 47% (spleen) and 37% (liver) of IL2RG genome targeted engrafted cells was achieved at four months following primary transplantation into NSG mice and a median of 9.5% - 20% was detected at 8 months from secondary transplants of IL2RG targeted HSPCs, thus achieving clinical levels of editing of LT-HSCs. Lastly, our observation of (1) an intact hematopoiesis derived from IL2RG targeted CD34+ HSPCs combined with (2) a normal karyotype analysis and (3) our deep analysis of potential off-target activity that showed only 2 off-target sites of no known functional significant with < 0.3% frequency of off-target activity presents strong evidence for the safety of our genome editing approach.
Conclusions: In sum, this pre-clinical study provides specificity, toxicity and efficacy data supportive of continued development of genome editing to treat SCID-Xl.

**(75) Submission ID#428044**

**Graft Versus Host Disease Following HLA- Matched Sibling Donor Compared with Matched Related Donor for Hematopoietic Stem Cell Transplantation in the Treatment of Severe Combined Immunodeficiency Disease**

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Introduction/Background: One of the limiting factors for successful hematopoietic stem cell transplantation (HSCT) is graft versus host disease (GVHD). The EBMT/ESID guidelines for HSCT in severe combined immunodeficiency (SCID) recommend no GVHD prophylaxis for matched sibling donor (MSD).
Objectives: To determine the risk of GVHD in MSD HSCT for SCID patients compared to matched related donor (MRD).
Methods: Retrospective cohort study comparing MSD with MRD and the outcome of GVHD in all SCID patients who underwent HSCT between 1993 and 2013. All statistical analysis was done using IBM SPSS statistic software.
Results: 145 SCID patients underwent 152 HSCT, 82 (54%) received GVHD prophylaxis. GVHD occurred in 48 (31.5%); 20/48 (42%) had GVHD prophylaxis compared to 28/48 (58%) that did not, P value = 0.022. Acute GVHD occurred at a higher rate in MSD 22/120 (18.3%) compared to MRD 2/32 (6.2%) P value = 0.095. We analyzed outcome also according to period of HSCT. First periods was 1993 to 2003; 48 HSCT, MSD: 43, MRD: 5; all had GVHD prophylaxis and there was no difference in GVHD. The Second period was 2004 to 2013:104 HSCT, 77 had MSD and 27 had MRD, GVHD prophylaxis was used in 22.1% in MSD and 63% in MRD, P value = 0.000. GVHD was significantly higher in the MSD (40.2%) compared to MRD (18.5%) odds ratio of 2.9 (95CI 1.01 to 8.66) P value = 0.041.
Conclusions: GVHD prophylaxis in MSD transplant may have a role to be considered in SCID patients.

**(76) Submission ID#428685**

**Gut Microbiota in a XIAP Deficient Patient with Severe Inflammatory Bowel Disease**

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Introduction/Background: XIAP deficiency (also known as X-linked lymphoproliferative disease type 2 XLP2; MIM: 300635) is an X-linked primary immunodeficiency associated with mutations in the gene encoding the X-linked inhibitor of apoptosis (XIAP; MIM: 300079). The pathophysiology is characterized by immune dysregulation, usually triggered by Epstein-Barr virus (EBV) infection. Primary EBV infection is followed by hemophagocytic lymphohistiocytosis (HLH) with high grade persistent fever, splenomegaly, hematologic cytopenias and hepatitis. Most patients die during this acute phase, and those who survive usually evolve with hypogammaglobulinemia, recurrent infections, cytopenias, inflammatory bowel disease (IBD) and low counts of iNKT cells. Dysbiosis of intestinal microbiota is believed to fuel IBD and possibly contribute to the initiation and/or perpetuation of the disease. Experimental studies have provided solid evidence to support a role for the indigenous gut microbiota in the pathogenesis of autoimmune diseases, thereby raising the possibility that an altered gut microbiota is an environmental risk factor for XIAP disease.
Objectives: In this study, we sought to investigate the identity and abundance of the bacteria in gut microbial communities in a 28 years old male patient with XIAP deficiency.
Case presentation: At 15 years of age, the patient presented with positive serology of active EBV infection, HLH, severe hepatitis, encephalitis and myocarditis. After recovery, the patient evolved well with few manifestations for several years. Approximately 3 years ago, the patient showed slow progression of hypogammaglobulinemia predisposing him to infections of the upper and lower respiratory system that required intravenous immunoglobulin replacement. Immunological evaluation revealed reduction (but not absence) of iNKT cells. At that time, the patient presented with intermittent diarrhea and abdominal pain that became more frequent and severe. After evaluation as IBD, the patients had been treated but without much improvement of diarrhea or resolution of his pain. After approximately 18 months, the patient presented noted pain and fistulas lesions at the scrotal and gluteal regions. The exact causes of IBD in XIAP deficiency are not known, but the abnormal activation of the mucosal immune system due to exaggerated response to the commensal bacteria associated to the dysregulation of NOD1 and NOD 2 signaling might play an important role in the development and maintenance of the inflammatory status.
Methods: The gut bacterial composition was assessed by targeted metagenomic from the patients stool sample, collected before initiation of IBD antibiotics therapy. The 16S rRNA amplified and its sequences were analyzed using a bioinformatic pipeline based on mothur software. We determined the bacterial community composition using 100,000 filtered reads using Illumina MiSeq platform.
Results: According to the EzBioCloud database, the obtained dataset included 548 operational taxonomic units at 3% dissimilarity, distributed among the following groups: Bacteroidetes (67.3%) with 37.5% of B. dorei, 18,1% B. vulgates, and 15.3% B. fragilis, Firmicutes (24.8%), Proteobacteria (7.7%), Bacteria_uc (0.06%), Actinobacteria (0.04%).

Conclusions: Increased abundance of Bacteroides species including B. dorei and B. vulgatus have been implicated in inflammation in several gut diseases such as ulcerative colitis, irritable bowel disease, and celiac disease. Although this experience is limited to a single patient, the results of the present study suggest an association between altered gut microbiota and the pathogenesis of IBD in XIAP disease and may be of relevance to the future development of novel therapeutic strategies for XIAP deficiency.

**(77) Submission ID#427718**

**Hematopoietic stem cell transplantation in patients with primary immune regulatory disorders: a Primary Immune Deficiency Treatment Consortium (PIDTC) and Inborn Errors Working Party (IEWP) Study**

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Introduction/Background: Primary Immune Regulatory Disorder (PIRD) is a newly recognized group of immune-mediated diseases with prominent features of autoimmunity, autoinflammation, and non-malignant lymphoproliferation in addition to immunodeficiency. The clinical manifestations of PIRDs are frequently difficult to manage and hematopoietic cell transplantation (HCT) can be considered as a treatment option, often in those with the most severe disease. We sought to aggregate data from patients who have undergone HCT for genetically defined PIRD or features of immune dysregulation.
Objectives: We sought to aggregate data from patients with PIRD who have undergone HCT in order to determine the quantity of patients, clinical manifestations, indication and HCT, and overall outcome.
Methods: A questionnaire based survey was sent to all Primary Immunodeficiency Treatment Consortium sites and 3 HCT referral centers in Europe to determine the quantity and characteristics of patients with PIRDs who have undergone HCT. The survey captured clinical manifestations, timing and indication of HCT, strategy of HCT, and outcomes from 1982-2017.
Results: 224 patients from 34 centers (31 in North America and 3 in Europe) were included with either known genetic defects or considered to have immune dysregulation regardless of gene defect. Known genetic defects were identified in 170 subjects, while 54 had symptoms of immune dysregulation but lacked a genetic diagnosis. The mean age of onset of disease was 2 years (range 0-20 years). Clinical manifestations included gastrointestinal disorders (69%), failure to thrive (67%), dermatitis (51%), hematologic cytopenias (49%), and lymphoproliferative disease (39%). Recurrent infections (66%), immunodeficiency (63%), autoimmunity (48%), and autoinflammation (38%) were also common. Organ specific autoinflammation occurred most commonly in the lung (39%) and brain (17%). The median age of HCT was 9 years (0-64 years). Graft sources included matched unrelated donors (47%), matched related donors (20%), mismatched unrelated donors (16%) and haploidentical donors (3%). Reduced or minimal intensity conditioning was used in 44% of transplants. Five-year overall survival was 61% and the majority of survivors had resolution of symptoms that led to transplantation. Among those patients that died, infection was the most common cause.

| Genetic defects | Number of Subjects |
|---|---|
| FOXP3 | 62 |
| APDS (PIK3CD, PIK3R1) | 23 |
| NFkB defects (IKBKG, IKBa, IKBKB) | 17 |
| CTLA4 | 13 |
| STAT3-GOF | 12 |
| Il-10R | 7 |
| LRBA | 4 |
| CD40L | 4 |
| C1q | 3 |
| STAT1-GOF | 3 |
| ZAP70 | 3 |
| CD18 | 3 |
| DADA2 | 2 |
| TTC7A | 2 |
| DOCK8 | 2 |
| STAT1-LOF | 1 |
| TACI | 1 |
| RAG1 | 1 |
| MVK | 1 |
| PSTPIP-1 | 1 |
| XIAP | 1 |
| ITK | 1 |
| SAP | 1 |
| MALT1 | 1 |
| CD25 | 1 |
| Unknown | 54 |

Conclusions: Based on our survey data, PIRD patients commonly develop clinical features of autoimmunity, autoinflammation, and susceptibility to infection at a young age. HCT can be successful and lead to disease resolution. However, further studies to define the appropriate patient, timing of HCT, donor selection and pre-HCT conditioning regimen are necessary to improve outcomes of patients with PIRD.

**(79) Submission ID#428660**

**Heterozygous pathogenic TNFRSF13B (TACI) variant in a patient with pediatric onset, difficult-to-treat inflammatory bowel disease**

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Introduction/Background: Pathogenic variants in TNFRSF13B (TACI) are relatively common (found in about 1% of the population) but have been seen in about 7-10 % of CVID patients, interestingly in both homozygous and heterozygous states. Recent articles suggest that the heterozygous state increases the risk of developing autoimmunity due to an effect on autoreactive B cell selection and activation.
Objectives
1) Illustrate the importance of genetic testing in patients with difficulty to treat inflammatory disease
2) Present a case report of inflammatory bowel disease associated with a pathogenic mutation in the TNFRSF13B gene
Results: 16 yo WF was diagnosed with Crohns disease due to the chronic abdominal pain, vomiting, and bloody stools since 13 years of age. Intestinal biopsy revealed inflammatory changes in the entire intestine with active, submucosal lymphoid hyperplasia, neutrophilic cryptitis with focal areas of crypathic damage, and submucosal epithelioid granulomas more predominantly seen in the colon and rectum. She was started on immunosuppressant medications but developed anaphylactic reaction to both infliximab and adalimumab. She was then treated with azathioprine, mesalamine, methotrexate, and oral budesonide. Despite these medications, she continued to have frequent relapses, 4-5 episodes a year and required periodic systemic corticosteroid bursts. Other biologics, vedolizumab and ustekinumab, were also tried without success, and she subsequently underwent a colectomy. Her postoperative course was complicated by ARDS, poor abdominal wound healing,

and sepsis. Due to her complicated clinical course, immune work up was performed which revealed a normal CBC and lymphocyte subpopulations, but hypogammaglobulinemia with low isohemagglutinin titers and specific antibody levels. Comprehensive genetic testing ruled out chronic granulomatous disease and other known primary immunodeficiencies but revealed a rare missense mutation in TNFRSF13B (TACI). This variant, c310T (p.Cys104Arg) (rs34557412, ExAC 0.5%) is likely pathogenic. This heterozygous variant has been seen in both CVID cases and unaffected relatives but significantly more common among CVID patients. Moreover, the studies on B cells of these relatives showed impaired function. Increased number of autoreactive B cells were also found in the bone marrow of heterozygous individuals and these cells could give a risk of developing autoimmunity.
Conclusions: In difficult-to-treat autoimmune diseases, identifying the underlying immune defect may aid in the treatment decision. In this case, B cell targeted treatment such as anti-CD20 monoclonal antibody could be beneficial.

**(80) Submission ID#428725**

**Heterozygous truncating variants in POMP that escape nonsense-mediated decay and cause a unique immune dysregulatory syndrome**

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Introduction/Background: Defects in immunoproteasome caused by biallelic or digenic loss-of-function mutations in proteasome catalytic subunits cause an autoinflammatory disease identified as Chronic Atypical Neutrophilic Dermatosis with Lipodystrophy and Elevated temperature (CANDLE) associated to an increased interferon type I gene signature. The proteasome maturation protein (POMP) is a chaperone for both standard and immuno-proteasome assembly and is critical for the incorporation of catalytic subunits. Here, we characterize and describe POMP-related autoinflammatory immunodeficiency disease (PRAID) in two unrelated patients and identify the underlying genetic mechanism of disease.
Objectives: Determine the genetic cause and mechanism of disease in two patients with POMP variants .
Methods: Whole-exome sequencing (WES) was performed to identify a genetic cause of our patients dysregulatory syndrome. Proteasome assembly and catalytic function was assessed by SDS-PAGE and native gel respectively, using patient derived cell lines. Expression of interferon type I- induced genes was measured by RT-qPCR. POMP protein was identified by Western blot.
Results: We identified two unrelated individuals with a unique syndrome characterized by neonatal onset autoinflammation, neutrophilic dermatosis, autoimmunity, and combined immune deficiency with severe systemic viral and bacterial infections. Immunologic evaluation for both individuals revealed elevated immunoglobulins, low CD8+ T cell numbers and extremely low B cell counts with persistently high titers of autoantibodies and increased expression of interferon type I-induced genes. In both individuals, truncating heterozygous de novo frameshift variants in POMP were identified by WES and confirmed by Sanger sequencing. Most mRNA transcripts with premature termination codons should undergo nonsense-mediated decay (NMD), however in both of our patients, cDNA sequencing revealed these transcripts escaped NMD. The expression wildtype and truncated versions of POMP protein was further confirmed by Western blot. Transfection of mutant constructs into an otherwise healthy cell line recapitulated an increased interferon signature suggesting a dominant negative mechanism.
Conclusions: We define PRAID in two unrelated individuals characterized by neonatal onset immune dysregulation and combined immunodeficiency caused by truncating variants in POMP in which transcripts that escape NMD result in a truncated protein that leads to a dominant negative (i.e antimorphic) allele. To our knowledge, PRAID is the first inherent defect of immunity mechanistically characterized by NMD escape.

**(81) Submission ID#419375**

**High Dose Immunoglobulin Treatment Options for Chronic Parvovirus Viremia in Immunosuppressed and Thymectomized Pediatric Heart Transplant Patients in a Tertiary Care Center**

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Introduction/Background: Parvovirus viremia may occur in pediatric heart transplant patients who underwent thymectomy and developed secondary T cell lymphopenia. High dose intravenous immunoglobulin (HDIvIg) has been used to treat parvovirus infection in these cases.
Objectives: We aim to review different routes of immunoglobulin treatment in pediatric cardiac transplant patients with parvovirus viremia and compare the patients immunological phenotype.
Methods: Data from three pediatric heart transplant patients with parvovirus viremia in a tertiary care center was reviewed including T cell counts, parvovirus viral load, route, dosage, and frequency of immunoglobulin treatment.

Results: All three patients received HDIvIg. Patient 1 and 2 tolerated the treatment and viremia improved. Patient 3 developed recurrent aseptic meningitis from HDIvIg treatment and his viral load remained >1 million copies/mL. Compared to the other two cases, patient 3 had a much lower T cell count that likely contributed to the persistence of viremia. To improve his quality of life and reduce healthcare costs, a facilitated subcutaneous immunoglobulin (SCIG) treatment option was explored. SCIG treatment was well tolerated and led to a dramatic decrease in parvovirus viral loads in patient 3.
Conclusions: Most pediatric cardiac transplant patients with persistent chronic parvovirus viremia respond well to HDIvIg, SCIG may serve as an alternative treatment option in refractory cases, especially in those with severe T cell lymphopenia.

**(82) Submission ID#428163**

**High Throughput Triplex TREC, KREC, RNAseP Assay in Newborn Screening in New York State**

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Introduction/Background: All but four states in the United States (US) screen for Severe Combined Immunodeficiency (SCID), by detecting T cell receptor excision circles (TRECs) from dried blood spots (DBS) on routine newborn screening (NBS). This has lead to improved estimates of the incidence and prevalence of SCID, decreased diagnostic delay, and improved patient outcomes. Preliminary studies outside the US have demonstrated that NBS can be adapted to include screening for B-cell deficient infants before symptom onset by quantifying kappa-deleting recombination excision circles (KRECs) on DBS.
Objectives: We report results of the initial characterization of a high throughput triplex TREC/KREC/RNAseP assay run in 9,994 samples in New York State (NYS).
Methods: DBS from 9994 anonymous, de-identified infants were included in the current study. DNA from patients with confirmed primary immunodeficiencies (n=32), including, but not limited to, X-linked agammaglobulinemia (n=11) and SCID(n=13) were obtained from the Centers for Disease Control and Prevention, and clinical immunologists working with the NBS programs in Massachusetts, Minnesota, Wisconsin, and NYS, were used as positive disease controls. All DBS were extracted and processed according to current NYS NBS protocols. A TREC/KREC/RNAseP triplex assay was designed and optimized to minimize reagent use, and maximize target amplification. Cycle threshold (Ct) was determined, and error detection cutoffs were identified to optimize sensitivity.
Results: PCR efficiency, assay quantification, intra-assay reproducibility, and error detection rates all met NYS NBS standards. Error detection rate for the triplex TREC/KREC/RNAse P assay is 6%, comparable to the current error detection rate of 5% for the current duplex TREC/RNAse P assay. Samples falling into the error detection range are repeated (analysis in process) to determine a receiver operating characteristic curve.
Conclusions: We show that the high-throughput TREC/KREC/RNAse P triplex assay is feasible in a large, racially and ethnically diverse population in NYS. Compared to the current duplex assay, this assay has favorable performance characteristics and provides additional immunologic characterization. Due to assay optimization, we were able to add the KREC test at no additional cost. Work is underway to further characterize other assay parameters such as sensitivity and specificity, in preparation for adoption of the triplex assay as part of routine NYS NBS.

**(83) Submission ID#409153**

**Highly accurate Wiskott-Aldrich syndrome diagnosis via rapid flow-based WAS protein staining**

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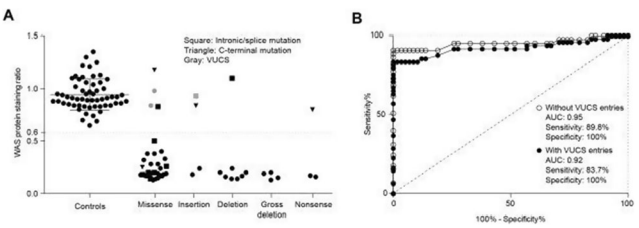
Introduction/Background: Wiskott Aldrich Syndrome (WAS) is a rare X-linked hemizygous disease commonly associated with symptoms of immune deficiency. Diagnosis is based on clinical parameters including thrombocytopenia and reoccurring infections, but currently does not include any disease specific marker. As the only permanent treatment for WAS is hematopoietic stem cell transplantation, it is imperative that a swift and accurate diagnosis be made. Absent or lowered WAS protein (WASP) levels have been reported in sporadic WAS cases. However, no systemic evaluation exists to date on the accuracy of WASP quantification for WAS diagnosis.
Objectives: To determine the accuracy of WASP staining in predicting WAS genetic abnormalities.
Methods: We retrospectively evaluated results from a rapid whole blood flow cytometry based assay on a cohort of suspected WAS patients and compared relative WASP staining levels to WAS genotype. ROC curves as well as accuracy calculations were generated.
Results: A total of 59 patients with normal and 49 patients with a genetic abnormality in WAS were collected. Missense mutations were most common but insertions, deletions, and gross mutations were also found (Fig A). Comparing WAS sequencing results to whole blood WASP expression levels provided an 82.6% sensitivity and 100% specificity for a combined accuracy of 95.3% when juxtaposed against genetic



sequencing. When 3 variants of unknown clinical significance (VUCS) were removed, the sensitivity improved to 89.1% (Fig B).
Conclusions: Staining for WASP is a quick, simple, and accurate assay for the prediction of genetic WAS defects.

**(84) Submission ID#426703**

**Hypereosinophilia, Eosinophilic Gastroenteritis, and Exocrine Pancreatic Insufficiency as Unique Manifestations of Cytotoxic T lymphocyte antigen 4 Haploinsufficiency.**

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Introduction/Background: Cytotoxic T lymphocyte antigen 4 (CTLA4) is an inhibitory co-receptor essential for regulatory T cell (Treg) function and a central regulator of T cell proliferation and expansion. CTLA4 haploinsufficiency is a recently described autosomal dominant disease, in which heterozygous CTLA4 mutations result in severe immune dysregulation with variable age of onset and a wide array of clinical manifestations. Herein we describe atypical findings in a patient with a novel pathogenic variant in CTLA4.
Objectives
1) To understand whether the novel CTLA4 variant identified is pathogenic and 2) to describe eosinophilic gastrointestinal inflammation and exocrine pancreatic insufficiency as possible manifestations of CTLA4 haploinsufficiency.
Methods: Next generation sequencing (Blueprint Genetics ©) was used to identify the CTLA4 variant. Polyphen and SIFT (Blueprint Genetics ©) were used for in silico analysis for the prediction of the effect of this genetic variant on protein structure/function. Flow cytometry was used to evaluate CTLA4 expression of regulatory T cells.
Results: A 10-year-old boy with type 1 diabetes mellitus and autoimmune thyroiditis presented with abdominal pain, diarrhea, and weight loss. Initial studies revealed markedly elevated peripheral blood eosinophils (>4000 cells/μL) and exocrine pancreatic insufficiency (<50 μg elastase per gram of stool). Prominent eosinophilic inflammation was appreciated in biopsies of the stomach, duodenum, jejunum, and terminal ileum. No parasitic infection or inciting drug/food trigger was identified. Additional blood studies revealed normal total quantification of T cells but with increased memory T cells (45%, CD3+CD4+CD45RO+) and decreased Treg (1%, CD3+CD4+CD25hiFOXP3hi). B cell quantification and serum immunoglobulin (Ig) levels were unremarkable, save a modestly elevated IgE level (74 IU/mL). Comprehensive next-generation sequencing of 232 genes associated with primary immune deficiency revealed a novel heterozygous missense mutation (c.457G>A, p.Asp153Asn) affecting the last nucleotide in the ligand binding domain (exon 2) of CTLA4. Subsequent analyses revealed decreased CTLA4 expression in the patients T cells compared to healthy controls as well as evolving hypogammaglobulinemia. Treatment consisted of methylprednisolone and parenteral nutrition followed by sirolimus and abatacept to which the patient responded favorably.
Conclusions: We report a 10-year-old boy with a history of type 1 diabetes mellitus and autoimmune thyroiditis presenting with hypereosinophilia, eosinophilic gastroenteritis, and exocrine pancreatic insufficiency as unique manifestations of CTLA4 haploinsufficiency. Although not previously reported in individuals with CTLA4 haploinsufficiency, peripheral blood eosinophilia and eosinophilic inflammation of the gastrointestinal tract have been observed in patients receiving ipilimumab (CTLA4 blocking antibody) suggesting a potential mechanism for the aforementioned findings. Severe exocrine pancreatic insufficiency is a rare but observed manifestation in individuals with type 1 diabetes mellitus. Whether severe exocrine pancreatic insufficiency would be expected to occur more frequently in individuals with CTLA4 haploinsufficiency and type 1 diabetes mellitus is unclear; however, our reported case and surveillance of others with CTLA4 haploinsufficiency could elucidate incidence and prevalence of this manifestation.

**(85) Submission ID#421449**

**Hypogammaglobulinemia Restricted to Pregnancy Requiring Immunoglobulin Replacement**

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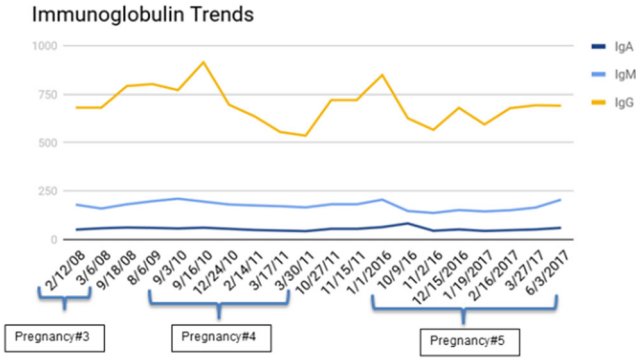
Introduction/Background: Mild asymptomatic hypogammaglobulinemia during pregnancy is a well-described phenomenon due to hemodilution. In patients with known humoral primary immunodeficiency such as common variable immunodeficiency, women require an upwards titration of their immunoglobulin replacement dose. Isolated symptomatic hypogammaglobulinemia during pregnancy in a patient is not well described in the literature.
Objectives
1. Understand the physiology of IgG during pregnancy.
2. Define a rare entity with a likely genetic predisposition that manifests as hypogammaglobulinemia isolated in pregnancy.
3. Management of this entity with defining goals of treatment.
Results: 33 year old gravida 5 para 4 female presented with progressive hypogammaglobulinemia restricted to pregnancy starting with 3rd pregnancy, recurrent otitis media requiring 3 sets of myringotomy tubes, recurrent sinusitis, and streptococcal pharyngitis. Her son has common variable immunodeficiency (CVID) requiring immunoglobulin replacement (IGRT). Prior to conception, her IgG was 849 mg/dL. During 13th week of pregnancy, her IgG level was 627 mg/dL. During 21 weeks of pregnancy, she complained of fatigue, and developed an episode of sinusitis that required 2 different antibiotic treatments. At that time, her IgG was 567 mg/dL. She was started on subcutaneous IGRT maintain IgG troughs of >650 mg/dL. She remained infection-free during her pregnancy and IGRT was stopped a few months after delivery with her serum IgG level returning to pre-pregnancy levels.
Patient was initially evaluated during her 3rd pregnancy with recurrent streptococcal pharyngitis. At that time, her IgG was 682 mg/dL. Diphtheria, Haemophilus influenza, Mumps, Measles and Rubella titers were protective, Tetanus titer was non protective with 1/14 antipneumococcal titers protective. She was vaccinated with PneumoVax and TDaP with development of protective titers and remained infection-free. IGRT was not given and post-delivery, her IgG levels improved to 792 mg/dL.
She was seen one and half years later, for prenatal counseling for her 4th pregnancy. Her immune evaluation included an IgG 915. She demonstrated protection to tetanus, Diphtheria and Streptococcal pneumoniae. At 16 weeks of gestation, she developed recurrent upper respiratory infections requiring antibiotics. Her IgG was 697 mg/dL. At 28 weeks of gestation, her IgG was 537 mg/dL. IGRT was recommended, but patient refused at that time. After delivery, IgG improved to 849 mg/dL.




Conclusions: Decreased immunoglobulin levels during pregnancy are well-described phenomenon which can be attributed to hemodilution of pregnancy. IgG transport to fetus generally begins in the second trimester and reaches its pinnacle in the third trimester. Patients with known CVID require dose adjustments (higher) during pregnancy as they can be more symptomatic during this time. Here we describe a patient who has an almost normal immune evaluation except for mildly low IgA during absence of pregnancy, but during pregnancy, develops recurrent infections with significantly low IgG level. Her family history of a son with CVID, new daughter with low IgG levels like to be transient hypogammaglobulinemia of infancy (THI), another daughter with THI suggests a genetic B- cell defect that manifests as CVID with mildly low IgA and hypogammaglobinemia during metabolic stress such as pregnancy. There is only one similar report of a single pregnancy of transient symptomatic hypogammaglobulinemia during pregnancy. Such patients should be adequately worked up and treated during pregnancy with IGRT to decrease maternal and fetal mortality.

**(86) Submission ID#426530**

**Hypomorphic CARD11 mutations associated with diverse immunologic phenotypes with or without atopic disease.**

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Introduction/Background: CARD11 encodes a scaffold protein in lymphocytes that links antigen receptor engagement with downstream signaling to NF-B, JNK, and mTORC1. Germline mutations in CARD11 are known to give rise to distinct primary immune disorders in humans, including SCID (null mutations), B cell Expansion with NF-B and T cell Anergy (BENTA; gain-of-function mutations), and severe atopic disease (loss-of-function, dominant interfering mutations).
Objectives: Here we report our experience with an expanded cohort of patients harboring novel heterozygous CARD11 mutations that extend beyond atopy to include other immunologic phenotypes not previously associated with CARD11 mutations.
Methods: Cell transfections and primary T cell assays were utilized to evaluate signaling and function of CARD11 variants.
Results: We demonstrate that in addition to severe atopy, heterozygous missense mutations in CARD11 associated with dominant negative activity can present with immunologic phenotypes similar to those observed in STAT3LOF, DOCK8 deficiency, common variable immune deficiency (CVID), congenital neutropenia, and immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome. Evaluation of rare or novel CARD11 variants found in affected patients showed that dominant negative activity was largely confined to the CARD or coiled-coil domains, but did not always manifest in atopic disease.
Conclusions: These results illuminate a broader phenotypic spectrum associated with CARD11 mutations in humans, and underscore the need for functional studies to demonstrate that rare gene variants encountered in expected and unexpected phenotypes must nonetheless be validated for pathogenic activity.

**(87) Submission ID#420723**

**Hypomorphic X-linked SCID: A Diagnostic Challenge in the Era of Newborn Screening**

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Introduction/Background: Increasing number of states have been screening for severe combined immune deficiency (SCID) as part of the expanded newborn screening program for nearly a decade. In the era of newborn screening, patients with SCID present often asymptomatically and are prepared for early hematopoietic stem cell transplantation (HSCT). With advances in genetic testing, mutations in over 20 genes have been associated with development of the SCID or leaky-SCID phenotype.
Objectives: To present 2 unique cases of hypomorphic X-linked SCID where no known pathogenic mutations were identified on initial genetic testing, the IL2R gamma-chain as protein was expressed, but subsequent testing months later revealed pathogenic IL2RG mutation affecting either translation or protein function. To expedite earlier identification of pathogenic IL2RG mutation, we propose screening with X-inactivation studies in maternal T lymphocytes and assessing gamma-chain function by evaluating IL21R signaling in select male infants with abnormal newborn screen for SCID, specifically those with SCID phenotype but no identified pathogenic gene mutation on initial genetic testing, and the presence of gamma-chain expression.
Male patient A presented during the first week of life after his newborn screen was found to be abnormal with undetectable T cell receptor excision circle (TREC) count. A phenotype of T-B+NK- SCID was established and the patient was sent for bone marrow transplant evaluation. Genetic testing revealed a novel hemizygous missense mutation in IL2RG (p.Glu59Gln), which was a variant of unknown significance. The patient had common gamma-chain expression by flow cytometry on B and NK cells. He underwent haploidentical HSCT with his father as donor. Unfortunately, his transplant was complicated by prolonged neutropenia, slow T cell reconstitution, and eventual graft failure. To review his next treatment options, it was necessary to prove that his IL2RG mutation was pathogenic.
Male patient B presented with concern for an underlying immune deficiency after being hospitalized for Peumocystis jirovecii pneumonia. His newborn screening for SCID was inadequate at birth and follow up was delayed. Retrospective analysis of the newborn screening card at birth confirmed absence of TRECs. His phenotype was also T-B+NK-, consistent with X-linked SCID. He also proceeded to HSCT with a haploidentical parent donor. Despite HHV-6 viremia pre-HSCT which persisted post-transplant, he has had appropriate T cell engraftment. Comprehensive genetic testing on whole exome level did not reveal any known mutations contributing to his phenotype. Patient did have expression of gamma-chain by flow cytometry on T, B and NK cells. However, further testing revealed an IL2RG 3 UTR deletion of AA that, based on similar findings from a prior study can possibly lead to mRNA abnormalities.1
To expedite the association of SCID phenotype with X-linked disease, implying gamma-chain pathology, we obtained X-inactivation studies in maternal T-cells that showed severe skewing in both cases. Furthermore, IL21R signaling was impaired on B-cells in each case. The combination of these two assays proved that both patients carry a pathogenic IL2RG mutation.
Conclusions: In the era of newborn screening for SCID, we are discovering that phenotypic variability of SCID patients can be very broad and caused by hypomorphic mutations in the common chain gene. Exon-based genetic testing cannot exclude all variants and novel variants of unknown significance have to be evaluated by additional assays, including functional studies for causal effect. It is important to expedite early proof of association with gamma-chain pathology, especially in the era of gene therapy. We propose that in male infants with abnormal SCID newborn screening and no known or previously described pathogenic mutation on genetic screen, evaluation continues for hypomorphic IL2RG mutations. The probability of this process can be increased by a simple screening test for X-inactivation of maternal T lymphocytes.
1. Hsu AP, Fleisher TA, Niemela JE. Mutation analysis in primary immunodeficiency diseases: case studies. Curr Opin Allergy Clin Immunol. 2009;9(6):517-524.

**(88) Submission ID#416317**

**IgG4-related Disease Concomitant With Humoral Immunodeficiency**

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Introduction/Background: IgG4-related disease (IgG4-RD) is an immunologic disorder with multiple clinical presentations previously thought unrelated. It is characterized by the frequent presence of tumor-like swelling of the affected organs and several histopathological findings including tissue lymphoplasmacytic infiltrates with predominantly IgG4-positive plasma cells and lymphocytes, storiform fibrosis and obliterative phlebitis. Humoral immunodeficiency is a term that encompasses several disease entities associated with impaired antibody production. It is suspected in patients who present with recurrent, frequently severe, sinopulmonary infections with encapsulated bacteria, which leads to evaluation of quantitative immunoglobulin levels and vaccine responsiveness. Despite a few reports of primary immunodeficiency in patients with serum IgG4 elevation, no adult case has been reported of IgG4-RD in a patient with concomitant humoral immunodeficiency.
Objectives: To present a unique case with the presence of concomitant IgG4-related Disease and Humoral Immunodeficiency.
Methods: Comprehensive chart review of our patient and all performed exams. Literature review for IgG4-related disease, IgG4 elevation in humoral immunodeficiency and concomitance of IgG4-related disease with humoral immunodeficiency.
Results: Our patient is an 84-year-old Caucasian male with relevant past medical history of chronic bronchitis who was referred to our practice after several episodes of pneumonia in the previous years, with six courses of antibiotics just in the year prior for recurrent sinopulmonary

infections. Blood tests revealed hypergammaglobulinemia and low level of vaccine responsiveness. Chest CT showed multiple bilateral pulmonary nodules and hilar and mediastinal lymphadenopathy. Sinus CT showed left maxillary sinus opacification. Pulmonary function testing was normal.
He later presented with left eye edema, proptosis, diplopia, and painless submandibular salivary gland enlargement. Laboratory investigation showed an IgG of 1730 mg/dL, IgG4 of 771 mg/dL. The patient denied any history of pancreatitis or abdominal pain and abdominal ultrasound was normal. Biopsy of a salivary gland was normal. MRI of the left orbit was obtained, showing lacrimal gland enlargement.
Based on the patients recurrent infections, lack of response to tetanus immunization, and limited, non-sustained response to pneumococcal immunization, the patient was started on IVIG therapy. The patient was also diagnosed with possible IgG4-RD based on his salivary gland enlargement and orbital disease in association with hypereosinophilia and increased plasmablast levels. Oral prednisone 40mg daily was started for four weeks, later followed by slow steroid taper (reducing 10mg every two weeks) with considerable improvement in left eye swelling and proptosis.
A few months after discontinuation of the steroids, the orbital disease returned to its previous severity. Left lacrimal gland biopsy confirmed IgG4-related Disease, with many areas showing greater than one hundred IgG4-positive plasma cells per high-power field. After another course of steroids, oral prednisone was weaned to a maintenance dose of 10mg daily and the patient became asymptomatic from his ophthalmologic complaints with normalization of his ophthalmologic exam. His last checked IgG was 1105 mg/dL. IgG4 was still elevated at 324.8 mg/dL , but given controlled symptoms the patient was spaced to monthly IVIG infusions and continued on that daily steroid dosage.
Conclusions: Our patient, initially diagnosed with a humoral immunodeficiency, was later also diagnosed with biopsy-proven IgG4-related disease, which is a novel association of this two diseases in an adult patient. Previous rare reports of association between elevated serum levels of IgG4 and patients with concomitant humoral immunodeficiency were in the presence of isolated IgG4 elevation and not in the presence of IgG4-related disease. This novel association creates a therapeutic dilemma since the patient in question is hypergammaglobulinemic, yet needs IVIG, which can lead to side effects such as thrombosis due to a hyperviscous state. The description of additional concomitant cases of both diseases and further understanding of their pathophysiology will be crucial to create awareness and obtain earlier diagnosis, to refine therapeutic options and design adequate treatment protocols.

**(89) Submission ID#428658**

**IgM and IgA anti-pneumococcal capsular polysaccharides as prognostic tool for common variable immunodeficiency: a longitudinal study.**

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Introduction/Background: The clinical spectrum of CVID ranges from a poorly symptomatic form to severe phenotypes characterized by high susceptibility to infections, autoimmunity, granulomatous inflammation, lymphoproliferative disorders, and malignancies. Due to high prognosis heterogeneity, prognostic factors are required.
Objectives: With the aim to identify additional prognostic factors, we evaluated the anti-polysaccharide IgA and IgM responses by Elisa assay in 75 CVID in a longitudinal study over a 6-year period.
Methods: Patients were immunized at baseline with the 23-valent pneumococcal polysaccharide vaccine (Pneumovax®). Twenty healthy donors (HD) were also included.
Results: As expected, CVID patient had lower IgM/IgA response than HD. For CVID, four immunological phenotypes were identified by post-vaccination IgM and IgA levels: IgM and IgA responders (11%), IgM-high responders (4%), IgM-low responders (20%) and non-responders (61%). To simplify, we analysed IgM-high group with IgM and IgA responders and IgM-low with non-responders. During the follow up, concomitant CVID-related conditions, immunoglobulin serum levels, respiratory infections and outcome were recorded by medical files. CVID IgM-low/non-responders developed more frequently respiratory, gastro enteric and autoimmune manifestation and malignancies in comparison to IgM-high/IgM and IgA responders (respectively, pneumonia: 62% vs 25% ; chronic diarrhea: 33% vs 18%; autoimmunity 38% vs 9%). Autoimmune cytopenias were not found in the IgM-high/IgM and IgA responders group. Eleven (15%) patients died during the study time. Survival analysis according to the IgM/IgA responder status showed that the 6-years estimated survival for IgM-high/IgM and IgA responders vs IgM-low/non-responders group was respectively: 100% vs 100%, 100% vs 95%, 100% vs 92%, 100% vs 89%, 100% vs 85%, 100% vs 85%, 100% vs 82%. Interesting, in our series only two deaths were due to infective complications: five were consequent to malignancies, one to autoimmune cytopenias and three to not-CVID related conditions.
Conclusions: In conclusion, even if patients could not raise the protective humoral level, in CVID the anti-polysaccharide IgA and IgM responses could represent a prognostic factor, individuating groups of patients with less immunological impairment, lower risk of comorbidities and better survival.

**(90) Submission ID#423933**

**Immune dyregulation pattern overlapping with ALPS in patients with Gaucher Disease. A single Center analysis**

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Introduction/Background: Gaucher Disease (GD) is a rare autosomal recessive disorder characterized by a defective function of the catabolic enzyme -glucocerebrosidase (GBA) leading to a progressive accumulation of its substrate- glucocerebroside (GC) -in various organs in particular in mononuclear phagocite system. Hepatosplenomegaly and cytopenia represent the most common features of the disease. Moreover, GD patients also show hyperinflammatory features -secondary to machrophages engorgement and actviation- hypergammaglobulinemia, and a immune-dysregulation involving B , T and NK cells. Since clinical phenotpye can be subdolous, symtoms can overlap with ALPS, however, few data are available on specific immunity pattern in these patients.
Objectives: To evaluate immune-phenotype and other ALPS parameters in a cohort of patients with GD
Methods: We evaluated lymphocytes subsets, immunophenotypic and serological features of ALPS (DNTs, TCR alfa/beta B220, B-memory cells, T-regs/HLA-DR ratio, IL-10, IL-18), and test of apoptosis in a cohort of patients with GD followed-up at IGG.
Results: 35 patients (28 in treatment, 5 not) were studied. DNTs and TCR alfa/beta B220+ resulted to be >1.5% of T-lymphocytes and > 60% in 6/32 (19%) and in 7/32 (22%), respectively. B-memory cells and T-regs/HLA-

DR ratio were <15% and <1 in 11/32 patients (34%). 3/32 evaluable (9%) had all these parameters concomitantly alterated. 4/19 (21%) evaluable patients were resistant to apoposis. IL-18 was pathological in 26/29 (89%) patients. All patients had normal levels of IL-10 and sFAS.
Conclusions: This study shows that some patients with GD may present an immune-dysregulation pattern that can overlap with ALPS features. Therefore, the differential diagnosis of GD should be taken into consideration by clinicians during diagnostic work-up of patients with an ALPS-like phenotype.

**(91) Submission ID#411329**

**Immune Reconstitution Following TCR-alpha/beta- and CD19-Depleted Hematopoietic Stem Cell Transplantation for Hematologic Malignancy in Children**

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Introduction/Background: Allogeneic hematopoietic stem cell transplantation (HSCT) using unrelated and haploidentical donors is complicated by increased rates of graft-versus-host disease (GVHD) and slow immune reconstitution. Selective depletion of alpha/beta T lymphocytes and B cells is a recently developed method of graft manipulation that retains mature natural killer (NK) and gamma/delta T lymphocytes, both of which may exert a graft-versus-leukemia effect and protection against life-threatening infections.
Objectives: To describe the rate and quality of immune reconstitution, incidence of transplant-related complications, including viral reactivation and GVHD, and overall outcomes following TCR-alpha/beta- and CD19-depleted HSCT for hematologic malignancy in pediatric patients.
Methods: Forty patients of median age 11.2 years (1.7-21.7) underwent HSCT for acute myeloid leukemia (n=25), acute lymphoblastic leukemia (n=12), and myelodysplastic syndrome (n=3). Grafts were from unrelated (n=29) and haploidentical (n=11) donors. TCR-alpha/beta and CD19 depletion was performed with the Miltenyi ClinicMACS Plus system. Median CD34+ cell dose was 10.1 x 106/kg (4.7-15), and median CD3+ cell dose was 2.3 x 107/kg (0.21-43.3). Conditioning was with myeloablative busulfan or total body irradiation, cyclophosphamide, and thiotepa. Twenty of 29 unrelated donor HSCTs and 11/11 haploidentical HSCTs also included antithymocyte globulin x 3. No patient received post-transplantation GVHD prophylaxis. All but 5 patients received rituximab on day +1 per protocol for recipient positive Epstein Barr Virus (EBV) serology.
Results: All patients engrafted. Median time to neutrophil engraftment was 13 (8-30) days, and median time to platelet engraftment was 16 (12-40) days. One patient experienced graft rejection on day +18, and twelve patients relapsed at a median of 173 (47-625) days. Overall survival was 28/40 (70%) at a median of 24.2 (5.8-34) months follow-up. Two (5%) patients developed Grade III or higher acute GVHD, and 2 (5%) patients developed extensive chronic GVHD. Cumulative incidence of cytomegalovirus (CMV) and adenovirus reactivation were 11/40 (27.5%) and 5/40 (12.5%), respectively. Nine (22.5%) patients developed BK hemorrhagic cystitis +/- viremia. EBV reactivation was not observed. Median total, myeloid, T cell, and B cell donor chimerism were all 100% (ranges 50-100%, 93-100%, 41-100%, and 99-100%) at 1 year post-HSCT.
Immune reconstitution of all cells lines was rapid (Table 1). Eighteen of 26 (69%) patients had detectable T cell receptor excision circles (TRECs) by 4 months with a median TREC count of 1226 (0-5713) per 10^6 CD3 T cells, and recovery of the naïve T-cell compartment was observed by 8 months in 19/26 (73%) of patients. T cell function as measured by response to PHA was normal by 8 months in 15/23 (65%) patients and continued to increase steadily with time. Despite rituximab on day +1 for 35/40 (87.5%) patients, there was rapid B cell reconstitution. Nineteen of 23 (82.6%) patients had present switched memory B cells at 8 months, and 22/28 (78.5%) surviving patients are off immunoglobulin replacement at a median of 4 (4-18) months.

| | 4 mo | 8 mo | 12 mo | 18 mo | 24 mo |
|---|---|---|---|---|---|
| CD3+ T cells/μL | 348 (9-1770) | 1003 (30-1855) | 1213 (97-2639) | 955 (65-2342) | 1202 (852-2380) |
| CD4+ T cells/μL | 160 (4-475) | 362 (19-1118) | 551 (11-1583) | 467 (9-1287) | 691 (297-1370) |
| CD8+ T cells/μL | 91 (1-579) | 298 (8-1113) | 400 (79-1159) | 473 (37-948) | 588 (246-839) |
| CD4+/CD45+ T cells/μL | 15 (1-138) | 159 (1-776) | 339 (2-1096) | 260 (4-878) | 440 (134-888) |
| CD3-/CD16+ &/or 56+ cells/μL | 194 (72-779) | 175 (64-673) | 226 (38-2269 | 209 (98-302) | 229 (108-411) |
| CD19+ B cells/μL | 75 (0-355) | 327 (0-714) | 468 (0-946) | 625 (0-1136) | 499 (210-1239) |
| CD19+/CD27+/IgD- B cells/μL | 1 (0-17) | 5 (0-45) | 14 (0-39) | 15 (3-61) | 37 (0-60) |

Conclusions: Selective TCR-alpha/beta and CD19 depletion of haploidentical and unrelated grafts results in high engraftment and rapid immune reconstitution with low incidence of GVHD in children with hematologic malignancy. CD19 depletion and routine post-HSCT rituximab on day +1 are effective at preventing EBV reactivation.

**(92) Submission ID#428714**

**Immunodeficienciies and Autoinflammatory diseases: A propos of a mevalonate kinase patient with very unusual manifestations**

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Introduction/Background: Hyper-IgD syndrome (HIDS; 260920) is an autosomal recessive disorder characterized by recurrent episodes of fever associated with lymphadenopathy, arthralgia, gastrointestinal disturbance, and skin rash. The diagnostic hallmark of HIDS is a constitutively elevated level of serum immunoglobulin D (IgD), although patients have been reported with normal IgD levels. The disease is associated with mutations in the gene of Mevalonate kinase.
Objectives: Male patient, 30 years old, born to non-consanguineous parents, with a history of severe diarrhea since childhood, followed by respiratory infections and pneumonias. Moreover he presented several episodes of severe abdominal pain, with intestinal obstruction since seven months old, needing surgical intervention due to acute abdomen. This picture repeated several times until 10 years of age, being submitted to new surgeries due to intestinal suboclusion. At 16 and 17 years he had gastroenteritis and then pancreatitis. 2 years ago new severe acute gastroenterocolitis with suboclusion, submitted to laparotomy and resection of a little part of the gut. He has frequent diarrheas, triggered by coffee and tea. Usually evacuates 5 times a day. He was evaluated by several pediatric immunologists in childhood, who distrusted alpha heavy chain disease.
He had an intense reaction to the BCG vaccine, and then did not make any more vaccines (only OPV (Sabin) in the campaigns). He was tonsillectomized at 2 years of age. He had measles, chickenpox, and mumps (at 5 years old).
Cellulitis at 7 years, repeating several times since then.
He presented improvement of pneumonias but still has sinusitis and otitis (approximately 8 times a year).
Methods: Laboratory exams: Hemogram: Hm: 5.13; Hb: 13.8; Ht: 42.9; Platelets: 25,000; Leukocytes: 10530 (76.1, 0.5, 0.1, 16.0, 7.3);

Serum protein electrophoresis: total protein: 7.40; Alb: 4.13; Alpha1: 0.30; Alpha2: 0.66; Beta1: 0.46; Beta2: 1.18; Gamma: 0.67; Presence of a monoclonal component in a region of beta-2 globulins, identified by immunofixation as IgA kappa.
IgA: 1340; IgG: 869; IgM: 32; HbsAg: Negat; Anti-HBs: Negative; Anti-HBc: Negative; Serology to pneumococcal polysaccharide: Negative to 7 serotypes (without post-vaccination evaluation).
Results: Monoclonal gammopathy of undetermined significance; XIAP? TTC7A?
Genetic evaluation (whole exome sequencing) identified two allelic variants in MVK, one definitively pathogenic (p.Val377Ile) and one probably pathogenic with a premature stop codon (p.Leu230Pro fs*46).
Conclusions: We present herein a case that expands the spectrum of clinical manifestations of mevalonate kinase deficiency

**(93) Submission ID#428072**

**Immunodeficiency in a Patient with Pitt Hopkins Syndrome requiring Immunoglobulin Replacement Therapy**

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Introduction/Background: PittHopkins syndrome is a rare neurological disorder caused by mutations the TCF4 gene on chromosome 18q21. Clinical features include severe intellectual disability, constipation, microcephaly, and seizures. Features distinguishing it from other neurodevelopmental syndromes such as Rett Syndrome and Angelman Syndrome include breathing abnormalities (either apneic episodes or hyperventilation) and atypical facial features. Typical facial dysmorphism includes bitemporal narrowing, deep-set eyes, an M shaped upper lip, and widely spaced teeth. Although a very rare diagnosis (slightly over 200 reported cases), it is not known to be associated with underlying humoral or cellular immunodeficiency. There is only one report of IgM abnormalities described in a patient with Pitt-Hopkins Syndrome.
Objectives: To present a case of PittHopkins syndrome with humoral immunodeficiency.
Methods: This is a case presentation of a patient with Pitt-Hopkins Syndrome requiring Immunoglobulin Replacement Therapy.
Results: 15 year old female with genetically diagnosed Pitt-Hopkins Syndrome who presented to our office for immunological evaluation in the setting of recurrent sinopulmonary infections. She was placed on chronic antibiotics by the Department of Otholaryngology for approximately two years prior to presentation. She was found to be hypogammaglobulinemic (IgG: 398 mg/dL, IgA: 21 mg/dL, IgM: 40 mg/dL), had non-protective titers to streptococcus IgG Antibody (6/23 titers protective greater than 1.3 mcg/mL) despite booster vaccination with Pneumovax, and had borderline tetanus (0.18 IU/mL) and diphtheria titers (0.11 IU/mL). Given this laboratory evaluation and her recurrent illness, immunoglobulin replacement therapy (IGRT) was started. Whole exome sequencing was completed to assess for any other genetic cause of her immunodeficiency. The only abnormality was her previous known pathologic variant p.Q670HfsX40 c.2010_2011delGA (Gln670His) in exon 19 in the TCF4 gene. She continued to have infections despite therapeutic IGRT. Chronic antibiotic treatment was initially tapered, however needed to be reintroduced as IVIG alone was not stopping her infections, despite a IgG level over 1000 mg/dL.
Conclusions: Humoral immunologic deficits are not known to be associated with Pitt Hopkins Syndrome. There has been one case report of a patient with poliomyelitis-like syndrome following an asthma attack in a patient with Pitt Hopkins Syndrome, which was treated with IGRT and resulted in a nearly complete recovery. However, IGRT was not used for reasons of underlying immunodeficiency. To our knowledge this is the first patient with Pitt Hopkins syndrome with persistent hypogammaglobulinemia and frequent infections requiring immunoglobulin replacement therapy. It remains unclear why the patient continued to have infections despite IgG levels > 1000 mg/dL, yet with the combination of IGRT and prophylactic antibiotics the patient remains healthy.

**(94) Submission ID#427579**

**Immunologic abnormalities in GATA2 deficiency: A case review of 3 siblings and USIDNET registry cohort**

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Introduction/Background: GATA2 deficiency is a rare disease that typically presents in late childhood or early adulthood with heterogeneous phenotypes including Emberger syndrome. Emberger syndrome is characterized by lymphedema and predisposition for myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Over 75% of patients with GATA2 mutations have immune deficiencies. Definitive diagnosis of GATA2 deficiency is made by gene sequencing, and treatment includes infection control and potentially hematopoietic stem cell transplant (HSCT).
Objectives: The goal of this report is to contribute data to the small documented cohort of patients with GATA2 deficiency to aid in diagnosis and management of this rare, heterogeneous disorder.
Methods: We present a case series of three siblings with identical GATA2 mutations with variable phenotypes. A USIDNET query resulted 51 patients with GATA2 mutations.
Results: Three siblings (12 year-old female (a), 15 year-old male twin (b), and 15 year-old female twin (c)) and their mother had congenital deafness. Clinical symptoms include (a) H1N1 influenza requiring mechanical ventilation, warts, and hypogammaglobulinemia, (b) Streptococcus pyogenes neck abscess, warts, acne, and MDS, and (c) lymphedema and acne. All three patients had absolute monocyte count (AMC) of 0 and lymphopenia without documented lymphoid cell dysfunction. A mutation on exon 5, c.1062delG, confirmed the diagnosis. All three patients were started on mycobacterial prophylaxis with azithromycin and recommended HPV vaccination. The USIDNET query average age of symptom onset was 22 years, and average age at diagnosis was 31.5 years. 35.3% (18/51) of patients had a family history of GATA2 deficiency, 49% (25/51) of patients had warts, 7.8% (4/51) had lymphedema and only 1 patient had sensorineural deafness. 38% (19/50) of patients had AMC of 0. Functional data was limited.
Conclusions: Life threatening infections as well as hematologic malignancies have been reported in patients with GATA2 deficiency, which can be successfully treated with HSCT. To our knowledge, the GATA2 mutation detected in this family has not been previously reported. The clinical presentation in these three patients was heterogeneous despite identical genotypes, and diagnosis occurred years after initial symptoms. Variable phenotypes were found in the USIDNET GATA2 deficiency cohort as well. A high index of suspicion for the disorder and early recognition of clinical manifestations and laboratory abnormalities may aid in timely diagnosis of GATA2 deficiency, with potential for improved outcomes.

**(95) Submission ID#421984**

**Incidence of Herpes Zoster (Shingles) Vaccination and Diagnosis Among Older Persons with Primary Immunodeficiency**

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Introduction/Background: As individuals with antibody deficiencies age they are susceptible to developing shingles. Patients with antibody deficiencies are advised not to receive live viral vaccines such as Zostavax, the shingles vaccine.
Objectives: Our survey aimed to determine the frequency of shingles and use of Zostavax in Common Variable Immunodeficiency (CVID) and Hypogammaglobulinemia patients.
Methods: 11,533 email invitations delivered to members of the Immune Deficiency Foundation database requesting participation in an online survey about zoster, influenza and varicella experiences. Data from 881 individuals age 19 years old or older with CVID (N=760; mean age 54 years old; 83% Female; 92% White non-Hispanic) or Hypogammaglobulinemia (N=121; mean age 54 years old; 83% Female; 93% White non-Hispanic) were analyzed.
Results: Close to one fifth (18%, n=105) of adults age 50 or older with CVID or Hypogammaglobulinemia (N=568) had received shingles vaccination. The majority of those who were vaccinated reported receiving Zostavax once (92%, n=97), while 5% (n=5) received a booster vaccination as well. Mild side effects (e.g., skin rash and muscle pain) were only reported by 18% (n=18) of vaccine recipients after receiving their first vaccination. No side effects were reported after receiving the booster vaccination and no hospitalizations were reported as a result of receiving Zostavax. When comparing shingles diagnosis and shingles vaccination, of the 568 adults age 50 years old and older, 35% (n=196) had been diagnosed with shingles, and of those diagnosed 82% (n=160) did not receive Zostavax. Of adults age 19 years old or older, 32% (n=279) reported a shingles diagnosis. Similarly, the CDC reports almost 30% people in the United States will develop shingles during their lifetime. More than half (56%, n=157) of those ever diagnosed with shingles reported experiencing shingles once, 18% (n=51) had shingles twice and 24% (n=68) had shingles three or more times. Respondents with more than three shingles episodes were more likely to report their rash lasted more than two months and their blisters became infected.
Conclusions: Almost 20% of adults with CVID or Hypogammaglobulinemia reported receiving Zostavax despite recommendations against vaccinations for immunodeficient individuals. However, side effects in those PI patients who received the shingles vaccine appears minimal. Though it is possible these individuals were vaccinated prior to diagnosis of their PI; additional patient and physician education on live vaccines and immunodeficiency may be needed as well. The approval of the new non-live virus component varicella zoster vaccine may be of benefit to patients with PI.

**(96) Submission ID#424270**

**Inclusion Body Myositis as a Complication of Common Variable Immunodeficiency**

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Introduction/Background: Inclusion body myositis (IBM) is a rare disorder characterized as an inflammatory myopathy with endomysial inflammation and numerous red-rimmed vacuoles seen on biopsy. Five cases of IBM have been described in the literature in patients with common variable immunodeficiency (CVID).
Objectives: To make immunologists aware of IBM as a complication of CVID, that may be incorrectly diagnosed as myositis or autoimmune neuropathy.
Methods: Case Description
Results: We report a 62-year-old man with common variable immunodeficiency on gammaglobulin replacement who presented complaining of progressively worsening lower extremity pain, weakness, and fatigue. He states that over the last couple of years, it has become difficult climbing and descending steps, arising from a seated position, and has begun experiencing frequent falls. His creatinine kinase level was found to be elevated at 293U/L and he continued to be lymphopenic with total lymphocyte counts ranging from 300 to 800k/uL (CD4+ T-cells 34%, CD8+ T-cells 18%, CD19+ B-cells 2%). His ESR ranged from 25 to 60mm/h. Anti-glutamic acid decarboxy antibody (GAD) was initially elevated at 1.3U/mL and rose to 2.3U/mL within 6 months suggestive of a neuropathy. Based on an electromyography (EMG) and a muscle biopsy, he was diagnosed with polymyositis. He was treated with high dose steroids with no improvement. His intravenous gammaglobulin dose was then increased from 45mg every 3 weeks to 45mg per day for 3 straight days every 2 weeks. 4 months later, his creatinine kinase level dropped into the normal range (<200U/L), however, he continued to complain of worsening weakness. Physical exam showed decreased muscle bulk in forearms and quadriceps bilaterally, lack of a quadriceps tendon reflex, strength 4/5 in flexor digitorum profundus and 4/5 in hip flexors, and a broad-based gait. Histology and electron microscopy of a repeat muscle biopsy identified rimmed muscle vacuoles as typically noted in inclusion body myositis.
Conclusions: Inclusion body myositis is a potential rare complication of common variable immunodeficiency. It can mimic polymyositis and inflammatory demyelinating disorders. High dose steroids and IVIG are of no clinical benefit in IBM, despite decreasing serum creatinine kinase levels, which may raise a false impression of a clinical benefit.

**(97) Submission ID#427248**

**Infections in PIDs - Clinical Case Of Severe Combined Immunodefiency In Child**

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Introduction/Background: T-cell immunity disorders among primary immunodeficiencies (PID) are 9% in the registry of the European Society of Immunodeficiency (ESID) and 10.5% in the United States . T-cell disorders are characterized by the absence or presence of T-lymphocytes. Because T cells are important for the normal functioning of B cells, most PID with a T-cell disorder lead to combined T- and B-cell disorders. Disturbances of the T-cell link of immunity are clinically manifested in early childhood. The most serious form of PID with violation of the T-cell link of immunity is a severe combined immunodeficiency (SCID), the first symptoms of which are already observed in infants and are characterized by the development of life-threatening infections.
Objectives: In this abstract we present the case of the development of generalized CMV infection in a child with SCID.
Girl N. at the age of 3 months entered the Children's Infectious Clinical Hospital with complaints of cough, high febrile temperature for 5 days, refusal to eat. From the anamnesis of life the girl from the 1st pregnancy, 1 birth, was born full term in 40 weeks gestation, birth weight 4640g. For 3 months of life, a bad increase in body weight was noted and at the time of admission, the weight in 3 months was 5400 g. According to the parents, the child had atopic

dermatitis. From the anamnesis of the disease on 08.01.2017, the temperature rose to 38.2 ° C, there was a cough and a mucous discharge from the nose. Then the child refused to eat, the body temperature rose to 39.2 ° C. At this time, the girl's mom was borne by the ARI. January 14, 2017 patient was hospitalized in the hospital with a diagnosis: acute respiratory viral infection, acute rhinitis, pharyngitis, acute bronchitis, toxicosis of 1-2 degrees. Acute pneumonia? Atopic dermatitis, infant form.
On January 15, 2017, due to the worsening of the condition associated with the increase in oxygen (O2) -dependence, the child was transferred to the department of anesthesiology and resuscitation.
Methods: In the general analysis of blood upon admission, leukocytes are 14.2x10 9 / l, hemoglobin is 105 g / l, platelets are 172 x 10 9 / L, ESR is 3 mm / hour, stabs are 4% (abs. - 0.58 x 10 9 / l), segmented - 60% (abs- 8.64 x 10 9 / l), lymphocytes - 24% (abs 3.46 x 10 9 / l), monocytes - 10% (abs - 1.44 x 10 9 / l). In a biochemical study, the total protein is 51 g / l, total bilirubin is 4.7 mol / l, urea is 7.4 mmol / l, creatinine is 60 mol / l, lactate dehydrogenase is 2249 U / l, ALT is 131 U / l, AsAt - 257 E / L, CRP - 5.8 mg / l. Radiography of the lung from 14/01/2017 - data in favor of interstitial pneumonia. The study of the acid-base pH state is 7.367, pC02 is 34.3 mmHg, pO2 is 40.1 mmHg, lactate is 1.4 mmol / l.
A blood test was performed using the ELISA and PCR method for markers of HSV, CMV, enterovirus and toxoplasmosis. Ultrasound of the abdominal cavity revealed moderate hepatomegaly, signs of thickening of bile, splenomegaly. Moderate diffuse changes in the renal parenchyma (toxic-inflammatory?). The minimum amount of free fluid in the abdominal cavity.
Ultrasound of the brain revealed signs of subependimal microcyst on the right.
According to the immunogram, a sharp decrease in CD3 + 26% (58-85%) was detected, activated T-lymphocytes (CD3 + HLA-DR +) were 19.9% (3-15%), T helper / inducers (CD4 + CD8 - 26.6% (30-56%) and T suppressors / cytotoxic (CD8 + CD4-) 0.5% (18-45%), a high ratio of Tx / Tc (CD4 + CD8 +) was detected 53.2% (0.6-2.3), cytotoxic non-T cells (CD3-CD8 +) -1,2, an increase in the number of B-lymphocytes (CD19 +) - 58.9% (7-20%), natural killers (CD16 + CD56 +) - 6.6% (5-25%), natural T-killers (CD3 + CD16 + CD56 +) - 0.3 (0-5%), leukocyte gates (CD45 + CD14-) - 99% (95 - 100 %). The absolute content of T-lymphocytes was 0.15 x 10 9 / l, B - lymphocytes - 0.35 x 10 9 / l. The number of thymic migrants (CD45 + CD45RA + CD31 +) was not detected (0%). According to the results of the immunogram the diagnosis is made: Severe combined immunodeficiency (T-B + NK +).
01/17/2017 CT scan of the chest was diagnosed CT signs of a polysergic two-sided inflammatory process in the lungs (Figure 3).
When blood was sown for sterility on January 19, 2017, Staphylococcus epidermidis was isolated in an amount of 10 3, sensitive to linezolid, gentamicin resistant to amoxicillin, amoxicillin / clavulonic acid, and ciprofloxacin. On January 19, 2017, CMV DNA was detected in an amount of 7.6 × 10 6 copies / ml.
Results: Since the arrival clarithromycin was administered at a dose of 15 mg / kg per day in 2 divided doses from 14/01/2017 to 15/01/2017. From 16/01/2017 to 17/01/2017. change of antibacterial therapy for azithromycin intravenous at a dose of 10 mg / kg per day once a day.17.01.2017 - 01/18/2017 the state of the child is very severe with negative clinical and laboratory dynamics despite the ongoing therapy. Antibacterial therapy was changed to meropenem in a dose of 60 mg / kg intravenously every 8 hours, linezolid at a dose of 30 mg / kg per day and oseltamivir at a dose of 6 mg / kg per day from 17/01/2017 to 20/01/2017. 19.01.2017-20.01.2017 substitution therapy with an octagam in a dose of 0.4 g / kg was intravenously dripped. The patient's condition without significant dynamics. Based on the results of PCR on CMV, ganciclovir was administered at a dose of 10 mg / kg intravenously drip 2 times a day.
01/23/2017 due to a decrease in platelet count, the platelet mass is transfused and there was a rash all over the body at night, which is associated with the development of the "graft versus host" reaction (GVHR).
Despite the ongoing therapy, a fatal outcome occurred.
The main diagnosis: Primary immunodeficiency (Severe combined immunodeficiency, T0 B + Nk +).
Complications: Sepsis. Septic shock. SPON: ARDS, renal failure, DIS, thrombocytopenia, anemia 3. Two-sided lower-lobe pneumonia. Generalized CMV infection. GVHD, acute dermal form.
Concomitant: Atopic dermatitis, infant form.
Conclusions: The peculiarity of the described clinical case was that the patient's first symptoms of SCID developed in the first months of life and were manifested by a bad weight gain, atopic dermatitis and the development of a life-threatening generalized cytomegalovirus infection with the development of bilateral low-grade pneumonia, respiratory insufficiency and acute cutaneous GVHD form, after transfusion of unirradiated platelet mass. An expanded immunological study confirmed the diagnosis of SCID.

**(98) Submission ID#427267**

**Infections in PIDs - Infections as a trigger of primary immunodeficiencies**

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Introduction/Background: In 2015 was published the updated classification of primary immunodeficiencies compiled by the Primary Immunodeficiency Expert Committee (PID EC) of the International Union of Immunological Societies (IUIS). Within the two years since the previous version, 34 new gene defects are reported in the renewed version. The most common types are deficiency in antibodies production, combined B-cell and T-cell deficiency as well as phagocytic deficiency and system complement disorders. Children with these diseases tend to have untypical, unusually severe and recurrent infections as well as infections badly responsive to etiotropic therapy.
Objectives: In 2016-2017 years. In the departments of the Children's Infectious Clinical Hospital, several cases of congenital immunodeficiency in children treated with various diseases have been identified.
Methods: This study included 5 patients (2 boys and 3 girls) aged 2 months to 5 years. The reasons for entering the hospital were manifestations of severe hepatitis (in 2 children), acute respiratory infection (2 children) and 1 patient with symptoms of infectious mononucleosis.
All patients were examined according to clinical protocols and given the severity and atypicality of the course of any infectious diseases, patients underwent immunological examination of the blood and they were consulted by an immunologist. All children were diagnosed with congenital immunodeficiency.
Results: In 3 cases, the trigger for the realization of the immunodeficiency state was infection (E. meningoseptica + Kl. Pneumonia + B. Pertussis; CMV; VEB); in 2 patients, giant cell hepatitis occurred. In 2 patients, despite the ongoing therapy, the disease had an unfavorable (lethal) outcome (1 patient with hepatitis and 1 patient with generalized CMV infection).
Conclusions: Thus, it should be noted that timely diagnosis of a congenital defect of the immune system and thus timely therapy will avoid adverse outcomes.

**PROPOSAL ID: 422260**

**Interferon Gamma (Actimmune®) Effects on Severe Burkholderia Cepacia Pneumonia in Variant X-linked Chronic Granulomatous Disease**

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Introduction/Background: Interferon gamma (IFNγ; Actimmune®) has been proven to significantly decrease the overall number of infections in patients with chronic granulomatous disease (CGD) when given prophylactically (NEJM 324:408, 1991). Therapy with IFNγ has also been employed to treat severe overwhelming infections in some instances, such as severe aspergillosis with success (JID 613:908, 1991). We report here, two patients with very severe Burkholderia cepacia (B. cepacia) infection, one of whom was placed on a respirator for approximately two weeks and another who was on extracorporeal membrane oxygenation for an extended period of time. Both were treated with IFNγ (Actimmune®) in addition to appropriate antimicrobial therapy in an attempt to affect these life-threatening infections.
Objectives: The objective of this presentation is to describe two very severe variant X-linked CGD patients with B. cepacia pneumonia who were treated with IFNγ. In addition, we measured both super oxide production as well as nitric oxide production in the stimulated or unstimulated phagocytes from these patients in the presence or absence of interferon gamma.
Methods: Case histories of both patients were reviewed in respect to the severity of their infection, the time spent on a respiratory or extracorporeal membrane oxygenation, the antimicrobial therapy administered, and the clinical results following the administration of interferon gamma as adjunctive immunomodulatory treatment.
A standardized neutrophil oxidative burst assay was employed using cytochrome C reduction to measure super oxide production. In addition, nitric oxide was measured in the phagoctyes of the patients after stimulation with phorbol myristate acetate (PMA) in the presence or absence of IFNγ using DAF-2 fluorescence dye to detect the production of intracellular nitric oxide.
Results: A 2-year-old male developed a left lobar pneumonia and was admitted to Primary Children's Medical Center's Intensive Care Unit and treated with IV cefotaxime, clindamycin, later, vancomycin and azithromycin were added. CT scan revealed a left-sided pneumonia and moderate parapneumonic effusion. Subsequently, the patient decompensated, was intubated, and placed on respirator therapy. Broncheoalveolar lavage and blood grew B. cepacia. Neutrophil dihydrorhodamine fluorescence (DHR) demonstrated an intermediate broad peak of fluorescence with a small peak of unactivated cells, while the mother's DHR showed a broad intermediate peak suggesting the carrier state of variant X-linked CGD. Both the patient, a 2.5 month old younger brother, and the carrier mother were found to have a G to A splice site mutation in exon 3 of the gp91-phox gene at position c.252 confirming the diagnosis of X-linked variant CGD. After approximately 2 weeks on the respirator, IFNγ (Actimmune®) therapy was instituted with significant improvement of the patient's lung function. He was taken off the respirator approximately 3 days after the IFNγ therapy was instituted. Following addition of IFNγ to his PMA-stimulated neutrophil, there was a 22% increase in superoxide production and a 20 fold increase in nitric oxide in his monocytes.
The second patient was a 9-year-old male who presented with fever and cough and was diagnosed with right-sided middle and lower lobe pneumonia with cavitations. Bronchoalveolar lavage grew B. cepacia and nocardia. Following increasing ventilatory and circulatory collapse he was placed on extracorporeal membrane oxygenation and treated with 4-5 antimicrobial agents. After 9 days of such therapy, IFNγ therapy was initiated and he was weaned from ECMO after 3 days and has remained essentially healthy since then when he is on IFNγ prophylaxis. DHR revealed a broad intermediate peak in the patient and normal and intermediate peaks in the mother suggesting variant X-linked CGD in the patient and the carrier state of X-linked variant CGD in the mother. Targeted sequencing revealed a G to A splice site mutation in exom 3 of the CYBB gene at position c.266 confirming the diagnosis of X-linked variant CGD. Following addition of IFNγ to this patient's PMA stimulated phagocytes, there was a 150% increase in superoxide production and a 200% increase in monocyte nitric oxide production.
Conclusions: Two male variant X-linked CGD patients with splice site mutations in the CYBB gene and severe life-threatening B. cepacia pneumonia, one on a respirator and one on ECMO were administered IFNγ (Actimmune®) and each responded dramatically within 2-3 days recovering their respiratory capacity and coming off of assisted ventilation and ECMO, and have continued to do well when on IFNγ (Actimmune®) therapy.

**(100) Submission ID#428118**

**Interim Analysis of the Global Post Authorization Safety Study of Hyaluronidase-Facilitated Subcutaneous Immunoglobulin 10% Treatment in Patients With Primary Immunodeficiency Diseases**

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Introduction/Background: HyQvia is a recombinant human hyaluronidase (rHuPH20)-facilitated subcutaneous immunoglobulin (IGHy) 10% replacement therapy for patients with primary immunodeficiency diseases (PIDD).
Objectives: To acquire long-term safety data on IGHy, and assess prescribed treatment regimens and administration in routine clinical practice, a global postauthorization safety study (PASS) is being conducted.
Methods: This is an ongoing prospective, non-interventional, open-label, uncontrolled, multicenter study initiated in the United States in November 2015 to assess local and systemic effects of IGHy within a routine clinical setting. Patients aged 16 years with PIDD who have been prescribed and/or have started IGHy are eligible for enrollment. Patients are followed according to standard clinical practice and their treatment regimen is at the discretion of the treating physician. The presence of anti-rHuPH20 antibody titers is evaluated on a voluntary basis.
Results: As of August 2017, 175 patients had been enrolled at 26 US study sites. There were no serious AEs which were deemed treatment related. Sixteen patients experienced a causally related non-serious local AE (9.1%; 0.43 events/patient-year, 0.07 events per infusion) and 25 patients experienced a causally related non-serious systemic AE (14.3%, 0.88 events/patient year, 0.14 events per infusion). Of the 113 patients with immunogenicity data, 7 had 1 positive binding antibody test to rHuPH20 (titers 1:160); no neutralizing rHuPH20 antibodies were detected.
Conclusions: This interim analysis of prospectively-collected data of IGHy use in routine clinical practice indicates that IGHy is well tolerated with no treatment-related SAEs and has not been associated with neutralizing anti-rHuPH20 antibodies in patients with PIDD.

**(101) Submission ID#418858**

**Is pulmonary arterial hypertension a risk post-splenectomy in common variable immunodeficiency?**

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Introduction/Background: Idiopathic thrombocytopenic purpura (ITP) and/or hemolytic anemia accompanied by splenomegaly occurs in up to 25% of patients with common variable immunodeficiency (CVID). Treatments include steroids, other immune suppressants and rituximab. However, in some that do not respond, splenectomy may be performed. While splenectomy is known to be associated with an increased risk of infections or thromboembolic events, studies in other conditions (hemolytic disorders, hereditary spherocytosis, etc), also suggest an increased risk of pulmonary arterial hypertension (PAH) after this procedure.
Objectives: While splenectomy is known to be associated with an increased risk of infections or thromboembolic events, studies in other conditions (hemolytic disorders, hereditary spherocytosis, etc), also suggest an increased risk of pulmonary arterial hypertension (PAH) after this procedure.
Methods: We report three cases of PAH following splenectomy for cytopenias in patients with CVID.
Results: The first is a 40 yo female, with long standing CVID complicated by interstitial lung disease, nodular regenerative liver disease and ITP post splenectomy. The second is a 48 yo man with severe bronchiectasis, cirrhosis/nodular regenerative liver disease and ITP post splenectomy. The third is a 54 yo woman with CVID (TACI compound) complicated by cirrhosis/nodular regenerative liver disease, lung nodules and Evans syndrome. All developed severe PAH requiring chronic medications. PAH in these patients is best classified as multifactorial, group V.
Conclusions: Whether due to thrombus formation, continued cytopenias, and/or vascular changes, we suggest that PAH may be a long-term complication of splenectomy in complex CVID.

**(102) Submission ID#428282**

**Isoform specific mutations in STAT3 with Varied Hyper IgE Phenotype**

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Introduction/Background: Dominant negative missense or small, in-frame deletion mutations in STAT3 can cause Autosomal dominant Hyper IgE syndrome (AD-HIES), characterized by eczema, recurrent lung and skin infections, chronic mucocutaneous candidiasis (CMC), connective tissue, skeletal and vascular abnormalities. Two isoforms exist, STAT3, a 770 amino acid protein, and STAT3, a 722 amino acid protein produced by alternative splicing of exon 23 resulting in a frame shift and truncated protein at the C-terminus.
Objectives: We follow 4 patients from 2 families with STAT3 mutations leading to altered C-terminal proteins. The patients have high IgE but milder features of AD-HIES.
Methods: Clinical data were collected. STAT3 sequencing, STAT3 functional assays as well as lymphocyte phenotyping were performed.
Results: Patient 1 is a 58 year old man diagnosed with HIES as a child due to eczema, recurrent boils and high IgE (5000s IU/mL). He has tortuous and dilated coronary arteries, however denies lung infections, CMC, retained teeth, scoliosis, minimal trauma fractures, or hyperextensible joints. As an adult he developed avascular necrosis of both hips. Whole exome sequencing revealed a novel splice mutation, c.2144+1G>T at the end of exon 22 causing skipping of the 43 nucleotide exon as well as utilization of the STAT3 alternative splice acceptor in exon 23, resulting in a 93 nucleotide deletion. The mutant STAT3 protein product has a 31 amino acid, in-frame deletion encompassing both Y705 and S727 phosphorylation sites. No STAT3 is made from the mutant allele. Lymphocyte phenotyping was unremarkable, however total STAT3 protein levels were decreased in EBV transformed B cell lines and there was decreased Y705 phosphorylation after stimulation.
Patient 2 (family 2) was healthy until diagnosed with severe, refractory Coccidiodies pneumonia complicated by pneumothorax with prolonged bronchopleural fistulae at age 16 years. This led to an immune evaluation in which he was found to have elevated IgE (878 IU/mL, ref 0.0-91.0 IU/ml). He had one perianal abscess, primary teeth requiring extraction and mild scoliosis, but denies CMC, bacterial pneumonias, eczema, or minimal trauma fractures. STAT3 sequencing revealed a single base insertion in the transactivation domain, c.2185_2186insC causing a frameshift in the STAT3 isoform, p.R729PfsX11, occurring immediately after the S727 phoshporylation site. The deletion occurs within the alternatively spliced region of exon 23, providing an intact, wild-type STAT3. Stimulation with IL-6 or IL-21 showed reduced pSTAT3 at Y705, and elevated pSTAT1which is often seen in other AD-HIES patients. Lymphocyte phenotyping was unremarkable and Th17 cell analysis showed low-normal levels of Th17 cells while EBV transformed B cells showed reduced total STAT3 levels. His mother and infant sister also share this mutation - the 8 month old infant had normal IgE, and intermittent rashes; his mother has high IgE (1193 IU/mL), recurrent sinopulmonary infections (complicated by tobacco use) but without bronchiectasis or pneumatocele, and denies CMC with the exception of pregnancy related vaginal candidiasis.
Conclusions: Loss of function and gain of function mutations in STAT3 lead to distinct syndromes, but it appears that STAT3 mutations affecting the isoform expression, such as those reported here, can also lead to immune dysregulation with incomplete features of AD-HIES. These STAT3 mutations will allow us to better understand the relative roles of the isoforms STAT3 and STAT3 in somatic and immune cell signaling.

**(103) Submission ID#421091**

**Kabuki Syndrome Another Player on the Immunodeficiency Stage**

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Introduction/Background: Kabuki syndrome (KS) is a rare congenital disorder characterized by distinct dysmorphic facial features, intellectual disabilities, short stature and skeletal abnormalities. It was first reported in 1981 by Japanese

physicians and is associated with immunological defects as well. Mutations in the KMT2D and KDM6A genes are the most common genetic changes that lead to Kabuki Syndrome but for many cases the genetic basis remains unknown.
Objectives: Recognize the varied presentation for a unique immunodeficiency syndrome
Methods: This is a case series.
Results: This is a case series describing three patients with KS and their clinical presentations which predominantly involve immunodeficiency and autoimmunity. Our first patient is a 31-year-old male with autoimmune hemolytic anemia (AIHA) at a young age, recurrent respiratory infections and hypogammaglobinemia which led to a diagnosis of CVID at the age of six. In addition, he has dysmorphic facial features, intellectual disabilities, and short stature but it was not until his late twenties where he was found to have a missense mutation in KMT2D (p.Arg5048Cys) which has been described in patients with KS. The second patient is a 34-year-old female with hypogammaglobinemia, Evan's syndrome, short stature, and severe complications which include granulomatous-lymphocytic interstitial lung disease, pulmonary hypertension, and chronic kidney disease. She was also found to have a missense mutation in KMT2D (p.Arg5048Cys) in her early thirties and passed away from complications of her disease. The third patient is a 27-year-old female with a history of low IgA/IgG and poor vaccine titers, AIHA, neutropenia, pulmonary nodules, and developmental delay who was diagnosed with CVID in her mid-twenties. For her immunodeficiency and autoimmunity, she was treated with immunoglobulin replacement, rituximab and cyclosporine and was found to have a missense mutation in KMT2D (p.Cys1471Trp). This mutation was a de novo mutation in this patient and has also been reported in another patient with KS.
Conclusions: These cases highlight that the presentation of KS is varied and frequently includes immunodeficiency and autoimmunity in addition to the characteristic short stature and developmental delay. A diagnosis of KS remains challenging due the diversity of symptoms and disease severity, the need for genetic testing, and due to overlapping clinical presentations with other developmental conditions. Thus, often times, like in these cases, the diagnosis of KS is delayed.

**(104) Submission ID#420386**

**Leukocyte Adhesion Deficiency-I (LAD-I): A comprehensive review of all published cases 1975-2017**

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Introduction/Background: LAD-I is a rare disorder of leukocyte adhesion, resulting from ITGB2 gene mutations encoding for the Beta-2 Integrin component CD18. CD18 deficiencies prevent integrin dimerization and endothelial leukocyte adhesion, essential for extravasation and antimicrobial activity. Severe LAD-I (<2% of normal neutrophil [PMN] CD18 levels) is characterized by recurrent serious infections and early mortality unless treated by allogeneic hematopoietic stem cell transplant (HSCT). Mortality for severe LAD-I was reported as 75% by age 2 in an initial 1988 multicenter retrospective study. Moderate LAD-I (2-30% of PMN CD18 levels) is more indolent; although most patients (pts) survive childhood with recurrent skin and mucosal surface infections; mortality by age 40 can exceed 50%. LAD-I is characterized by umbilical cord complications (delayed separation and omphalitis), poor wound healing and leukocytosis.
Objectives: Reports regarding LAD-I have been published in recent decades but no recent comprehensive prognostic assessments are available. We sought an updated understanding of severe LAD-I with emphasis on prognosis in the absence of HSCT, HSCT outcomes and association of CD18 expression with clinical features.
Methods: We created a database of all published LAD-I cases via Pubmed searches and review of available references.
Results: Three hundred twenty-three LAD-I cases were reported between 1975-2017 in 107 publications (68 case-reports; largest series n=36). The nations reporting the most cases were Iran (n=65), USA (n=50), and India (n=45); the highest number of publications were from US centers (25). 113 pts were considered to have severe LAD-I, 63 moderate and 147 were not classified. PMN CD18 expression levels was reported for 265 cases and was <2% in 135 patients (51%) and >=2% in 130 pts. Four pts with CD18 >2% were considered to have severe LAD-I (CD18% range 2.4 17.3). Gender was noted for 282 pts; 148 (52%) were male. Age at presentation was reported for 146 cases. For 63 pts with CD18<2%, median presentation was age 1 m (range 0.03-18m); for 62 pts with CD18 >=2%, median presentation was age 6m (range 0.03-192m).
Infection details and CD18% were available for 154 (48%) cases. The most frequent infections in pts with CD18 <2% were respiratory tract (39%), sepsis (29%) and otitis media (27%) and for pts with CD18 >=2% they were periodontal (52%), otitis media (365%) and sepsis (25%). Perianal skin infections and necrotic skin ulcers were noted in >10%. Umbilical complications were more frequent in severe LAD-I (92 of 110 pts with CD18<2% [84%] and 47 of 81 with CD18 >=2% [58%; $p = 0.0002$]). For severe LAD-I pts with 2 years of follow-up (or death prior to 2y), there was correlation between absence of umbilical complications and survival to 24 m ($p < 0.001$). WBCs were reported in 143 cases (median 45 x 109/L; range 10 150 x 109/L). There were limited correlations between CD18 expression and WBC ($r < 0.1$) and between CD18 and CD11 expression ($r < 0.5$). Mutation analyses were reported in 139 cases with >20 gene locations noted and mutations on Exons 5, 6 and 7 accounting for 44% of specified cases. In 18 cases, CD18 expression was >30%; in 8 of 12 cases where CD11 expression was noted, at least one CD11 moiety was reported as <2%.
We sought to understand whether prognosis for severe LAD-I in the absence of HSCT is similar to the initially-reported 25% survival to age 2. There were 66 severe LAD-I cases (per investigator assessment or CD18 <2%) for whom survival to 2 years was reported, 40 of whom died prior to age 2 (61% mortality). Mortality was similar for the subset of 43 cases reported since 2000 (56%, 24 deaths). Early mortality was substantially lower in patients with CD18 >=2% and the majority of pts with CD18 >4% survived to adulthood. Outcomes for 101 pts who received HSCT were consistent with recent series; phenotypic correction was reported in 83% of pts with HLA-matched sibling donors. Mortality was 19% overall (11% for HLA-matched sibling recipients). For 22 pts receiving haploidentical HSCT there was 32% mortality and 55% received 1 subsequent HSCT.
Conclusions: Severe LAD-I remains a life-threatening condition with limited 2-year survival in the absence of allogeneic HSCT. Umbilical complications and granulocytosis are frequent early manifestations; respiratory tract, ear, sepsis, oral and skin infections are common. HSCT is potentially curative; transplant-mortality and other complications are frequent, especially in haploidentical recipients. Diverse ITGB2 mutations

result in LAD-I, and genetic evaluation may be valuable for diagnosis and prognosis. Rapid identification of pts with potential LAD-I (unusual or severe infections in infancy, granulocytosis and umbilical complications) is essential to enable referral to centers with disease expertise.

**(105) Submission ID#428539**

**Location of STAT3 hyper-IgE syndrome mutations does not invariably correlate with STAT3 phosphorylation potential**

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Introduction/Background: Autosomal dominant hyper-IgE syndrome (AD-HIES) is a primary immunodeficiency conventionally characterized by the triad of eczema, recurrent skin and pulmonary infections and elevated serum IgE. AD-HIES is caused by heterozygous mutations in the signal transducer and activator of transcription 3 (STAT3) gene. STAT3 is a transcription factor involved in numerous cytokine signals and is activated, in part, by phosphorylation of the tyrosine at amino acid 705 (Y705). Previous studies have suggested that AD-HIES mutations in the STAT3 DNA-binding domain (DBD) display normal phosphorylation of Y705 after activation, while AD-HIES mutations in the Src homology 2 (SH2) domain greatly impair phosphorylation.
Objectives: To determine the phosophorylation of STAT3 in AD-HIES patients with known STAT3 mutations.
Methods: Peripheral blood mononuclear cells (PBMCs) were collected from AD-HIES patients under IRB-approved institutional research protocols. PBMCs were then stimulated with IL-6 or IL-21 for 15, 30 and 60 minutes. Cells were surface stained for CD3, CD4, CD8, CD56 and CD19. They were then fixed, permeabilized and stained with anti-Y705-STAT3 to evaluate for phosphorylation of STAT3 at position Y705. Cells were then washed and data acquired using flow cytometry.
Results: PBMCs from one AD-HIES patient with a STAT3 SH2 domain mutation (p.Y657C) demonstrated normal Y705 phosphorylation after IL-6 stimulation. PBMCs from another AD-HIES patient with a DBD mutation (p.H437Y) exhibited highly reduced STAT3 phosphorylation after IL-6 stimulation and absent phosphorylation after IL-21 stimulation.
Conclusions: These findings highlight that, in the context of AD-HIES, the domain location of the STAT3 mutation does not predict STAT3 phosphorylation potential following stimulation and challenges the current paradigm.

**(106) Submission ID#426423**

**Low CD160 Expression Contributes to NK Cell Exhaustion and Predicts a Poor Prognosis of Patients with Liver Cancer**

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Introduction/Background: As the predominant lymphocyte subset in the liver, natural killer (NK) cells have been shown to be highly correlated with the outcomes of patients with hepatocellular carcinoma (HCC). Previously, we reported that NK cells were decreased and functional deficiency in HCC. However, the mechanism underline remains unknown.
Objectives: In this study, through the use of 23 healthy livers, paired peritumoural tissues (PT) and intratumoural tissues (IT) from 236 HCC patients,
Methods: we have evaluated the expression of CD160 and its co-ligand receptor BTLA on hepatic CD8+ T cells and NK cells.
Results: Decreased expression of CD160 on NK cells was observed in intratumoural but not PT regions, along with NK cell dysfunction, poor prognosis and tumour metastasis. Human CD160+ NK cells exhibited functional activated, high capacity of IFN- secretion and NK mediated immunity by global transcriptomic analysis of sorted CD160+ and CD160- hepatic NK cells. Blocking TGF-1 specifically reversed the IFN- production of CD160+ NK cells. In addition, this decreased CD160 expression is predominantly on CD56bright NK cells.
Conclusions: These findings indicate that CD160 expression reduction contributes to NK cell exhaustion and tumour immune escape, suggesting that CD160 has the therapeutic potential for fighting liver cancer.

**(107) Submission ID#426431**

**Lymphocyte Reference Values for Arab Children**

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Introduction/Background: The low number of circulating lymphocytes in the blood is a marker for cellular immunodeficiency in young children. Ethnicity also affects the lymphocyte count and ethnicity-specific lymphocyte norms have been used in many countries.
This study analyzed the lymphocyte counts in a large cohort of infants and young children from the Arabian Peninsula.
Objectives
1- To define the normal lymphocyte counts in Arab Children
2- To define the possible cutoff lymphocyte count that define lymphopenia.
Methods: This is a cross-sectional analysis of the lymphocyte counts in 11,237 Arab children. The age groups were: 1 day, 1-6 months, 6-12 months, 1-2 years, 2-3 years, 3-4 years, 4-5 years, and 5-6 years, 47% females.
We analyzed the first blood count performed during their visit to the Abu Dhabi SEHA Ambulatory Healthcare Services between April 2008 and October 2013. The median, 10th percentile and 90th percentile counts were calculated. The 10th percentile lymphocyte count was used to define lymphopenia. The Kolmogorov-Smirnov test, a non-parametric test, was used to compare lymphocyte counts between groups. Statistical significance was defined by a two-tailed $p<0.05$.
Results: The median counts were higher during infancy. The variability (disparity) of the counts (reference intervals) progressively decreased from birth to 6 years of life. The 10th percentile lymphocyte counts were relatively constant from birth to 2 years (2.5-3.0 x109/L) and from 2 to 6 years (1.4-1.8 x109/L), Table 1. The lymphocyte counts were similar in boys and girls. The lymphocyte counts were compared to those from five other studies.
Conclusions: Arab children have lower lymphocyte counts (10th Percentile) than children in the United States, Brazil and South Africa, but their counts are similar to children in China and Uganda.
Our study results support the development and use of ethnicity-specific lymphocyte count standards. The implication of our results is that using these lower cutoff values for lymphopenia will prevent a large number of Arab children from having unnecessarily investigated for immunodeficiency.

**(108) Submission ID#428581**

**Measurement of Endothelial Adhesion Markers as Biomarkers of Inflammation in Patients with Chronic Granulomatous Disease**

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Introduction/Background: Chronic granulomatous disease (CGD) is a rare phagocytic defect caused by mutations in the NADPH oxidase 2 system leading to reduced or absent reactive oxygen species production. In addition to specific infectious susceptibility, patients with CGD are predisposed to hyperinflammation in response to infectious agents, autoimmunity, colitis, and other forms of autoinflammation. CGD patients with mutations in p47phox are at increased risk of diabetes and cardiovascular disease. Hyperinflammatory and auto-inflammation responses are often difficult to predict and manage. Intracellular adhesion molecule-1 (ICAM-1) and E-selectin are endothelial adhesion markers that facilitate the adhesion and transendothelial migration of leukocytes and elevations in these markers have been associated with cardiovascular disease, glomerular injury, and thrombotic events. Expression of adhesion molecules is induced by pro-inflammatory cytokines and are associated with a hyperinflammatory state.
Objectives: To determine if E-selectin and ICAM-1 are elevated in patients with CGD and to determine of endothelial adhesion markers could serve as a biomarker of inflammatory disease in CGD patients.
Methods: Thirty-eight pediatric and adult subjects with CGD (14 X-linked (XL-CGD), 21 p47phox deficient and 1 p22phox deficient, and 2 X-linked CGD carriers were enrolled. E-selectin (pg/ml) and ICAM-1 (ng/ml) were measured from the plasma of patients via sandwich ELISA.
Results: All 38 CGD patients had histories of severe infection, active infection, chronic colitis, or other autoimmune disease at time of evaluation. Nine p47phox deficient CGD patients had history of diabetes and/or early onset cardiovascular disease. One subject with X-linked CGD had undergone hematopoietic stem cell transplantation (HSCT). Plasma levels of E-selectin were significantly elevated above healthy controls (median 19,648 pg/ml) in subjects with XL-CGD (median 45,990 pg/mL, $p<0.001$), p47phox deficient CGD (median 23,707 pg/mL, $p=0.0017$) and p22phox deficient CGD (80,108 pg/mL). Plasma levels of ICAM-1 were also elevated above healthy controls in subjects with XL-CGD (median 553.2ng/mL), p47phox deficient CGD (median 241.7ng/mL), and p22phox deficient CGD (383.1ng/mL), although none were statistically significant. Plasma quantities of E-selectin and ICAM-1 increased further in those p47phox deficient patients with diabetes and/or cardiovascular disease (median E-selectin 37,143 pg/mL, ICAM-1 356.7 ng/mL) but neither reached statistical significance. E-selectin and ICAM-1 quantities in female carriers of XL-CGD and in 1 XL-CGD cured by HSCT were similar to values found in healthy controls.
Conclusions: Immune dysregulatory features and hyperinflammation in CGD can be difficult to predict and manage. The endothelial adhesion markers E-selectin and ICAM-1 are elevated in patients with XL-CGD and p47phox deficient CGD that worsens with presence of early onset cardiovascular diseases and resolves post-HSCT. Elevations in E-selectin and ICAM-1 in the serum of CGD patients may serve as surrogate markers of inflammation and suggest a chronic endotheliopathy in CGD patients.

**(109) Submission ID#428732**

**Multilineage Cytopenias in CTLA4 Deficiency Due to Autoimmune Destruction: A Retrospective Review**

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Introduction/Background: The phenotypic presentation of CTLA4 haploinsufficiency was only recently described. The management of these patients in the medical literature is limited to anecdotal case reports. We aimed to detail our experience with short and long term immunomodulatory therapy in treating autoimmune cytopenias in the background of CTLA4 impairment.
Objectives: We aimed to assess the efficacy of mTOR inhibitors in treating autoimmune cytopenias in patients with CTLA4 haploinsufficiency.
Methods: We retrospectively identified 7 patients with proven CTLA4 mutations and documented refractory autoimmune cytopenias while receiving care at NIH clinical center (from July 2011 to August 2017). The complete (CR) and partial (PR) clinical response was assessed after six weeks of treatment and defined as Hgb >10 g/dL, platelets >100K/uL and Hgb >8g/dL, platelets >50K/uL, respectively without transfusion requirement.
Results: All analyzed patients failed or exhibited disease recurrence on at least one prior medical therapy, including: corticosteroids, rituximab, romiplostim and eltrombopag. The initial response rate to evrolimus and/or sirolimus was 71% (3 CR and 2PR). One of the partial responders had recurrence of idiopathic thrombocytopenic purpura at four months while on Rapalogs. Overall, we used mTOR inhibitors in 22 patients for a total of 37 patient years to treatm multiple modalities. The top three most common recorded adverse events were: Clostridium difficile colitis (n=9, in 4 patients), lipid abnormalities (n =7, 3 patients required treatment) and bacterial pneumonia (n=6, in 4 patients).
Conclusions: Our limited retrospective data suggests that mTOR inhibitors might be efficacious in the treatment of autoimmune cytopenias in CTLA4 haploinsufficent patients. Further prospective studies are required to assess safety and efficacy of mTOR inhibitors in this patient population.

**(110) Submission ID#428373**

**Mutation in NLRP12 responsible for Familial Cold Autoinflammatory Syndrome**

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Introduction/Background: The patient is an 11-year-old girl of non-consanguineous Quebecois origin. She presented with a history of recurrent febrile episodes from one year of age, initially described in

association with frequent upper respiratory tract infections and pharyngitis. From 2 years of age she continued to have recurrent febrile episodes in the absence of infection, with fever of 39-40 degrees Celsius on a monthly basis, normally lasting 1-2 days. She also developed episodes of urticaria, temporally unrelated to her febrile episodes. The rash was noted to be induced by exposure to the cold, and would generally last 1-3 days with some clinical response to antihistamines and Naproxen. Her clinical picture then progressed and she developed joint pain and swelling, particularly affecting her hands and feet. Over the following years the patient continued to have recurrent episodes of urticaria as well as progressive joint involvement and limitation.

There was some initial improvement with Naproxen and prednisone. Her symptoms were refractory to subsequent treatment with Methotrexate, Leflunomide, Infliximab and Tocilizumab and she remained corticodependent.

Immunological work up including lymphocyte phenotype, immunoglobulins, vaccine responses to both protein and live vaccines and CH50 were normal. There was no evidence of raised inflammatory markers with ESR 3-11mm/h, CRP <0.2mg/L and ferritin 39ug/L. Autoimmune workup including ANA, ENA, Anti-DNA, ANCA and C3 and C4 was normal. The initial differential diagnoses included Systemic Juvenile Arthritis, Periodic Fever Syndrome or Cryopyrin Associated Periodic Syndromes (CAPS).

The patient therefore had a Periodic Fever gene panel that identified a heterozygous mutation in Exon 3 of NLRP12, c.466C>T (p.Arg156Trp). Whilst mutations in NLRP12 are known to be associated with Familial Cold Autoinflammatory Syndrome, this was reported as a variant of unknown significance. We therefore proceeded with functional in vitro testing to demonstrate pathogenicity.

The patients monocytes showed increased secretion of IL1b upon stimulation with LPS as compared to healthy donors. When tested in a luciferase reporter assay, the mutated NLRP12 partially lost the capacity to inhibit the NFKB pathway. Overall these in vitro studies show that this NLRP12 mutation results in defective regulation of the inflammatory response.

The patient was commenced on Anti-IL1 therapy with Canakinumab, with no clinical improvement so was therefore discontinued.

We report a case of Familial Cold Autoinflammatory Syndrome due to a mutation in Exon 3 of NLRP12, that to this point has remained refractory to multiple treatment modalities. Functional testing was able to demonstrate mutation causality and defective regulation of the inflammatory response.

**(111) Submission ID#424446**

**Mutations in NFkB essential modulator in the USIDNET Registry: Spectrum of the Clinical Phenotype**

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Introduction/Background: Patients with hypomorphic mutations in NFkB essential modulator (NEMO) have a varied clinical phenotype characterized by ectodermal dysplasia with immune deficiency (EDA-ID) as well as inflammatory features.

Objectives: Authors aim to evaluate the spectrum of clinical phenotypes associated with NEMO hypomorphic mutations within the USIDNET registry.

Methods: Investigators obtained demographic, laboratory, and clinical data on patients with a defect in NEMO within the USIDNET registry.

Results: There were 19 male patients within the USIDNET Registry with a diagnosis of EDA-ID attributed to NEMO hypomorphic mutation. Of these, 9 were associated with a known variant in NEMO (E391X, F312L, M407V), and 4 were associated with previously unreported variants (D113V, E287del, c.1-16G>C). For 6 patients, a mutation was not specified. Most reported having an affected family member (n=14, 74%). Median age of symptom onset and diagnosis were 1 year (IQR 0.5-2y) and 2 years (IQR 1-10y), respectively. Median age at most recent visit was 11 years (IQR 9-16y). Infections, additional clinical features, treatments, and outcomes are summarized in Table 1. Skin manifestations (n=13, 68%) and pulmonary complaints (n=10, 53%) were common, with eczema (n=11, 58%) and asthma being most prevalent (n=5, 26%). Gastrointestinal conditions (n=9, 47%) were also frequently reported, and included non-specific diarrheal illness, enteropathy, colitis, enteritis, and inflammatory bowel disease. Neurologic features (n=8, 42%), including seizures, hearing defect, peripheral neuropathy, and encephalopathy, were unexpectedly common, suggesting a previously unrecognized disease association.

| Patient | Age (y, last entry) | Status | Variant | EDA | Infection | | | | | | Other clinical features | | | | | | Therapy | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Pneumonia | Disseminated | Bacterial | Atypical mycobacteria | Viral | Fungal | Skin | Lung | GI | Cytopenia | Neurologic | Endocrine/Growth | Ig | HSCT |
| 1 | 5 | ALIVE | E391X | x | x | | x | | | | x | x | x | x | x | | x | |
| 2 | 9 | ALIVE | E391X | x | x | | x | | | | | | | x | | | x | |
| 3 | 12 | ALIVE | E391X | x | | | x | | | | x | | x | x | | x | x | |
| 4 | 10 | ALIVE | | x | | | x | x | | | x | x | x | | x | x | x | |
| 5 | 11 | ALIVE | F312L | x | | x | x | x | | | x | x | | | | | x | |
| 6 | 6 | DECEASED | | | x | | x | | | | | | | | | | x | |
| 7 | 2 | ALIVE | E391X | x | | | x | | | | x | | | | | | x | |
| 8 | 11 | ALIVE | E391X | x | x | | x | | | | | | | | x | | x | |
| 9 | 19 | ALIVE | | | x | | x | | | | | x | | | | | x | |
| 10 | 5 | DECEASED | | x | | x | x | x | | x | x | | x | x | x | x | x | x |
| 11 | 41 | DECEASED | c.1-16G>C | | x | x | x | x | | x | x | x | x | x | x | x | x | |
| 12 | 34 | DECEASED | | | | | x | x | | | | | | x | | x | | |
| 13 | 13 | ALIVE | D113V | x | x | | x | | x | | x | | x | x | | x | x | |
| 14 | 10 | ALIVE | D113V | x | | | x | | x | | x | x | x | x | | x | x | |
| 15 | 12 | ALIVE | F312L | | | x | x | | | | x | x | | | | | x | |
| 16 | 11 | ALIVE | F312L | | | x | x | | | | x | | | | x | | x | |
| 17 | 22 | ALIVE | M407V | | x | x | x | | x | x | x | x | x | | x | x | x | x |
| 18 | 12 | ALIVE | E287del | | x | | x | | | | x | x | | | | | x | |
| 19 | 19 | DECEASED | | | | x | x | x | x | x | | | x | x | x | | x | x |
| Median /Sum | 11y | 14 A | | 10 | 11 | 7 | 19 | 6 | 4 | 4 | 13 | 10 | 9 | 10 | 8 | 8 | 18 | 3 |
| IQR/% | 9-16y | 74% | | 53% | 58% | 37% | 100% | 32% | 21% | 21% | 68% | 53% | 47% | 53% | 42% | 42% | 95% | 16% |

EDA, ectodermal dysplasia
EDA-ID, ectodermal dysplasia with immune deficiency
GI, gastrointestinal
HSCT, hematopoietic stem cell transplant
Ig, immunoglobulin replacement
A, alive
Y, year

Conclusions: We observed that allergic diseases, including asthma and eczema, were common in patients with NEMO mutation. Notably, varied neurologic features were more prevalent than previously reported. This study highlights the potential of cross-institutional registry analysis to deepen our understanding of extremely rare genetic diseases. Coordinated effort across institutions is required to better characterize the spectrum of clinical phenotypes associated with hypomorphic mutations in NEMO.

**(112) Submission ID#428416**

**Mycobacterial lysate (ML) and purified protein (PPD) in the diagnosis of patients with Mendelian Susceptibility to Mycobacterial Disease (MSMD)**

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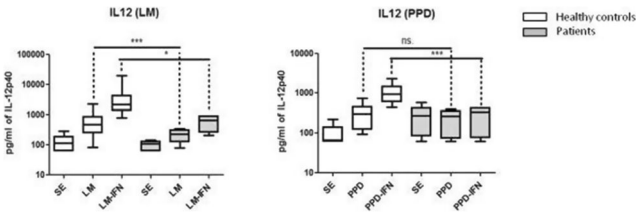
Introduction/Background: Patients with primary immunodeficiency (PID) have an increased susceptibility to mycobacterial infections. The

elimination of this infection depends mainly on the success of the interaction between macrophages and infected T lymphocytes. Patients with Mendelian Susceptibility to Mycobacterial Disease (MSMD) present severe and recurrent infections due to impaired signaling of the IFN/IL-12 axis.
Objectives: Our aim was to evaluated the IFN/IL-12 axis of patients with clinical history suggestive of MSMD using mycobacterial lysate (ML) and purified protein (PPD) by ELISA assay.
Methods: Samples of patients (N=43) with a clinical history suggestive of MSMD arrived in our laboratory. For the diagnosis, 3 mL of blood diluted (1:2) in RPMI 1640 culture medium supplemented were used. The samples were distributed in two different plates, one used in the dosage of IL12 (24 h) and the other in the IFN dosage (48 h). Thus, they were stimulated with ML (2 ug/ml for the 24 h plate and 10 ug/ml for the 48 h plate) and with PPD (10 ug/ml for the 24 h plate and 2 ug/ml for plate of 48 h). At the same time, in half of the wells stimulated with LM and PPD, the cytokine IFN (1000 IU/mL) or IL12p40 (2 ng/mL) were added in the plate. Next, the plates were incubated at 37°C and 5% $CO^2$ and the supernatant were collected and quantified by the ELISA assay. The results were evaluated by the statistical Mann Whitney U test.
Results: Six of the 43 patients presented alterations in the evaluation of the IFN/IL-12 axis. The age of the diagnosis of male patients ranged from 2 to 16 years. The only two female patients diagnosed were 36 and 40 years old. The clinical history was heterogeneous: 4 had lymph node hyperplasia, 2 pneumonia, 1 colitis, 1 Herpes zozter, another had a urinary tract infection and 1 BCGitis. The pathogens isolated were the M. tuberculosis, M. abscessus, M. gordonae, M. genavense and M. konsossi species found in 5 different patients. Statistical analysis of the IFN-/IL-12 axis evaluation by ELISA was performed.The results of the dosage of IFN were significant in samples with LM and LM plus IL-12 (***). Similar results were observed in samples treated with PPD and PPD plus IL-12 (**) when compared with healthy controls. In the IL-12 dosage, statistical difference was observed in the samples with LM (***) and with LM plus IFN (*). In samples stimulated with PPD, the results did not show statistical differences (ns.) but PPD + IFN (*) were significantly different.




Conclusions: Six patients were diagnosed by the evaluation the IL-12/IFN axis. The use of micobacterial lysate (ML) showed reliable results to the diagnosis of patients with IL12/IFN pathway defects. The genetics diagnosis will be performed.

**(113) Submission ID#420624**

**Mycobacteria-Specific T-Cells can be Expanded from Healthy Donors and are Absent in Primary Immunodeficiency**

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Introduction/Background: Invasive infections due to mycobacterial species are a feared complication in patients with T-cell deficiency or phagocyte disorders, and treatment is frequently complicated by antimicrobial resistance. CD4+ Th1 T-cell immunity is known to be critical to anti-mycobacterial defense; accordingly, adoptive T-cell immunotherapy with mycobacteria-specific T-cells (MST) may be a beneficial therapy for combating these infections.
Objectives: To determine if ex vivo expansion of T-cells targeting common mycobacterial antigens is feasible from healthy donors, and whether the same antigens are recognized by patients with primary immunodeficiency (PID) and invasive mycobacterial infections.
Methods: Peripheral blood mononuclear cells (PBMC) from healthy donors were pulsed with overlapping 15-mer peptide libraries encompassing five mycobacterial antigens (AG85B, PPE68, ESAT-6, CPF10, ADK) and expanded for 10 days with cytokines IL-4 and IL-7. Expanded MSTs were tested for specificity against the targeted antigens via IFN-g ELISpot, multiplex cytokine analysis, and flow cytometry. PBMCs from PID patients with invasive mycobacterial infections were similarly tested for presence of T-cells recognizing the tested mycobacterial antigens. A minimum of 20 spots per 1x105 cells above negative control was considered specific on ELISpot.
Results: Ten healthy donors and eight patients with PID were tested. Specificity against 1-5 mycobacterial antigens (median 3) was confirmed in all ten healthy donors, with a mean 6.6-fold cellular expansion during the 10-day culture. MSTs were predominantly CD4+ T-cells (mean/SD: 55 +/- 6%), with both central memory (mean/SD 17.7 +/ -6.4%) and effector memory (mean/SD 77.4 +/- 7.6%) populations. There was no clear difference in antigen specificity between BCG immunized (n=4) and BCG naïve (n=6) healthy donors. Six of 8 PID patients had no detectable immunity to tested mycobacterial antigens. One patient with combined immunodeficiency has a low detectable specificity to AG85B (mean 47 spot forming colonies[SFC]), and a patient with NFKB1 haploinsufficiency mounted a response against AG85B (mean 388 SFC) and PPE68 (mean 40 SFC).
Conclusions: Mycobacteria-specific T-cells can be rapidly expanded from healthy donors utilizing a protocol that could easily be translated to a Good Manufacturing Practices facility. The majority of tested PID patients lacked immunity to the targeted antigens. Adoptive immunotherapy with MSTs derived from third-party healthy donors may be a beneficial adjunctive therapy for PID patients with invasive mycobacterial infections.

**(114) Submission ID#423264**

**NAPDH oxidase-specific Flow Cytometry allows for Rapid Genetic Triage and Classification of Novel Variants in Chronic Granulomatous Disease.**

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Introduction/Background: Chronic granulomatous disease (CGD) is an innate immune deficiency, primarily affecting the phagocytic compartment, and presenting with a diverse phenotypic spectrum ranging from severe childhood infections to monogenic inflammatory bowel disease. Dihydrorhodamine (DHR) flow cytometry is the standard diagnostic test for CGD, and correlates with NADPH oxidase activity. While there may be partial genotype correlation with the DHR flow pattern, in several patients, there is no correlation.
Objectives: In such patients, assessment by flow cytometric evaluation of NADPH oxidase-specific (NOX) proteins provides a convenient and rapid means of genetic triage (Table).
Methods: We performed DHR flow cytometry and NOX flow cytometry on granulocytes and monocytes of CGD patients.
Results: Phenotypic and laboratory patient data shown in Table.
*P9 had decreased p22phox (% and MFI) monocytes, but not in granulocytes. All other siblings (P6, P7, and P8), and mother (P10) had relatively higher p22phox (%) monocytes, but still lower than the healthy control. P7 had normal %p22phox monocytes, comparable to control. However, P6-P8, and P10 had normal p22phox (MFI) in monocytes and granulocytes. P6-9, and P10 have normal %gp91phox+ granulocytes, while P6and P9 have modestly decreased %gp91phox in monocytes. P6-9, and P10 have all moderately decreased gp91phox protein (MFI) in granulocytes and monocytes compared to healthy control, with a single population for protein expression. P6, P9 and P10 have bimodal populations for gp91phox in monocytes but not in granulocytes, with a larger positive population, and a much smaller negative population.
The data from P6-9 suggest that the amount of gp91phox does not necessarily correlate with neutrophil oxidative burst, as measured by DHR. Also, not all CYBB variants affect p22phox protein expression, though both proteins are membrane-bound. The CYBA VUS in P8 does not appear to have affected p22phox protein expression in either monocytes or granulocytes.

| Patient ID | Age (years) | Gender | Clinical Presentation | DHR Flow (%, MFI) Ref. values (>=95%, >=60) | NOX Protein Absent by flow (grans, monos) | Genetic Variant |
|---|---|---|---|---|---|---|
| P1 | 3.5 | M | Early-onset colitis, no infectious history | Tested once (93.1, 6.35) | gp91$^{phox}$ | *CYBB*, c.338-3 C>A |
| P2 | 3 | M | Very early-onset IBD, granulomas from esophagus to colon | Tested x3 in 3 months (49.3, 6.04) | gp91$^{phox}$ | *CYBB*, c.37_45+2del |
| P3 | 19 | F | Self-limiting Miliary pattern on CXR at 5 years, no hospitalizations | Tested once (4.9, 0.71) | p47$^{phox}$ | *NCF1*, c.75_76 delGT |
| P4 | 14 | M | Crohn's disease, liver abscess s/p lobectomy | Tested x4 in 10 months (2.3, 0.74) | p47$^{phox}$ | *NCF1*, het. c.75_76 delGT |
| P5 | 8 | M | *Serratia marcescens* abscess at 4mo, forehead osteomyelitis | Tested once (0.51, 0.47) | gp91$^{phox}$ | *CYBB*, c.805-4_802del |
| *P6 | 8 | F | Failure to thrive, Wt & ht <5$^{th}$%ile | Tested once (91.2,2.86) | gp91$^{phox}$monos bimodal | *CYBB*, c.*1702G>A* |
| *P7 | 1 | M | *Chromobacterium violaceum* cellulitis | Tested once (93.2,6.02) | ?p22$^{phox}$monos | *CYBB*, c.*1702G>A* |
| *P8 | 4 | M | Failure to thrive, Wt & Ht <5$^{th}$%ile | Tested once (93.6,56.9) | | *CYBB*, c.*1702G>A* *CYBA*, c.203+5G>A VUS |
| *P9 | 7 | M | *Burkholderia cepacia* adenitis, MSSA septicemia at 7 months | Tested once (72.93, 2.93) | gp91$^{phox}$monos bimodal | *CYBB*, c.*1702G>A* |
| *P10 | Mother of P6-P9 | | *CYBB* carrier | 2 cell populations (69, 15) (31, 172) | gp91$^{phox}$monos bimodal | *CYBB*, c.*1702G>A* |

Conclusions: The atypical clinical presentation of some CGD patients can make genotype-phenotype correlation with DHR flow data challenging. Genetic testing, while necessary, can take several weeks. However, NADPH-oxidase specific-protein flow assessment offers a rapid alternative to identification of the underlying genetic defect, and can be utilized as a reflex test to an abnormal DHR flow. Further, it can provide insight into correlation between oxidative burst relative to protein expression in granulocytes and monocytes.

**(115) Submission ID#420588**

**Neutrophils Development and Function in an Animal Model of Adenosine Deaminase Deficiency**

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Introduction/Background: Adenosine deaminase (ADA) is a ubiquitous enzyme important for purine metabolism. Few studies have indicated that ADA deficiency, in addition to causing profound lymphopenia and susceptibility to infections, is associated with neutrophils abnormalities.
Objectives: Determine whether ADA deficiency directly affects neutrophils and what are the mechanisms involved.
Methods: Peripheral blood (PB) and bone marrow (BM) from 2.5 weeks old ADA-deficient (ADA-/-) mice that closely recapitulate the phenotype observed in ADA-deficient patients, as well as ADA+/- littermates were used to study neutrophils development and function. Some experiments were supplemented with 6-week old ADA-/- mice, maintained until 4 weeks of age with ADA enzyme replacement, and littermates.
Results: The number of neutrophils in PB of ADA-/- mice at 2.5 and 6 weeks of age was similar to ADA+/- mice. The function of PB neutrophils from ADA-/- mice, determined by oxidative burst, was also normal. The percentage of Lin-/C-Kit+/Sca1+ hematopoietic progenitor cells in BM demonstrated significant reduction in ADA-/- mice compared to littermates (0.93±0.23% and 1.21±0.33%, respectively, p=0.013). Moreover, expansion of BM isolated from ADA-/- mice in methylcellulose resulted in significantly less CD11b+/Ly-6G+ neutrophils compared to healthy controls (13.6±8.4% compared to 25.3±10.3%, respectively, p=0.003). Proliferation of BM cells, determined by BrdU incorporation into cells DNA, was higher in ADA-/- mice than in littermates, possibly contributing to the normal neutrophil numbers in PB of ADA-/- mice.
Conclusions: ADA deficiency directly affects neutrophil development. Further studies will help understand the significance of these effects and potential therapies for ADA-deficient patients.

**(116) Submission ID#427052**

**New diagnosis of combined immune deficiency in a 10-year-old female with Fanconi anemia due to FANCD2 gene deletion.**

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Introduction/Background: Fanconi anemia (FA) is spectrum of inherited blood disorders where mutations in DNA repair enzymes lead to genomic instability. Mutations in many of the 17 FA genes are well characterized

biochemically and clinically. Little is known about the FANCD2 gene which acts downstream of the FA complexes.
Objectives: We present here the clinico-pathologic features of a 10-year-old female with a heterozygous FANCD2 gene deletion/mutation with evidence of cellular and humoral immune dysregulation.
Methods: We evaluated the patient using standard immunology anatomic, cellular, and biochemical functional assays.
Results: The patient has multiple dysmorphias including total anomalous venous return (repaired), mesomelia, absent ear canal, radial ray dysplasia, and short stature. Her medical history is significant for an episode of pneumococcal sepsis despite adequate vaccination. Whole exome sequencing demonstrated deletion of exons 2-17 and a pathologic mutation (c.2444G>A, p.Arg815Gln). Repeated blood samples and immunophenotyping demonstrated severe lymphopenia. There were markedly low CD4+ T-cell counts with a low CD4:CD8 ratio (0.42). Changes in the composition of the B-cell population included: significantly diminished absolute total B-cells, elevated immature cells, low levels of transitional cells, and undetectable advanced B-cell populations. There was no immunogenic response to PCV-13 or varicella/tetanus/diphtheria vaccination. The NK-cell count was unaffected and demonstrated normal spontaneous and stimulated cytotoxic response. Bone marrow analysis demonstrated hypocellularity without dysplasia.
Conclusions: We report here a pediatric patient with a novel FANCD2 deletion/mutation presenting with severe lymphopenia in two cell compartments (B and T cells) and susceptibility to invasive bacterial infection. The findings are suggestive of combined immune deficiency. The cellular immune profile suggests that FANCD2 may be involved in the transition of immature B and T cells to mature cells, a process that requires substantial DNA recombination. Additional genetic and biochemical evaluation is needed to further characterize this rare clinical finding.

**(117) Submission ID#418732**

**New Diagnosis of Familial Hemophagocytic Lymphohistiocytosis Type 2 in Adolescent Presenting with Acute Lower Extremity Paralysis**

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Introduction/Background: Familial hemophagocytic lymphohistiocytosis type 2 (HLH) is a rare fatal condition due to a mutation in the PFR1 gene on chromosome 10q21-22 inherited in an autosomal recessive pattern which results in overactivation of the immune system. Symptoms usually manifest before 1 year of age.
A 17-year-old previously healthy male presented with acute onset of bilateral lower extremity pain and weakness, with subsequent inability to ambulate over a 10-day period. Neurological exam demonstrated decreased lower extremity power, sensation, absent patellar and achilles reflexes, and a wide-based gait.
Objectives: Not applicable
Methods: Not applicable
Results: Laboratory data was significant for neutropenia and thrombocytopenia, elevated levels of ferritin and serum CD25, and a positive EBV PCR. Patient was initially treated with IVIG for possible EBV-driven Guillain-Barre Syndrome with some improvement in neurologic symptoms as well as thrombocytopenia. A mass of retroperitoneal lymph nodes were noted on spinal MRI. Testing was negative for ALPS and bone marrow biopsy was negative for leukemia/lymphoma.
Further work-up revealed absent perforin expression in cytotoxic cells and normal SAP protein expression on staining, with poor NK function. Genetic testing revealed a pathogenic mutation in the PFR1 gene with 1 additional variant of unknown significance. The patient was treated with Rituximab for persistent EBV and returned with worsening lower extremity weakness. On MRI, focal enhancements were found in the brain as well as worsening mass compression of the lumbosacral nerve roots. Nerve root biopsy showed histiocytes with hemophagocytosis and a dense lymphohistiocytic infiltrate which stained positive for CD3, CD2, and granzyme B, with some loss of CD5 and no perforin. Bone marrow biopsy was negative for hemophagocytes.
Conclusions: The patient was diagnosed with worsening familial HLH with CNS involvement. HLH-directed chemotherapy (dexamethasone, cyclosporine, etoposide, intra-ommaya methotrexate/hydrocortisone) was started and HSCT was performed.
Familial HLH is a rare and often lethal disorder that generally presents at a very young age. The acute onset and severity of presentation as occurred in this previously healthy adolescent is uncommon. Familial HLH should be considered even in older patients with unexplained overactivation of the immune system.

**(118) Submission ID#421026**

**Newborn Screening for IKBKB Severe Combined Immune Deficiency Using Genetic Mutation Analysis**

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Introduction/Background: Severe combined immune deficiency (SCID) is the most profound form of primary immune deficiency, and is usually fatal in the first year of life without treatment. Newborn screening for SCID using quantitative analysis of T-cell receptor excision circles (TRECs), has become the accepted method to facilitate early diagnosis and treatment in most of the United States, as SCID babies typically do not make TRECs. IKBKB deficiency is a rare form of autosomal recessive SCID found in the Northern Cree First Nations people of Canada, where T cells develop normally but are non-functional. TREC analysis is expected to be normal in IKBKB SCID, and does not identify these cases.
Objectives: The objective of our study was to determine the feasibility of targeted genetic newborn testing for IKBKB deficiency.
Methods: We implemented a pilot project of prospective targeted genetic testing for the previously described homozygous IKBKB mutation (c.1292dupG) in newborns from 2 small northern Manitoba communities. Between 2013 and 2017, DNA was extracted from dried blood spots of 724 newborns, and targeted Sanger sequencing of the mutation-harbouring IKBKB exon 13 was performed.

Results: All 724 infants born in the 2 selected communities underwent testing. Fifty-five infants (7.6%, or 1/13.5) were found to be heterozygous carriers. One affected infant was identified, and underwent hematopoietic stem cell transplant before onset of infections. Our findings are consistent with the predicted homozygosity for this mutation (1/13.5 x 1/13.5 x 1/4 = 1/729 births).
Conclusions: We demonstrated that targeted newborn testing for IKBKB deficiency was feasible, and provided the first prospective estimate of the IKBKB mutation carrier frequency in select Manitoba Northern Cree First Nations populations. We suggest that if we are to capture all babies with SCID in Manitoba, future newborn screening should be universal and include both TRECs and direct mutation testing for population-specific mutations, including the First Nations IKBKB mutation. High throughput analysis for TRECS and targeted mutations will be introduced for universal newborn screening in Manitoba.

**(119) Submission ID#428017**

**No clinical signs of Hyper-IgM or other relevant primary immunodeficiency syndrome in novel patients with constitutional mismatch repair deficiency (CMMRD)**

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Introduction/Background: Immunoglobulin class-switch recombination (CSR) and somatic hypermutations (SHM) are prerequisites of antibody and immunoglobulin receptor maturation and diversity within the adaptive immune system. The mismatch repair (MMR) machinery, consisting of homologues of MutSa, MutLa, and MutSb (MSH2/MSH6, MLH1/PMS2, and MSH2/MSH3, respectively) and other enzymes, is involved in CSR, e.g. as backup of nonhomologous end-joining repair of activation-induced cytidine deaminase-induced DNA mismatches, and furthermore, in addition to error-prone polymerases, in the repair of SHM-induced DNA breaks. In line, a varying degree of antibody deficiency, from IgA or selective IgG subclass deficiency, to common variable immunodeficiency and hyper-IgM syndrome have been shown in small numbers of patients with constitutional MMR deficiency (CMMRD) in addition to the known severe cancer predisposition due to genomic instability of patients with biallelic loss-of function mutations in one the MMR components.
Objectives: To elucidate the clinical relevance of primary immunodeficiency (PID) in CMMRD, we collected history and laboratory data of a novel cohort of 14 consecutive patients from 14 families with homozygous mutations in PMS2 (n=7), MSH6 (n=5), and MLH1 (n=2) reported to the consortium Care for CMMRD (C4CMMRD) between 2014 and 2017, most of whom manifested with typical malignancies during childhood.
Methods: Retrospective chart review according to a specific questionnaire and extended routine immunological analyses were performed with IRB approval from the Medical University of Graz, Austria.
Results: None of the presented patients fulfilled any classical or extended clinical warning signs of PID (infections, immune dysregulation, inflammation). Furthermore, analyzing multiple specific laboratory parameters of the humoral and cellular immune system, we could not detect a uniform pattern of abnormalities. Importantly, our data do not confirm previous suggestive evidence of IgA or IgG subclass deficiency, a specific antibody formation, or a B memory cell maturation defect. Results of next generation sequencing-based detection of impaired class switch recombination and somatic hypermutations are pending. The T cell subsets and receptor repertoires were unaffected. Together, neither clinical nor laboratory parameters were suggestive of PID in the present series of novel CMMRD patients.
Conclusions: We conclude that patients with CMMRD do not generally show a clinically relevant PID that could facilitate early diagnosis. On the contrary, these data support the prospect of potentially successful immune therapy of malignancies in the context of CMMRD.

**(120) Submission ID#428691**

**Novel CECR1 gene mutation causing ADA-2 deficiency**

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Introduction/Background: ADA-2 deficiency is immune dysregulation diseases caused by an autosomal recessive mutation on CECR1 gene characterized by polyarteritis nodosa, childhood-onset, early-onset recurrent ischemic stroke and fever.
Objectives: Report a new mutation on the CECR1 gene resulting in ADA-2 deficient children.
Methods: Female, 6-year-old, presented history of multiple ischemic strokes at one-year-old associated with recurrent fever and livedo racemosa. She has no siblings and parents are not consanguineous.
Results: The laboratory evaluation shows red cell=4.91x106/mL, hemoglobin=12.4g/dL, leucocytes=6.1x103/mL, neutrophils=4.3x103/mL, lymphocytes=1.2x103/mL and plateletes=217 x103/mL. IgE<25UI/mL, IgG=467mg/dL, IgM=13.8mg/dL and IgA=21.3mg/dL. Subsets lymphocytes shows CD3+=81.2%, CD4/CD8=1.41, CD19+ =10% and CD16/56=8.8%. Due the clinical history, we performed CECR1 gene sequence homozygous substitution located at position -2 of acceptor splice site intron 6, c.847-2 G>A. This is a high conservative region with no alteration along phylogenetic studies and predicted to be pathogenic. To confirm the functional alteration, the ADA-2 activity was tested in dried plasma spot showing 0.0 mU/g protein confirm the gene loss of function and the mutation pathogenicity.
Conclusions: The authors presented a novel mutation of CECR1 gene, the first one described in splice site causing gene loss of function and confirmed by extremely reduced ADA2 activity.

**(121) Submission ID#428738**

**Novel genetic variants in a cohort of severe combined immunodeficiency from India**

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Introduction/Background: Severe combined immunodeficiency (SCID) is the most severe form of primary immunodeficiency characterized by severe, life threatening infections during early infancy. SCID is a medical emergency associated with significant mortality if hematopoietic stem cell transplantations is not instituted early in the course of the disease. SCID is a genetically heterogeneous disease caused by mutations in more than 30 different genes. Different genes are implicated in different ethnic populations and geographical locales depending on the rates of consanguinity and endogamy in these populations. However, following the institution of newborn screening of SCID in almost all state of the US and the widespread use of next generation sequencing in primary immunodeficiency diseases AR-SCID due to mutations in RAG1 and RAG2 genes are found to be more prevalent than reported earlier.
Objectives: We performed a retrospective analysis of SCID cases diagnosed at our centre and referred to us from other centres to determine the clinical, immunological and genetic basis of the disease in these cases. Genetic variants both recurrent and novel were analysed in detail.
Methods: Fifty six (56) of the 70 suspected patients met the ESID diagnostic criteria. The clinical features, immunological defects and the gene sequencing results of these patients were analysed. Gene sequencing was performed at the our centre and other collaborative centres at Dept of Pediatrics and Adolescent Medicine, Queen Mary Hospital, Hong Kong, National Defense Medical College, Saitama Japan, Kazusa DNA Research Centre, Chiba, Japan and Duke Medical University Centre, USA. Mutations were detected in 36 of the 56 patients. Mutations were classified as recurrent or novel after checking different databases such as Exome Aggregation Consortium (ExAc), Human Gene Mutation Database (HGMD) and other relevant SCID databases. The effect of novel, previously unreported mutations was determined using in-silico prediction tools such as SIFT and PolyPhen2. Functional studies were also performed in few cases to determine the effect of novel mutations.
Results: Mutations were detected in 36 patients. Mutations were more common in genes causing autosomal recessive form of SCID than the X-linked variant. Mutations were detected in IL2RG gene in 8 patients followed by mutation in the RAG1 gene in 7 patients, DCLRE1C in 6 patients and RAG 2 in 5 patients. Mutations were also detected in ADA gene (4 patients, 6 mutations), IL7R (3 patients), STIM1, PNP and NHEJ1 (1 patient each). Nine novel mutations were detected. Three in IL2RG gene, 2 in RAG1, 2 in ADA and one each in the NHEJ1 and IL7R gene.
Conclusions: Autosomal recessive form of SCID was more common in our cohort compared to X-linked form of the disease. Mutations in RAG1 and RAG2 genes were the commonest (12 patients) followed by mutation in IL2RG gene (8 patients). Nine novel mutations in 5 different PID genes were detected in our cohort of SCID patients

**(122) Submission ID#425243**

**Novel mutation in RASGRP1 presenting with EBV-driven lymphoproliferative disease and combined immunodeficiency**

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Introduction/Background: RASGRP1 is a guanine-nucleotide exchange factor which phosphorylates RAS-GDP to the activated form RAS-GTP in response to T-cell receptor stimulation, resulting in Ras activation. Mutations in the gene coding for RASGRP1 have been recently described in four patients with profound T-cell deficiency, resulting in recurrent bacterial and viral infections, autoimmunity and malignancy. Here we describe a two-year-old male presenting with recurrent sino-pulmonary infections, found to have two variants in RASGRP1, one not previously described.
Objectives
1. To describe a case of combined immunodeficiency with two pathogenic compound heterozygous RASGRP1 mutations.

2. To compare the clinical phenotype of RASGRP1 deficiency of our patient with that of previously described cases.
3. To argue for early hematopoietic stem cell transplantation in view of the increased susceptibility to Epstein Barr virus (EBV) induced lymphoma in patients with RASGRP1 deficiency.
Methods: A two-year-old male was referred to Seattle Children's Immunology Clinic for recurrent otitis media and two episodes of pneumonia. The diagnosis of combined immunodeficiency was considered based on a profound T-cell deficiency during immune evaluation and he was started on azithromycin and TMP/SMX prophylaxis. At age 3 ½ years he was hospitalized with a bladder outlet obstruction and found to have two abdominal masses. Biopsies were obtained and he was diagnosed with an EBV driven B cell lymphoproliferative disorder. In addition, his CSF and bone marrow were considered positive based on PCR and staining, respectively. Treatment with cyclophosphamide, prednisone, rituximab, and intrathecal methotrexate was initiated. Due to poor CSF EBV clearance, intrathecal therapy was escalated to rituximab.
Results: Initial laboratory evaluation showed elevated IgG (2290 mg/dL), normal number of CD19 B-lymphocytes, and adequate response to tetanus, Prevnar-13 and varicella vaccine. B-cell phenotyping showed elevated immature/transitional B-cells. A profound T-cell defect was identified with CD4 T-cell lymphopenia (361/mm3), elevated CD8 T-cells (2074/mm3), inverted CD4/CD8 ratio (0.3) and absent proliferation in response to mitogens (PHA, anti-CD3) and antigen (tetanus). Forty-three percent of peripheral blood T-cells were / positive. T-cell phenotyping revealed decreased CD4 and CD8 naïve T-cells with elevated proportion of CD8+ T-effector memory T-cells. Cervical lymph node and retroperitoneal mass biopsies showed atypical lymphoproliferation without malignant transformation. Exome sequencing revealed two variants in RASGRP1. The first variant (c.1428+1G>A) is located at a splice site predicting an unstable transcript targeted for degradation. The second variant (c.1780C>T) is a novel mutation resulting in a stop codon.
Conclusions: Recurrent sino-pulmonary infections are often a presentation of antibody deficiency. In this case, further investigation showed a profound T-cell defect, resembling that reported in 2 patients with homozygous nonsense mutations in the catalytic domain of RASGRP1 and 2 patients with homozygous insertion mutations leading to a premature stop codon at the bZIP domain. Our patient had biallelic mutations in RASGRP1 downstream of the catalytic domain, both leading to unstable transcripts. He developed an EBV induced atypical lymphoproliferative disorder, a complication reported in one RASGRP1 deficient patient whose disease progressed to B cell lymphoma and unsuccessful HSCT. Another patient developed EBV induced lymphoproliferative disorder after HSCT for EBV-positive Hodgkin lymphoma. Two additional patients presented with recurrent infections, developed B-cell lymphoma and one was successfully transplanted. We are preparing the patient for HSCT after chemotherapy for his lymphoproliferative disease to correct the underlying immune defect given that these patients are at high risk of developing lymphoma following EBV infection.

**(123) Submission ID#421328**

**Novel Presentation of Immunodeficiency-Centromeric-Instability-Facial Anomaly Syndrome 4: CD8 T Cell Lymphopenia, Neuroblastoma, and Neutropenia**

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Introduction/Background: Immunodeficiency- centromeric instability-facial anomaly is a group of rare genetic disorders typically involving agammaglobulinemia. Type four is caused by variants in the HELLS gene. The five patients previously reported with ICF4 have fit the phenotype of agammaglobulinemia. Here we report a patient with novel phenotype including neutropenia and neuroblastoma.
Objectives: Describe a unique presentation of immunodeficiency-centromeric-instability-facial anomaly syndrome 4 to further expand our understanding of this disease.
Methods: Retrospective chart review
Results: Six-month old male was transferred to our tertiary care facility for ongoing chronic respiratory infection, chronic diarrhea, and failure to thrive. His past medical history was significant for 31-week prematurity due to rupture of membranes requiring a two month NICU stay for bronchopulmonary disease. Upon discharge, he was bottle feeding and on room air. He had recurrent congestion for three months with two courses of antibiotics and one and a half weeks of diarrhea leading up to admission for difficulty breathing. He was found to have multiple infections including rhinovirus and parainfluenza virus on nasal wash, PJP pneumonia, norovirus, and pseudomonal cellulitis of his nose causing significant destruction.
Although previous laboratory studies revealed a normal absolute neutrophil count (ANC), his ANC quickly dropped to 170 cells/uL. His IgG, IgA, and IgM were undetectable. While his total B cell count (451 cells/uL) was normal, he lacked any switched memory B cells. He had near normal total T cell count (CD3 2579 cells/uL) and CD4 count (2373 cells/uL) and a markedly decreased CD8 count (181 cells/uL) and poor proliferative response to low concentrations of phytohaemagglutinin and pokeweed mitogen. A 0.9cm x 1.6cm x 1.9cm paraspinal mass was found on chest CT, which was subsequently characterized as MIBG-avid with a Curie score 1 neuroblastoma. Metastatic evaluation including bone marrow aspirate and biopsy was negative for malignancy. However, marked granulocytic hypoplasia and maturation arrest were present suggesting severe congenital neutropenia or, less likely, immune-mediated. Whole exome sequencing detected homozygous variant of unknown significance in the HELLS gene (p.M223T).
He was treated with intravenous immunoglobulin and G-CSF with clinical and laboratory improvement. His neuroblastoma was initially observed, then subsequently removed due to a >50% increase in size. Pathology confirmed MYCN non-amplified favorable histology. He remains in remission 4 months after resection. He is currently awaiting bone marrow transplant for his immunodeficiency.
Conclusions: The significance of this case report is the novel presentation of ICF4. Neutropenia and malignancies have been reported in immunodeficiency-centromeric-instability-facial anomaly syndrome 2 (ICF2) but not ICF4. This case report thus expands upon the clinical picture of ICF4 patients to include neutropenia and malignancies, and further describes the immunodeficiency.

**(124) Submission ID#426339**

**Overcoming Health System Barriers to Provide Ideal Care to Infants with SCID Identified through Newborn Screen**

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Introduction/Background: Identification of newborns with severe combined immunodeficiency using state wide newborn screening (NBS) began in Florida in 2012. Abnormal results require extensive confirmatory diagnostic testing and prophylactic antimicrobial medications are needed to effectively evaluate and treat the infant. Several barriers have been identified within government-sponsored health insurance programs that impede delivery of these evaluations and medications, often resulting in delays and/or inpatient hospitalization in order to provide timely and appropriate care.
Objectives: Determine the cost differential between the initial evaluation and treatment of a SCID patient detected by newborn screening in the inpatient versus outpatient setting.
Methods: The cost utilization of inpatient versus outpatient management of newly identified SCID patients from NBS were analysed to include the cost of confirmatory testing and initiation of prophylaxis within the inpatient versus outpatient setting. Laboratory tests included assessment of T cell immunity with quantitative and functional assessment, immunoglobulin measurement, genetic testing for SCID variants, evaluation for maternal engraftment, and HLA typing. Medications included Ig supplementation, pentamidine, fluconazole, and acyclovir. We compared the actual cost of inpatient stay and inpatient evaluation versus the approximate cost that would have been accrued if the patient were not admitted to the hospital.
Results: From 2012-2016, 4 infants with government-sponsored health insurance had abnormal NBS and were confirmed to have SCID after evaluation at our institution. All 4 infants were admitted into the hospital for initial evaluation and initiation of appropriate medications for an average of 7 days. Total average cost of medication administration for 7 days was $1,623, total cost of laboratory testing was $12,297, and average inpatient stay averaged $22,158 per patient. Conversely, the cost that would have been accrued in the outpatient setting for medication would have been $1,097 for 7 days. Laboratory testing costs would be no different as an outpatient. In total, the cost for inpatient evaluation was $36,708 versus $13,389 as an outpatient.
Conclusions: Standard laboratory assessments and medications are necessary for infants identified with SCID by population based NBS. Despite government sponsoring of the Florida NBS program, unnecessary barriers exist by government sponsored insurers that lead to a delay appropriate care. Inpatient admission alleviates these barriers, but significantly increases cost. We advocate that standard ambulatory SCID outpatient evaluation and initial treatment be authorized in children identified with SCID through NBS without delay.

**(125) Submission ID#415721**

**Patients with CD3G mutations reveal a role for human CD3G in Treg diversity and suppressive function.**

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Introduction/Background: Integrity of the TCR/CD3 complex is crucial for positive and negative selection of T cells in the thymus, and for effector and regulatory functions of peripheral T lymphocytes. Genetic defects that reduce, but do not abrogate TCR signaling, are associated with a variable degree of immune deficiency and immune dysregulation. In particular, while CD3D, CD3E, and CD3Z gene defects in humans present mainly with severe immune deficiency, CD3G mutations lead to milder phenotypes, mainly characterized by autoimmunity. However, the role of CD3, encoded by CD3G, in establishing and maintaining immune tolerance has not been elucidated.
Objectives: We aimed to investigate abnormalities of Treg cell repertoire and function in patients with genetic defects in CD3G with evidence of clinical autoimmunity.
Methods: High throughput sequencing (HTS) was used to study composition and diversity of the T cell receptor (TRB) repertoire in Treg, conventional CD4+ (Tconv), and CD8+ cells from 6 patients with CD3G mutations and in healthy controls. Treg function was assessed by studying their ability to suppress proliferation of Tconv cells.
Results: Treg cells of patients with CD3G defects had reduced diversity, increased clonality, and reduced suppressive function. The TRB repertoire of Tconv cells from patients with CD3G deficiency was enriched for hydrophobic amino acids at position 6 and 7 of the CDR3, a biomarker of self-reactivity. Overlap between Treg and Tconv cell repertoires was observed in CD3G mutated patients.
Conclusions: The Treg and Tconv cell repertoire of patients with CD3G mutations is characterized by a molecular signature that may contribute to the increased rate of autoimmunity associated with this condition.

**(126) Submission ID#428223**

**Persistent Hypogammaglobulinemia Following Rituximab Treatment in Pediatric Patients**

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Introduction/Background: A common concern with B cell-depleting therapies is their potential effect on humoral immunity. Although there have been reports of prolonged hypogammaglobulinemia in adult patients receiving rituximab, little is know about this phenomenon in children.
Objectives: We sought to assess humoral immunity in children receiving rituximab and determine risk factors leading to low immunoglobulin levels and infections.
Methods: We conducted a retrospective study on all pediatric patients ( 18 years) who received rituximab for the first time between January 2014 to December 2016 in a single tertiary pediatric hospital. Charts were reviewed and data was collected prior to rituximab treatment and at 6, 12, and > 12 months after treatment. Patients who received rituximab after hematopoietic cell transplantation (HCT) or for a malignancy and those with an underlying primary immune deficiency (PID) at the time of treatment were excluded.
Results: In total, 106 patients received rituximab during the study period. Of those, 38 were excluded (HCT: n=31, lymphoma: n=6, PID: n=1). Sixty-eight patients were eligible. Indications for rituximab treatment were renal disease (n=23), neurologic disease (n=18), hematologic disease (n=12), rheumatologic disease (n=10), EBV control (n=3), other (n=1). One patient who died from autoimmune encephalitis 8 days after rituximab was excluded from the follow-up study. At any time after rituximab treatment, low IgG was present in 24/62 (38.7%), low IgA in 6/61 (9.8%), and low IgM in 36/61 (59.0%) of patients. Over a year after their last rituximab dose, 12/29 (41.4%) of patients still had low B cell counts for age, and 10/10 (100%) had low memory B cell counts (CD27+ among CD19+ cells: mean value = 2.9% +/- 1.9% SD). Hospitalisation for infection was required in 13/67 (19.4%) patients in the year following rituximab treatment, which was associated with having either low IgG (33.3% vs 15.2%, p=0.03) or low IgA (50.0% vs 18.2%, p=0.04), but not with low IgM levels (19.4% vs 24.0%, p=0.33). Also, receiving a treatment with more than one rituximab cycle was a risk factor for low IgG (63.0% vs 20.0%, p=0.0003).
Conclusions: Hypogammaglobulinemia following rituximab treatment was frequent, and the presence of low IgG and IgA were associated with a higher risk of serious infection in this context.

**(127) Submission ID#394101**

**Pharmacokinetic Analysis of Biweekly Administration of Hizentra® in Patients with Primary Immunodeficiency**

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Introduction/Background: Subcutaneous immunoglobulin (SCIG) replacement therapy for patients with primary immunodeficiency (PID) is usually administered once a week. However, a variety of dosing regimens can be used to provide flexibility for patients.
Objectives: We used pharmacokinetic (PK) analysis to evaluate the PK characteristics of weekly and biweekly (once every 2 weeks) SCIG administration in patients with PID.
Methods: This PK substudy was part of a prospective, open-label, phase 4 study (NCT02711228) in patients with PID treated with IgPro20 (Hizentra®, CSL Behring, Bern, Switzerland). A noncompartmental analysis of serum IgG concentrations was used to calculate PK parameters and compare PK outcomes on weekly and biweekly dosing.
Results: Of the 17 patients included in the PK substudy, 15 provided samples for both weekly and biweekly regimens. The dose-adjusted area under the concentration-time curve was comparable for both treatment regimens: 0.24 and 0.25 (h*g/L)/mg for the weekly and biweekly regimens, respectively. The IgG clearance was also similar, being 4.41 for the weekly and 4.14 mL/h for the biweekly regimen. Median peak IgG concentrations occurred later with the biweekly regimen (3.02 days) compared to 2.00 days for the weekly regimen. IgG trough levels were close for both treatment regimens, with arithmetic means slightly lower for biweekly than for weekly regimens, at 10.13 vs. 10.21 g/L respectively. The minimum IgG concentrations within a dosing interval were also comparable, with arithmetic means of 9.60 and 9.83 g/L for the weekly and biweekly treatment regimens, respectively.

Mean (CV%/SD) of serum IgG PK parameters by treatment regimen
(PK Set, subjects who have completed both PK parts only)

| PK Parameter | Summary Statistic | Treatment Regimen | |
|---|---|---|---|
| | | Weekly (n=15) | Biweekly (n=15) |
| $AUC_{0\text{-}tau}$ (h*g/L) | Geometric mean (CV%) | 1681 (15.1) | 3562 (15.8) |
| | Arithmetic mean (SD) | 1699 (256) | 3601 (531) |
| dAUC ((h*g/L)/mg)[a] | Geometric mean (CV%) | 0.24 (18.0) | 0.25 (14.3) |
| | Arithmetic mean (SD) | 0.24 (0.05) | 0.25 (0.04) |
| $C_{max}$ (g/L) | Geometric mean (CV%) | 10.79 (15.5) | 11.82 (17.0) |
| | Arithmetic mean (SD) | 10.91 (1.67) | 11.97 (2.02) |
| $t_{max}$ (day) | Median | 2.00 | 3.02 |
| | Min, max | 0, 5.1 | 2.0, 7.1 |
| $C_{trough}$ (g/L) | Geometric mean (CV%) | 10.08 (16.3) | 9.96 (19.7) |
| | Arithmetic mean (SD) | 10.21 (1.63) | 10.13 (1.94) |
| $C_{min}$ (g/L) | Geometric mean (CV%) | 9.43 (19.9) | 9.69 (17.8) |
| | Arithmetic mean (SD) | 9.60 (1.93) | 9.83 (1.73) |
| $CL_{ss}$ (mL/h) | Geometric mean (CV%) | 4.35 (18.5) | 4.08 (17.2) |
| | Arithmetic mean (SD) | 4.41 (0.77) | 4.14 (0.73) |

(d)AUC, (dose-adjusted) area under the concentration-time curve; $C_{max}$, maximum serum concentration; $C_{min}$, minimum concentration during dosing interval; $C_{trough}$, trough concentration; $CL_{ss}$, clearance at steady state; CV%, percent coefficient of variation; n, number of patients; PK, pharmacokinetic; tau, 7 days (weekly regimen) or 14 days (biweekly regimen); $T_{max}$, time to reach $C_{max}$.

Conclusions: Biweekly and weekly Hizentra® administration at the same total monthly IgG doses resulted in similar IgG exposures.

**(128) Submission ID#421971**

**Pharmacokinetics, Efficacy, Tolerability and Safety of a New Subcutaneous Human Immunoglobulin 16.5% in Primary Immune Deficiency**

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Introduction/Background: Patients with primary immune deficiencies (PID) require life-long replacement therapy with immunoglobulins (Ig) to prevent severe infections and irreversible complications. In addition to safety and efficacy, tolerability and convenience of administration of Ig products are essential factors in patient acceptance. A new 16.5% Ig preparation (Octapharma, Lachen) was developed for subcutaneous administration (SCIG) derived from the established manufacturing process of Octapharmas intravenous Ig (IVIG) brand octagam®.
Objectives: Primary outcome was to assess efficacy of a new 16.5% subcutaneous human immunoglobulin preparation in preventing serious bacterial infections. Secondary endpoints included evaluating tolerability and safety, determining the PK profile, the number and rate of other infections and changes in quality of life measurements.
Methods: A prospective, open-label, single-arm phase 3 study involving 61 patients was conducted at 18 centers in North America and Europe. PID patients who were stable on IVIG treatment for at least 6 months and with IgG trough levels 5.0 g/L underwent a 12-week wash-in/wash-out period consisting of weekly SCIG doses 1.5 times the previous IVIG dose (based on published conversion rates for marketed SCIG products), followed by a 52-week efficacy period (64 SCIG infusions in total). 22 of 61 patients enrolled had complete pharmacokinetic assessments at different time points: before the switch from IVIG to SCIG (PKIV), after the wash-in/wash-out phase (PKSC1) and at week 16 of the efficacy period (PKSC2).
Results: 61 patients (age: 2-73 years; mean age 32.2 years; 54.1% female) receiving a total of 3,497 SCIG infusions (0.135 g/kg/week in young children (2 years and <5 years of age) and 0.185 g/kg/week in adults; average overall: 0.175 g/kg) were included in the Full Analysis Sets.
No serious bacterial infections were recorded. Among the 188 other infections observed during the efficacy period only one infection was graded as severe (bronchiolitis due to RSV virus), which led to hospitalization (2 days). All other infections were mild (72.3%) or moderate (27.1%) in intensity. Infection rate per person-year was 3.43.
Of the 233 reported adverse events, only 14 were assessed as being related to the study drug; all of these events were non-serious. Five non-study drug related serious adverse events were reported in 4 patients (6.6%).
Serum IgG trough levels were nearly constant during the study with a minimum trough level of 6.1 g/L and mean trough plasma concentrations of 11.4 ± 3.3 g/L and 11.6 ± 3.4 g/L for PKSC1 and PKSC2. Median IgG trough levels after SCIG treatment were 1.9 to 2.6 g/L higher compared to IVIG treatment prior to enrollment. A dosing conversion factor (DCF) of 1.37 was determined by AUC (area under the curve) measurements, allowing dose adjustment to achieve bioequivalence between IVIG and SCIG dosing.
Improved quality of life measurements, utilizing SF-36v2, were observed in both physical and mental health parameters when compared from the first to last SCIG infusion.
Conclusions: This study demonstrated that the new subcutaneous human normal immunoglobulin 16.5% is well tolerated, safe and effective in patients with PID.

**(129) Submission ID#423418**

**Phenotype of Transient Hypogammaglobulinemia of Infancy Associated With Severe Atopic Dermatitis**

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Introduction/Background: Atopic dermatitis (AD) is a chronic, relapsing, inflammatory skin disorder with associated pruritus that affects 11 percent of children in the United States. Severe atopic dermatitis refractory to conventional therapy can be concerning for an underlying immunodeficiency, especially in infants. Increased incidence of hypogammaglobulinemia has been associated severe AD. A handful of cases describe a correlation with transient hypogammaglobulinemia of infancy (THI), but a thorough immunological evaluation is often missing to further understand this relationship between AD and characterization of THI.
Objectives: Define various phenotypes of THI with their clinical presentation and laboratory findings.
Compare THI vs. THI associated with severe A.D.
Methods: A case series of six patients was conducted at a single academic center from 2/1/2014 to 12/1/2017 for patients with severe atopic dermatitis with low IgG levels. All available immunological laboratory data were retrospectively collected during this time period. Descriptive statistical analysis was utilized for data comparison.
Results: Of the six patients, four had no infectious history. Of the remaining two, one had recurrent skin abscesses associated with his poorly controlled atopic dermatitis requiring oral antibiotics and one patient had two episodes of Staphylococcus aureus superinfection of the eczema. At the time of presentation, the mean age was 7 months with mean IgG of 164 mg/dL and mean IgE of 2,273 KU/L. IgA and IgM were within normal age cut offs. All patients had normal protein and polysaccharide specific antibody titers after completion of vaccination series. Mean CD19+ count was 2,200/uL with normal CD3+, CD4+, CD8+ and CD1656+ cell counts. Three patients were tested for lymphocyte mitogen proliferation and complement function which were normal. Two patients, who were tested, had normal phagocyte work up. Mean age of IgG improvement to IgG> 217 mg/dL, was 10 months. Patients had total protein and albumin levels with mean 5.1 KU/L and 3.5 KU/L, respectively which eventually normalized. Four patients improved with skin care and dietary modification to hypoallergenic formula. One patient improved with extensively hydrolyzed formula and three patients improved with amino acid formula. One patient improved with aggressive skin care. One patient, who was noncompliant with dietary recommendations and aggressive skin care, did not improve.
Conclusions: One prospective study described an increased incidence of hypogammaglobulinemia in patients with atopic dermatitis compared to controls regardless of the severity. No further prospective studies have characterized hypogammaglobulinemia in this population. According to the Primary Immunodeficiency Practice Parameters, children with THI often present with frequent viral and bacterial respiratory illnesses, low IgG levels and normal vaccine responses. In one study, the period of hypogammaglobulinemia spontaneously corrects to normal by mean age of 27 months with all patients reaching normal levels by 59 months. These patients may have low IgM or IgA and decreased T or B cells, which eventually normalize. Management is often with antibiotic prophylaxis and if refractory to prophylaxis or unable to tolerate, IgG administration (IGRT) based on severity of symptoms is recommended. We describe a less severe variant of THI associated with severe atopic dermatitis and characterized by very transiently decreased IgG levels with earlier resolution than typical THI, normal IgM and IgA levels, normal specific antibody levels and normal T, B and NK cells. These patients typically do not manifest recurrent viral or bacterial respiratory illnesses. They also do not require antibiotic prophylaxis or IGRT. Severe atopic dermatitis maybe a positive prognostic indicator for patients with THI and is associated with an earlier self-resolution of

hypogammaglobulinemia, lack of typical infections, normal IgA, IgM and normal T and B cell numbers. A possible mechanism for THI associated with severe AD may be transdermal loss of protein which was supported with mildly low total protein and albumin levels rather than immaturity of the immune system in patients with true THI.

**(130) Submission ID#428704**

**PIK3CD mutation in a patient with common variable immunodeficiency**

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Introduction/Background: In recent years it was found that heterozygous mutation in PIK3CD gene produces an autosomal dominant primary immunodeficiency characterized by onset of recurrent sinopulmonary and other infections in early childhood with defects in both B- and T-cell populations and a special susceptibility to uncontrolled viral infections. Many patients develop chronic lymphoproliferation and there is also an increased susceptibility to B-cell lymphomas. Here we present a patient assumed as a common variable immunodeficiency with heterozygous mutation in PIK3CD gene.
Objectives: To describe a case of a patient assumed as a common variable immunodeficiency with heterozygous mutation in PIK3CD gene.
Results: 60 year old woman with personal history of severe and recurrent upper and lower respiratory infections, chronic pulmonary disease with bilateral bronchiectasis, chronic diarrhea without diagnosis, mild osteopenia, focal lesion in right hepatic lobe, atopic dermatitis and anemia. She was following up in other center and in 1996 she was diagnosis with common variable immunodeficiency (CVID) and started treatment with intravenous immunoglobulin (IVIG), but she referred low adherence to it. She did not referred history of lymphoproliferation nor significant viral infections.
She has a daughter with spherocytosis who required esplenectomy and also had bronchiectasis and CVID, she deceased at 28 years old because pulmonary infection. Other daughter and 2 sons referred healthy.
In our first immunologic studies we found severe hypogammaglobulinemia (IgG 428 mg%, no dosable IgA and IgM) with absent of B cells in peripheral blood.
We started with high doses of IVIG (800 mg/k/month) and antibiotic prophilaxis with improvement of the functional respiratory test and without new infections. We are planning colonoscopy to study her chronic diarrhea. Thinking that her clinical picture could be other than CVID we order a genetic study. A Nextera Exome Capture and Next Generation Sequence with Illumina HiSEq was made and an heterozygous mutation in PIK3CD gene (chr1:9.775.746, p.Pro97Ala) was found. Family and functional studies are still pending.
Conclusions: Due that clinical presentations of primary immunodeficiencies are becoming more complex, its diagnosis is a challenge for immunologist now a days. Studies with Next Generation Sequence is a very useful tool in indefinite cases, especially when more than one member in the family are involved.

**(131) Submission ID#417198**

**PIK3R1 mutation in mother and child with disseminated and congenital toxoplasmosis**

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Introduction/Background: Heterozygous gain-of-function mutations in PIK3CD as well as heterozygous PIK3R1 mutations that affect interaction of p85 with p110 lead to constitutive hyperactivation of the PI3K pathway and cause activated PI3K delta syndrome type 1 or type 2 (APDS1, APDS2), respectively.
We describe a female with APDS2 with short stature, diffuse lymphadenopathy, recurrent upper respiratory tract infections, elevated IgM, persistent EBV and CMV viremia and disseminated toxoplasmosis who gave birth to a genetically affected daughter with severe congenital toxoplasmosis.
Objectives: To characterize the molecular and cellular defects underlying severe toxoplasmosis in this family
Methods: Investigation of the molecular basis of the disease was performed through Whole Exome Sequencing, and results were validated by Sanger sequencing.
Functionality of the PI3K pathway was assessed by analyzing AKT and S6 phosphorylation in freshly isolate B cells with and without stimulation with anti-IgM.
An excisional lymph node biopsy from the affected mother was stained with anti-PD1 antibody to detect T follicular helper cells, and with IgM and IgG specific antibodies to analyze the proportion of isotype-specific B and plasma cells
Results: Whole exome sequencing of maternal DNA with targeted analysis of 362 PID genes identified a heterozygous mutation at an essential donor splice site of PIK3R1 (NM_181523.2:c.1425+1g> a). Sanger sequencing confirmed the presence of this mutation in both the mother and her child.
Functional studies on B cells freshly isolated from both patients confirmed an increase in baseline AKT and S6 phosphorylation, suggesting constitutive activation of the PI3K-mTOR signaling pathway. A lymph node biopsied from the mother contained numerous PD-1+ TFH cells and IgM+ plasma cells
Conclusions: Toxoplasma gondii is an obligate intracellular parasite that is usually only symptomatic in immunocompromised hosts. Severe toxoplasmosis has been reported in the following primary immunodeficiencies: CD40 Ligand deficiency, TAP deficiency, CVID, NFKB2 deficiency, and immunodeficiency due to anti-IFN- autoantibodies. Importantly, Toxoplasma infection was previously reported in a 9-month-old infant with APDS1, and ocular involvement has been described in a 36-year-old patient with APDS2. This, however, is the first report of systemic and severe congenital toxoplamosis in a mother and child with APDS.
To evade innate host defenses, T. gondii induces the activation of the PI3K/AKT signaling pathway, reducing intracellular reactive oxygen species and creating an intracellular environment that is hospitable to parasite survival and proliferation. Therefore, we postulate that the PIK3R1 mutation may lead to a hospitable cellular environment for toxoplasmosis replication.
This work was partially supported by the Division of Intramural Research, NIAID, NIH (protocol # 05-I-0213).
Additional authors of this work are: Ottavia Delmonte and Kerry Dobbs, from the Laboratory of Clinical Immunology and Microbiology, NIAID, NIH.

**(132) Submission ID#427397**

**PLCG2 gene calcium-binding C2 domain variant results in autoinflammation and phospholipase C-gamma-2-associated antibody deficiency and immune dysregulation phenotype**

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Introduction/Background: APLAID (autoinflammation and PLC-gamma-2-associated antibody deficiency and immune dysregulation) is a term that was proposed for the newly discovered autoinflammatory condition resulting from a pathogenic missense variant, Ser707Tyr, in the C-terminal SH2 (cSH2) domain of PLC-gamma-2 in order to distinguish it from PLAID, a distinct clinical entity that results from intragenic deletions of portions of the cSH2 domain of the same protein. PLC-gamma-2 is a phosphodiesterase that is predominantly expressed in hematopoietic cell lines and acts on PIP2 to produce IP3 and DAG in the PKC and Ras/Raf/Erk pathways. The only formally published case of APLAID described a father-daughter pair with an autoinflammatory clinical syndrome affecting the skin, mucosa, eyes, pulmonary and gastrointestinal systems.
Objectives: To describe the APLAID phenotype resulting from a novel genetic variant of the phosphodiesterase PLC-gamma-2 in two unrelated families
Methods: Clinical review and case presentation
Results: We report a 33-year-old female with a long-standing history of recurrent pneumonia, cellulitis and cystitis with obstructive lung disease characterized as bronchiolitis and dynamic airway collapse, with negative alpha-1-antitrypsin testing. She had a history of childhood onset granuloma annulare and pressure-induced urticaria, as well as episcleritis. Immune testing revealed low IgM (37 mg/dl), elevated BAFF levels and a low percentage of CD27+ memory B-cells, prompting sequencing of PLCG2, which identified a c.3422T>A, p.Met1141Lys variant in the calcium binding C2 domain. Her 15-month-old daughter, who has a history of bullous skin lesions, failure to thrive, febrile episodes and recurrent respiratory infections was likewise found to have this PLCG2 variant. Like her mother, the daughter also has low IgM and elevated BAFF levels. Similar symptoms of recurrent sinopulmonary infections and hypogammaglobulinemia, have been described in an unrelated family that shares this same PLCG2 variant in a doctoral thesis by Rozmus. This work also describes functional analysis, in which this particular variant of PLC-gamma-2 was demonstrated to have dysregulated PLC-gamma-2 activity leading to aberrant intracellular calcium signaling and increased apoptosis of immature B-cell subsets.
Conclusions: We describe our experience in evaluation and treatment of this family with a previously undiagnosed disorder. Together, these new cases add to the expanding body of knowledge regarding PLC-gamma-2 and its importance for the development of autoinflammatory and primary immunodeficiency conditions.

**(133) Submission ID#427357**

**Present a case.Non-celiac gluten sensitivity is an emerging entity with symptoms similar to celiac disease, but without positivity in specific diagnostic tests. It is considered more common than Celiac Disease**

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Introduction/Background: selective immunoglobulin A deficiency(SIgAD) is the most common primary antibody deficiency with a frequency of 1 in 600 in Caucasians.
Patients with SIgAD have a greater risk of concomitant autoimmune disorders than health individuals. SIgAD was previously to be associated with Celiac Disease, but not usually in Non -Celiac gluten sensitive.
Objectives: Describir a case ataxia Non Celiac gluten Sensitivity,
Methods: clinic case description.
Results: He patient has negative serology test for gluten, but clinically respond as Celiac Disease.
Conclusions: All patient has negative serology test for gluten, but clinically respond as Celiac Disease, could has Non-Celiac Disease, This case described here ,it suggest than this entity should to thought before than the Celiac Disease, since not-Celiac disease is it more common.

**(135) Submission ID#427873**

**Primary Cutaneous Actinomycosis Caused By Sacharopolyspora Sp. In A Patient Under Anti-Tnfalpha Therapy**

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Introduction/Background: Primary cutaneous actinomycosis is a rare condition caused by Gram-positive filamentous bacteria and generally occur after traumatic inoculation.
Objectives: To report an unusual etiology of skin lesions in a patient under anti-TNF-alpha therapy.
Methods: We report the case of a 30-years-old female receiving conventional doses of adalimumab for ankylosing spondylitis who presented, two weeks before referral for dermatological assessment, with an erythematous nodule with purulent discharge on right pretibial region and pruritic erythematous plaques with scaling and peripheral pustules in the trunk and nose tip.
Results: A fungal etiology was suspected and scraping specimens from several cutaneous lesions were submitted to direct microscopic examination and culture, which were negative for fungi. Cutaneous biopsy of the pretibial lesion was sent for histopathology and microbial cultures. Adalimumab was interrupted and empirical therapy with oral terbinafine (250 mg/day) was started with close clinical follow-up.
A folliculitis reaction pattern without granulomas was observed during histopathological examination and gram-positive cocci were isolated from biopsy sample and further identified as Saccharopolyspora sp. by molecular typing. Sulfamethoxazole+trimethoprim was added and discontinued after one week due to a cutaneous rash. Terbinafine (250 mg/day, p.o) was used for 12 months with complete clearing of all skin lesions and very good tolerability. Spondyloarthritis signs and symptoms were unremarkable and no anti-inflammatory or immunomodulating treatment was necessary.
Conclusions: Increased risk of skin actinomycotic infections in patients under anti-TNF therapy is not consistently reported in the literature. Nevertheless, they should be included as a differential diagnosis of atypical skin lesions in individuals under anti-TNF therapy.

**(136) Submission ID#393777**

**Primary Immunodeficiency Diseases in Qatar: First Report from the Qatar National Primary Immunodeficiency Registry (QNPIDR)**

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Introduction/Background: This is the first report of the Qatar National Primary Immunodeficiency Registry (QNPIDR), established in 2015, where we aim to determine the prevalence, incidence, characteristics, treatment and outcomes of Primary immunodeficiency Disease (PID) patients in Qatar. PIDs are rare heterogeneous disorders of the immune system that result in an increased susceptibility to infection, immune dysregulation and occasionally, to cancer.
Objectives: Determine the range of PIDs with important epidemiological data in Qatar after analyzing the database and creating a registry.
Methods: This is a retrospective study of PID patients followed at Hamad Medical Corporation from 1989 to 2017 using medical records. All patients who were diagnosed with a PID irrespective of age were included. Patients were classified according to the International Union of Immunological Societies Expert Committee on PID. The data is captured under 5 sectionsa) Patient demographics including age, gender, ethnicity b) Clinical presentation, c) Immunodeficiency profile including age at diagnosis, type of immunodeficiency, family history d) Treatment modality and e) Lab/genetic data.
Results: We registered 150 patients (60 females and 90 males) over a span of four years. Mean age at onset, diagnosis and diagnostic delay were 2.6, 4.6 and 2 years respectively. Majority of the patients were Arabs 77% followed by people from the Asian subcontinent 12%. Antibody deficiency was seen in 32%, immune dysregulation 28%, well defined Immunodeficiency (AT, HIgE, Digeorge,Wiskott Aldrich) 25% and T/B cell CID 12%. Rare diagnoses (IPEX, MSMD) were recorded whereas no cases of Toll like receptor and complement deficiency were seen. Consanguinity rate was (N = 97, % = 65) and first degree cousin marriage (N = 38, % = 40). Family history was positive in 50% (N= 75) of the patients. Maximum diagnostic delay was seen in SCID (33% >3 months) and Agammaglobulinemia (66% >3 months).During the patients life, infection was the most common presenting complaint (47%) followed by sinopulmonary disease (16%) and GI-tract manifestations (7%). The most common infections were pneumonia (46 %), otitis media and conjunctivitis (42 % each) followed by failure to thrive (30%) and sepsis (21%).Microbial isolates particularly seen as causative agents of infections were P.aeruginosa and salmonella (14%) each, MRSA and E.coli (9%) each. A genetic defect was confirmed in 57% of Ataxic Telangiectasia and 65% of SCID patients. Active infections were treated and prophylactic antibiotics were prescribed in 76 cases (51%). Prophylactic antibiotics were prescribed to 34% of patients with immune dysregulation and to 25% of well-defined syndromes. Out of 18 patients who had HSCT, 72% had successful transplants. IVIG was given to 42% of the total PID patients with humoral immunodeficiency patients receiving the most (45%). One patient had gene therapy and two required interferon gamma treatment for MSMD. Mortality rate at 1st year of life was 4% whereas total mortality rate was 12%, excluding cases that passed away before PID was diagnosed.
Conclusions: The estimated prevalence of PID in Qatar is found to be 6 per 100000. Over the years, physicians have become increasingly aware of PID and survival rate has improved. Initiation of newborn screening for SCID and Agammaglobulinemia will lead to earlier diagnosis and initiation of therapy with better outcomes.

**(137) Submission ID#427142**

**Progressive hypogammaglobulinemia with T cell abnormalities in two adult females with infections and autoimmunity: a diagnostic dilemma**

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Introduction/Background: Agammaglobulinemia is typically associated with a near absence of B cells secondary to a developmental block in bone marrow, and, usually but not always, manifests in early life. Most of such patients are males with X-linked agammaglobulinemia (BTK deficiency).
Females with agammaglobulinemia, of either autosomal recessive or dominant inheritance, are rare.
Objectives: We present and discuss the differential diagnosis for the conflicting clinical and immunological phenotypes of two adult females who present with infections, cytopenias, and agammaglobulinemia with low to normal B cell count.
Methods: Retrospective chart review of clinical and laboratory data
Results
Case 1: A 22-year-old female who, at age 17 years, had Evans syndrome (autoimmune thrombocytopenia and hemolytic anemia) that resolved after steroid and high-dose gammaglobulin treatment. At age 21 years, immunologic workups revealed a complete absence of serum immunoglobulins (IgG, IgA, IgM) and low B cells 43 (3%) (normal range 100-500). However, three years prior, patient had detectable IgM (218 mg/dL). B cell subset analysis showed an expansion of CD19hi21lo B cells (30%, normal 0.2-8.6%), with a marked decrease in switched memory B cells (0.2%, normal 7-61%). Additional immunophenotyping revealed a reduced frequency of naïve CD4+ (3.6%, normal >50%), CD8+(42%, normal >50%) T cells, and normal lymphocyte proliferative responses to mitogens and anti-CD3, anti-CD3/anti-CD28, and anti-CD3/IL-2.
Case 2: A 30-year-old female with recurrent upper respiratory tract infection, undetectable IgG, IgA, IgM and IgE, and no response to vaccinations (tetanus and pneumococcal). Thrombocytopenia (38x103 count/microliter) was noted once during her third-trimester pregnancy without evidence of pre-eclampsia. Postpartum CD4+ T cell count was low (442 cell/microliter, normal 500-1400 cell//microliter). Immunophenotyping revealed normal B cell count with reduced frequency of naïve CD4+ (17.6%, normal >50 %) and CD8+ (18%, normal >50 %) T cells.
Conclusions: We report two adult females who present in early adulthood with recurrent infections and cytopenias. Both had agammaglobulinemia and decreased naïve T cells suggestive of late-onset combined immunodeficiency (LOCID).
The presence of peripheral B cells makes autosomal recessive defects in B cell receptor signaling (lamda5, Iga, Igb, Igm, BLNK) less likely. Differential diagnosis of LOCID with cytopenias includes CTLA-4 haploinsufficiency, gain-of-function PIK3CD mutations, IKAROS defects and autosomal recessive RAG deficiency. Both patients remain at risk for developing autoimmune complications. A molecular diagnosis will facilitate targeted therapy for the underlying defect.

**(138) Submission ID#427420**

**Properdin deficiency in a child presenting with recurrent lower respiratory tract infections.**

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Introduction/Background: Complement factor properdin (CFP) is a soluble glycoprotein which has a unique known role as a positive regulator of the alternative complement pathway by binding and stabilizing the inherently labile C3/C5 convertase enzymes. Mutations in the CFP gene lead to aberrant protein expression or to expression of a dysfunctional protein which results in high susceptibility to pyogenic infections especially Neisseria meningitidis. Properdin-deficient individuals are at greater risk of fulminant meningococcal disease, with mortality rates as high as 75%.
Objectives: Case report: A 5 year old Belgium boy from non-consanguineous parents presented with achronic purulent cough and recurrent infections of upper and lower airways. He suffered from a severe pneumonia at the age of 5. CT thorax showed bronchiectasis of the right middle lobe and left lower lobe. In addition, he had recurrent acute otitis media since the age of 1 year old. Immunological work-up showed an absent AP50 activity, with normal CH50, C3 and C4.
Results: Genetic and functional analysis: Sanger sequencing of CFP gene identified a hemizygous c.961T>G, p.Y321G (CADD score 8.081, MSC_CADD 0.102) mutation in the patient and his mother. Properdin Elisa (Hycult Biotech) showed absent and 50% of the normal healthy control value of serum properdin concentrations in patient and the healthy mother, respectively.
Conclusions: Conclusion: We diagnosed properdin deficiency in a 5y old boy presenting recurrent lower and upper respiratory tract infections. AP50 testing, added to CH50, should therefore be part of initial work-up for patients with recurrent severe respiratory tract infections. Indeed early diagnosis allows for appropriate prolonged antibiotic prophylaxis and immunization to reduce the risk of fatal meningococcal disease, to reduce or prevent organ damage and to allow for genetic counselling in the family.

**(139) Submission ID#423568**

**Psychiatric aspects of patients with primary immunodeficiency and their parents**

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Introduction/Background: As a result of early diagnosis of primary immunodeficiency and starting treatment early and reducing the frequency of infections, the life expectancy of these patients is extended. However, this led to social, psychological, developmental and environmental problems in patients with primary immunodeficiency depending on various factors.
Objectives: We aimed to investigate the impact of psychiatric symptomatology, quality of life and care burden as well as related sociodemographic features and treatment variables in children diagnosed with Primary Immunodeficiency and their parents.
Methods: 164 patients in 2-18 age group who are followed up with primary immunodeficiency diagnosis at zmir Dr. Behçet Uz Childrens Hospital and their parents and 124 healthy volunteering children and their parents are included in our study. 78 of the patients were having IVIG replacement treatment. While Pediatric Quality of Life Inventory (Ages 2-4, 5-7, 8-12 and 13-18) and Child Depression Inventory (CDI) were implemented to both children and parents in addition to Sociodemographic Data Form; Beck Depression Inventory (BDI), Beck Anxiety Inventory and Zarit Caregiver Burden Scale were implemented only to parents.
Results: The depression inventory parent and child form values of the patient group with primary immunodeficiency were significantly higher than the control group (p=0,004 and p=0,032). According to Beck Depression and Anxiety inventories, it was seen that the depression and anxiety inventory scores of the parents of the patient group were higher than the control group (p=0,009 and p=0,022 respectively). It was determined that the CDI Parent Form scores of the patients with hospitalization history were statistically higher than the patients without hospitalization history (5,4±0,5 and 4,8±0,6; p=0,009). BDI scores were significantly higher in the group which received IVIG (p=0,024). While the quality of life of the patients compared to their parents was perceived as worse than the healthy children in all dimensions (p=0,003 p<0,0001 and p<0,0001), the quality of life of the children was worse only in psychosocial and total quality of life fields (p<0,0001 p=0,002 and p=0,158). It was seen that Quality of Life of Children Physical Health scores of only the patients with hospitalization history were statistically significantly lower (15,5±1,9 and 14,3±2,3; p=0,04). While no statistically significant difference was found in terms of quality of life of the child scores between the groups which received and did not receive IVIG replacement treatment, psychosocial and total qualify of life scores of parents were statistically significantly lower in the group which received IVIG replacement treatment (p=0,017 p=0,033). Zarit Care Giver Burden Scale scores were similar in patient and control groups. Although both groups were on the limits of mild to moderate caregiving burden, it was seen that the scores of the group which received IVIG were significantly higher than the group which did not receive IVIG (p=0,026).
Conclusions: As a conclusion, we think that it will be appropriate to inform and monitor the entire family in relation to psychosocial difficulties and care giving burden they may experience in time as well as the medical aspects of the disease in order to develop a holistic approach to children with primary immunodeficiency.

**(140) Submission ID#420232**

**Pulmonary complications in hyogammaglobulinemia**

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Introduction/Background: Chronic lung disease is the most common complications of CVID, affecting 30-60% of patients. It includes bronchiectasis affecting 50% of patients and granulomatous lymphocytic interstitial lung disease (GLILD) in 10 to 55%. Both are associated with an increased morbidity and mortality. Pulmonary functional studies and CT scans have been proposed as screening procedures for lung involvement in CVID. The definitive diagnosis of GLILD is established histologically, but it is too invasive in patients with radiological abnormalities and no symptoms.
Objectives: We aim to describe the pulmonary complications of our cohort of patients with CVID comparing them with subjects affected with other types of hypogammaglobulinemia.
Methods: We reviewed all clinical records of the patients with a diagnosis of hypogammaglobulinemia of any cause until December 2016. We looked at all pulmonary function tests (PFT), 6-minute walk tests and CT scans performed. We classified patients according to the CT scan pulmonary disease pattern. We compared the demographic data and pulmonary characteristics of each group and intended to describe similarities and differences between them.
Results: We collected 34 patients and 70 CT scans from 26 patients. Patients were grouped for further analysis as follows: no CT performed:

8; normal CT: 9; bronchiectasis: 9; GLILD: 7 and bronchiectasis + GLILD: 1. Eight patients (24%) had no CT including six with CVID, one with X linked lymphoprolipherative disease (XLP) and one had a syndromic deletion in chromosome 11. The median age of symptom onset was 23.5yo, with a median delay to diagnosis of 1 year. Nine subjects (26%) had CT scans with no chronic disease, six had CVID, 2 rituximab induced hypogammaglobulinemia (RIH) and 1 MGUS with hypogammaglobulinemia (MGUSwH). The median age of symptom onset was 58 years old with a median delay to diagnosis of 2 years. Nine patients (26%) presented persistent bronchiectasis, 7 with CVID, 1 with an IgG3 deficiency (sIgG3d) and 1 with XLP. Median age at symptoms onset was 14yo and median delay to diagnosis 13 years. Seven patients (21%) had GLILD; all afected with CVID. Median symptoms onset was 30 years old, and median delay to diagnosis was 10 years. One patient (3%) was included in the bronchiectasis and GLILD group. She was referred with a diagnosis of RIH, in the context of a pulmonary MALT lymphoma. However, her CT did not show a typical CT lung lymphoma pattern, lymphocyte monoclonality was never shown and she had a reduced pre-treatment low gamma globulin concentration and a history of respiratory infections since adolescence, we believed her diagnosis is CVID. PFT with an obstructive pattern identified patients with bronchiectasis ($p<0.01$) and patients with GLILD had a significantly lower basal and post 6 minute walk O2 saturation ($p<0.01$, n=22)
Conclusions: Using a systematic approach, we identified that roughly 50% of patients with hypogammaglobulinemia have chronic pulmonary CT abnormalities. Half of them had bronchiectasis, that were associated to hypogammaglobulinemia of any cause, a longer disease course and a reduction in FVC and FEV1 with a shorter distance reached in the 6 minute walk test. The other half had GLILD, spirometries in this group were useless, but O2 saturation was significantly lower, basal and after the 6 minute walk test. We found a high frequency of pulmonary disease in our cohort and a disease progression study is now in place.

**(141) Submission ID#428733**

**Pulmonary Disease in Autosomal Dominant Hyper IgE Syndrome**

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Introduction/Background: Autosomal dominant Hyper IgE Syndrome (AD-HIES) is a primary immunodeficiency characterized by eczema, sinopulmonary infections, and musculoskeletal and vascular abnormalities. Care of patients with this disease is largely supportive with the use of prophylactic antibiotics and topical eczema therapies. The use of replacement immunoglobulin is increasing. Hematopoietic stem cell transplant (HSCT) is being considered more frequently, but many questions remain regarding which patients should undergo transplant. As pulmonary complications are a leading cause of morbidity and mortality in this disease, and potentially improved with replacement immunoglobulin and HSCT, we sought to examine more closely the patients with more frequent pulmonary hospitalizations and structural lung disease.
Objectives: To determine the rates of pulmonary complications in our large cohort of AD-HIES patients, and examine the relationship between immunologic markers and pulmonary disease.
Methods: We retrospectively reviewed the records of 110 AD-HIES patients seen more than one time at NIH between 2010 and 2016. There were 36 pediatric patients under the age of 18 years. We reviewed the number of and cause for hospitalizations, and reviewed radiology reports for structural lung disease including bronchiectasis and pneumatoceles. We correlated these findings with specific antibody responses and lymphocyte phenotyping, such as the number of memory B lymphocytes.
Results: The patients range in age from 2 years to 66 years, with a median age of 23 years. 106 patients had Chest CTs, with 42 percent having bronchiectasis, and 41 percent having cavitary lesions. 31 percent of patients had both. These abnormalities were more prevalent in patients with low memory B cells. 31 percent of patients receive replacement immunoglobulin. In patients not receiving replacement Ig, 42 percent had appropriate response to pneumococcal vaccine. During this time period, there were 190 hospitalizations, the majority of which were associated with pulmonary infections. The rate of overall hospitalization was higher in the group with low memory B cells ($p=0.006$), but there was no difference associated with age.
Conclusions: Immunologic abnormalities may assist in determining the long-term prognosis of patients with AD-HIES, and can be considered in management plans such as the use of immune globulin and consideration for HSCT.

**(142) Submission ID#428713**

**Purine Nucleoside Phosphorylase Deficiency in Three Adults with Recurrent Infections**

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Introduction/Background: Purine nucleoside phosphorylase (PNP) deficiency is an autosomal recessive disorder affecting the purine salvage pathway causing a (severe) combined immunodeficiency disorder, autoimmunity, and neurological symptoms. Patients typically present in the first few years of life with frequent infections and a failure to thrive. Decreased plasma levels of uric acid are suggestive, while neutropenia is a rare complication. Prognosis is generally poor with few patients making it to adulthood without treatment. Treatment options are limited to general supportive care and hematopoietic cell transplantation.
Methods: We present the cases of three patients, one sister and two brothers, from a consanguineous French Canadian family. They presented in early adulthood with nearly identical histories of repeated pneumonias and chronic rhinosinusitis. One patient had necessitated filgrastim to treat a parainfectious neutropenia. They did not report any neurological abnormalities or symptoms of autoimmunity.
Results: A primary immune deficiency was suspected. Initial evaluation showed normal immunoglobulin levels, a lack of response to vaccination, positive EBV EBNA IgGs, and auto-antibodies. The patients were severely lymphopenic with reduced numbers of T, B and NK cells ( L: 300, CD3+81%, CD4+71%, CD8+5%, CD19+12%, CD3-CD56+7%). In particular, they had a severe depletion of naive T-helper recent thymic emigrant cells (CD4+CD45RA+CD31+2%). Additional studies revealed increased urinary inosine, guanosine, deoxyinosine, and deoxyguanosine, severely reduced erythrocyte PNP activity (3% residual activity), and a normal uric acid level. A homozygous, missense mutation, c.769 C>G (p.His257Asp) was found and described as pathogenic in silico. The diagnosis of PNP deficiency was made; TMP-SMX prophylaxis and IVIGs were started.

Conclusions: Theses cases are atypical for a number of reasons. The first is the relatively benign immunological course, the lack of neurological symptoms, and the advanced age at the time of the diagnosis, possibly due to residual enzymatic activity. In addition, PNP deficiency has been classically described as causing a marked reduction of T cells with a relative sparing of B cells. However, B cell lymphopenia have been reported, particularly with latter presentations.
While this specific mutation had been identified once previously in the literature, as a compound heterozygote, these are the first
confirmed cases of homozygotes. The pathogenicity of the mutation was not only suggested in silico, it was confirmed biochemically. In addition, in vitro functional studies have demonstrated the importance of His257 for the binding of PNP to its substrate, particularly in the formation of the early transition state. Indeed, PNP (p.His257Asp) has been shown to have a greatly reduced affinity for its substrates and catalytic activity.
Further study is needed to identify the optimal management for these patients.

**(143) Submission ID#428090**

**Rare case of late presentation of Tetratricopeptide repeat domain 7A (TTC7A) deficiency with severe NK cell dysfunction**

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Introduction/Background: Whole Exome Sequencing has become an integral part of diagnosis and treatment of rare immunodeficiency diseases. One of these diseases is Tetratricopeptide Repeat Domain 7A(TTC7A) mutations; a rare disorder associated with multiple intestinal atresias and severe combined immunodeficiency. Histologic assessment of organ biopsies of patients with TTC7A deficiency suggest it may play a role in multiple organs as it is expressed in the cytoplasma of intestinal, thymus and pancreatic cells. Many patients with TTC7A deficiency have moderate to severe combined immunodeficiency. Nine patients described in the literature with TTC7A mutations have been treated with hematopoietic stem cell transplant, mostly at a young age.
Objectives: This case report describes a rare presentation of an adolescent with TTC7A deficiency, her clinical presentation, immune evaluation and treatment with eventual referral to bone marrow transplant at the age of 16.
Methods: Retrospective chart review under IRB approval was performed. Whole exome sequencing performed at Baylor Texas Childrens Hospital and mutations were confirmed by Sanger sequencing. Extensive immune and NK cell phenotyping were performed by flow cytometry and NK cell function was tested using standard Cr51 release cytotoxicity assays.
Results: A 13 year old female was referred to immunology for severe refractory warts, history of severe diarrhea with epithelial dysplasia but no atresia, failure to thrive requiring parenteral nutrition until 6 years old and multiple infections with Staphylococcus aureus and Candida. At 16 years of age, she developed rapidly worsening pulmonary function that improved with monthly pulse methylprednisone. Her immune phenotype demonstrated a combined immunodeficiency including severely low CD4, CD8, B and NK cells. She had no IgD-CD27+ memory B cells and undetectable IgG, IgA and IgM, isohemagglutinins and vaccine titers to diphtheria and tetanus. Her mitogen proliferation response was low and she had abnormal TCR V-beta repertoire. She was referred to the Texas Childrens Hospital Center for Human immunobiology for the NK cell Evaluation and Reasearch Clinic (NEAR)
The patient was found to have impaired NK cell lytic function and terminal maturation. This was demonstrated by the decreased frequency of cells that matured to the CD56dim subset and was accompanied by decreased expression of the lytic effector molecule perforin and the Fc receptor CD16. This phenotype was conserved when we isolated CD34+ hematopoietic precursors and performed NK cell differentiation in vitro.
Whole exome sequencing demonstrated a compound heterozygous mutation in the TTC7A gene, including a c.1001+3_1001+6AAGT mutation, which has been previously described as a pathologic founder mutation in the French Canadian population. The second mutation is a previously unreported c.211G>A (p.E71K), a variant of unknown significance. Mutations were confirmed by Sanger sequencing, and parents are carriers of the mutations. She was referred for hematopoietic stem cell transplantation with a 10/10 unrelated donor option.
Conclusions: TTC7A deficiency is a genetic mutation associated with high morbidity and mortality leading to gut atresia and dysfunction in combination with severe immunodysfunction. We have described a unique case of TTC7A deficiency with a novel mutation which is associated with intestinal epithelial dysplasia with no atresias and a late presentation and evolution of combined immune deficiency. NK cell assessments show significantly impaired terminal maturation suggesting a critical role for TTC7A in human NK cell development.

**(144) Submission ID#409560**

**RAS-associated autoimmune leukoproliferative disorder in a 4 month-old male infant**

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Introduction/Background: RAS-associated autoimmune leukoproliferative disorder (RALD) is a rare condition with significant overlap of clinical and laboratory findings with malignant disorders, notably juvenile myelomonocytic leukemia (JMML) and chronic myelomonocytic leukemia (CMML), but with much better prognosis without specific therapy. We report a case of RALD in a 4-month-old male infant originally suspected to have JMML.
Results A 4-month-old male infant, born to an unrelated Ethiopian father and Turkish mother, presented with splenomegaly and leukocytosis (26,000/l), monocytosis (7,800/l), and thrombocytopenia (14,000/l); hemoglobin was 10 gm/dl and fetal hemoglobin concentration 1.9%. Family history indicated early death of three paternal uncles, two at age 6-7 months and one at age 10 years. JMML was suspected and bone marrow aspiration was performed; however, results of immunophenotypic testing with flow cytometry analysis and cytogenetic testing with FISH did not support this diagnosis. Patient had a normal B12 level. Serum IgG (1884.8 mg/dl [nl 694.0 - 1618.0 mg/dL]) and IgM

(139.0 mg/dl [nl 35.0 - 102.0 mg/dL] levels were elevated and IgA was normal. Lymphocyte subset analysis showed 37% CD3 [nl 51-74%], 20% CD4 [nl 34-53%], 51% CD19 [nl 17-37%] positive cells with normal percentages of CD8 and CD16/CD56 cells; there were no CD4-/CD8- T cells detected. Given the findings of leukocytosis, monocytosis, splenomegaly, hypergammaglobulinemia, and a dearth of CD4-/CD8- T cells, the patient appeared to meet criteria for RALD. Mutation testing with next generation sequencing showed an NRAS missense mutation [c.37G>T; pGly13Cys] in 46% of T cells and 48% of myeloid cells. The presence of the NRAS mutation, in the absence of other typical JMML findings or RASopathy syndrome features, supported the diagnosis of RALD. There was no therapeutic intervention and, at one year of age, the patient was reported to remain clinically well; he relocated to Ethiopia with his parents.

Conclusions RALD is a nonmalignant clinical syndrome originally classified as a subtype of autoimmune lymphoproliferative syndrome (ALPS) but subsequently distinguished to be a separate entity due to lack of double negative T-cells, a mutation in FAS/FASL/Caspase-10, or consistently elevated serum B12 levels. Though also present in 25% of JMML patients, a somatic mutation in RAS signaling protein (KRAS or NRAS), which controls B-cell tolerance and production of autoantibodies, is present in all reported cases of RALD. While all previously reported cases of RALD had somatic KRAS or NRAS mutations, our patient's NRAS mutation was present in more than 40% of T cells and myeloid cells, suggesting a heterozygous germline mutation; this has not previously been reported in RALD and needs to be further confirmed

**(145) Submission ID#421315**

**Recent outcome of hematopoietic cell transplantation for Wiskott-Aldrich syndrome is excellent in all donor types: A Primary Immune Deficiency Treatment Consortium (PIDTC) Study**

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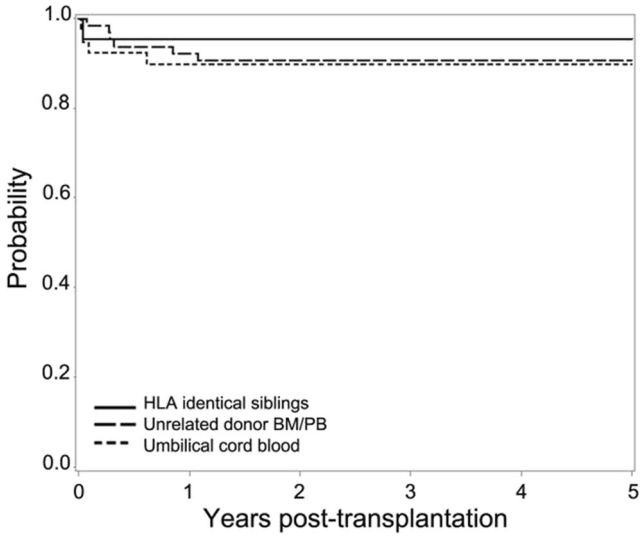
Introduction/Background: Outcomes of hematopoietic cell transplantation (HCT) for Wiskott-Aldrich syndrome (WAS) appear to be improving over time; however, multi-institutional results have not been evaluated in North America. In a previous collaborative study of mostly European centers, mismatched related donor and umbilical cord blood donor HCT was associated with poorer survival (Moratto et al, Blood, 2011)

Objectives: The primary objective of this study was to evaluate HCT outcomes in patients with WAS who underwent HCT since 2005 in North America. We hypothesized that survival after HCT from alternative donors such as cord blood has improved compared to published results, but that overall survival would be superior in patients who undergoing HCT at a young age.

Methods: Patients were enrolled on PIDTC protocol 6904, a multicenter retrospective natural history study of patients treated for WAS in North America since 1990. Clinical features, disease status, HCT type and post-HCT outcomes were analyzed in 129 patients who underwent HCT at 29 PIDTC centers between 2005-2015. Descriptive statistics such as median and range for continuous variables and counts and percentages for categorical variables were used to summarize characteristics of the study population. In addition, Kaplan-Meier curves were used for estimating survival probabilities

Results: Diagnosis of WAS was confirmed by an expert review panel for eligibility. Mutation in the WAS gene was available for 118 patients, including nonsense (n=27, 23%), frameshift (n=32, 27%), missense (n=31, 26%), splicing (n=20, 17%), gross deletion (n=5, 4%), in-frame deletion (n=2, 2%), pr complex (indel) (n=1, 1%). Donor types included matched sibling (n=22, 17%), unrelated adult volunteer bone marrow or peripheral blood stem cells (n=66, 51%), umbilical cord blood (n=39, 30%) and other related donors (1 each, phenotypically matched related, haploidentical). Median age at time of HCT was 13.6 months (range, 2.1 260.5 months). The vast majority of patients received busulfan containing conditioning regimens (87%), with some receiving other myeloablative (2%) or reduced intensity regimens (11%). With a median follow-up of 4.6 years, overall survival was excellent with 1-year and 5-year survival probabilities of 92% (95% CI, 86-96%) and 91% (95% CI, 84-95%), respectively. Survival was similar at 1 year for recipients of HCT from matched sibling (95%, 95% CI, 72-99%), matched unrelated donor (92%, 95% CI, 82-97%) or umbilical cord blood (90%,95% CI, 75-96% see Figure). Importantly we confirmed that survival at 1 year was better in patients who were <5 years old (n=118) compared to those who were 5 years old (n=11) at the time of HCT (95% versus 62%, respectively p=0.032). This difference persisted when only unrelated donor recipients were analyzed (97% vs 57%, p=0.036). Overall the percentage of patients in our study who underwent HCT at a young age was high (91%) compared to the literature (84% in Moratto et al, 2011, Blood). The rate of second HCT was only 4% (n=5). Cumulative incidence of acute grade 2-4 at 100 days, acute grade 3-4 at 100 days and chronic graft-versus-host disease at 1 year were 26% (95% CI 18-34%), 15% (95% CI 9-22%) and 14% (95% CI 8-21), respectively.




Conclusions: Outcome of HCT for WAS since 2005 shows excellent overall survival for all donor types, including umbilical cord blood with very low rates of second HCT. Importantly, HCT at a younger age (<5 years old) continued to be associated with superior survival supporting the provision of HCT earlier in the course of the disease. Further analysis of the complete cohort is planned to determine whether age at HCT has decreased in the modern era compared to pre-2005 and to analyze factors associated with platelet and immune reconstitution, donor chimerism and autoimmunity.

**(146) Submission ID#424627**

**Recurrent Infections, Congenital Ichthyosis and Atopy: A Mixed Genotype**

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Introduction/Background: Comel-Netherton syndrome is a rare disease hallmarked by congenital ichthyosis, atopy, trichorrhexis invaginata (bamboo hair) and, within the past 10 years, has been defined as a primary immunodeficiency. Specific, rare genetic polymorphisms (c.230T>A, pL66H) in the Fc receptor IIIa (FCGR3A) on natural killer (NK) cells have been shown to decrease NK cell function. Patients with these defects present with susceptibility to severe and recurrent viral infections, however, not all FCGR3A mutations result in this clinical phenotype. Here we report on a child with a mixed genotype, presenting with congenital ichthyosis, atopy and recurrent bacterial and viral infections.

Methods: Immunophenotyping of lymphocyte subpopulations were evaluated by flow cytometry. Genomic DNA was sequenced using Next generation sequencing. All detected variants were then sequenced using Sanger sequencing. CD16 dual epitope assay and evaluation of NK cell maturation was also performed. Intravenous immunoglobulin replacement therapy was initiated.
Results: Our patient presented at 9 months of age for evaluation of food allergy. She has congenital ichthyotic erythroderma with pruritic atopic dermatitis-like skin eruptions and a history of hypernatremic dehydration immediately following birth, thin and easily broken hair and a history of failure to gain weight with appropriate catch-up growth following formula fortification. She has had multiple episodes of acute otitis media and recurrent upper respiratory tract infections associated with wheezing. T-, B- and NK cell quantitation by flow cytometry was normal, including naïve and memory B cells. Immunoglobulins and vaccine antibody levels to Haemophilus influenza type b, Tetanus and Streptococcus pneumoniae were normal. Lymphocyte proliferation to mitogens was normal. Natural NK cell cytotoxicity was initially decreased, however, subsequent cytotoxicity testing performed at 13 months of age was normal. Whole exome sequencing revealed a homozygous mutation in SPINK5 (c.795-11A>G) and a homozygous missense mutation in the first immunoglobulin domain of FCGR3A (c.305T>A), both variants of unknown significance and under additional investigation. This homozygous mutation in SPINK5 was not detected in the patients healthy, unaffected sibling.
Conclusions: This is a case of an infant with a clinical phenotype consistent with Comel-Netherton syndrome, however genotyping has revealed homozygous mutations in SPINK5 and FCGR3A, suggestive of a possible mixed genotype. A recent large study investigating the use of whole exome sequencing to identify variants implicated in primary immunodeficiency found that in 11% of families, more than 1 gene contributed to the immunodeficiency phenotype. It should therefore be kept in mind that variability in an individuals clinical phenotype may be attributable to the presence of a mixed genotype.

**(147) Submission ID#418677**

**Reduced Intensity Conditioning Allogeneic HCT with Alemtuzumab, Fludarabine and Melphalan: Preferred HCT Approach for SCID due to Cartilage Hair Hypoplasia in the Era of Newborn Screening?**

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Introduction/Background: Cartilage-hair hypoplasia (CHH) is a rare autosomal recessive disease caused by mutations in the RMRP gene and can manifest with SCID. Allogeneic HCT is a curative therapeutic option for SCID associated with CHH. Bordon et al. reported an overall survival of 62% (10/16 patients) following HCT with a predominantly myeloablative conditioning regimen with busulfan and cyclophosphamide. Data on outcomes of HCT using a RIC regimen are limited.
Objectives: We herein report our experience with allogeneic RIC HCT for SCID associated with CHH.

Methods: We reviewed records of all patients who underwent allogeneic HCT for SCID associated with CHH at our institution, with a RIC regimen containing alemtuzumab, fludarabine and melphalan.
Results: Five patients (3 male, 2 female) underwent allogeneic RIC HCT for CHH at median age of 8 months (range, 4 months 4 years). All patients had biallelic mutations in the RMRP gene, and met PIDTC criteria for SCID, prior to HCT. Two patients were diagnosed by newborn screening for SCID. One patient received serotherapy only (rituximab 375 mg/m2 daily x 3 days, anti-thymocyte globulin 1.5 mg/kg daily x 4 days) conditioning for initial HCT but developed graft failure and subsequently received a RIC regimen for second HCT. All patients received a RIC regimen consisting of alemtuzumab 1 mg/kg over 5 days(n=4) or 3mg/kg over 4 days (n=1) and fludarabine 5 mg/kg (weight <10 kg, n=4) or 150 mg/m2 over 5 days (n=1), and single dose of melphalan 4.7 mg/kg(weight <10 kg, n=4) or 70 mg/2(n=1, dose reduced by 50% for pre-existing sclerosing cholangitis with grade 3 liver fibrosis). Patients received matched (n=3) or 1-2 allele mismatched (n=2) bone marrow grafts and all but 2 grafts were from unrelated donors. All patients received cyclosporine and steroids for GVH prophylaxis.
All patients engrafted with full donor chimerism. Three patients developed mixed chimerism, but continue to maintain donor T cell chimerism > 90%. Two patients developed VOD of the liver (one patient developed mild VOD whereas the patient with pre-existing sclerosing cholangitis developed severe VOD). One patient developed grade 2 skin GVHD and one patient developed limited chronic skin GVHD. None of the patients developed liver or GI-GVHD. All patients remain alive at a median follow up of 4 years (range 11 months-11 years) with good T-cell and B-cell immune reconstitution.
Conclusions: Our experience suggests that allogeneic RIC HCT with alemtuzumab, fludarabine and melphalan for SCID associated with CHH is curative, offers durable T-cell engraftment, low GVHD along with excellent survival and might be preferable over a myeloablative conditioning regimen, to further limit toxicity in young infants, especially in the era of newborn screening.

**(148) Submission ID#420730**

**Refractory thrombocytopenia in a patient with Wiskott-Aldrich syndrome despite hematopoietic stem cell transplantation: eltrombopag as a therapeutic option.**

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Introduction/Background: Wiskott-Aldrich syndrome (WAS) is an X-linked disorder characterized by: immunodeficiency, eczema and hemorrhage due to thrombocytopenia[1]. Hematopoietic stem cell transplantation is the treatment of choice, with an overall survival of approximately 90% regardless of the source of the stem cells [2]. Eltrombopag has been used as a pre-transplant stabilization therapy[3]. In our knowledge, there are no reports of its use for the management of post-transplant autoimmune cytopenias in WAS.
Objectives: to present a clinical case in which the complexity of diagnosis and management of Wiskott-Aldrich syndrome is highlighted, as well as the usefulness of eltrombopag in post-transplant persistent thrombocytopenia.
Methods: Clinical case presentation and review of literature.
Results: Clinical case: 9-month-old infant with a history of thrombocytopenia identified at the third day of life (positive serology and viral load for CMV), bacteremia due to Serratia marcescens at 4 months, intermittent diarrhea from 4 months, multiple platelet transfusions, IVIG cycles, and ambulatory management with corticosteroids; who consulted the ER for a 5-day of bloody diarrheic stools, generalized petechiae and fever. He was irritable with generalized petechiae in the lower extremities, diaper area dermatitis with signs of superinfection. Admission CBC: WBC: 20180/mm3, neutrophils 330/mm3, lymphocytes 17250/mm3, monocytes 2360/mm3, Hb 11.2 g/dL, Ht 34.1% MCV 80.2fL, MCH 26.3 g/dL, MCMH 32.8%, Platelets 15000/mm3 MPV 8 fL. Bone marrow aspiration was performed that ruled out proliferative syndrome. Immunological profile with normal immunoglobulins and flow cytometry showed T lymphocytosis with CD4/CD8 inversed ratio, direct Coombs was positive. He progressed to refractory thrombocytopenia, with intracranial bleeding and transfusion platelet requirement every 12h. Molecular diagnosis of Wiskott-Aldrich syndrome was made and it was decided to carry out an allogeneic transplant of unrelated umbilical cord. He showed adequate response, 100% chimerism; however, he persisted with important cytopenias, without response to management with IVIG and corticosteroids, so that on day +68 he restarted eltrombopag that he received prior to transplantation. From day +97 he received Rituximab for 6 weeks. He continued with eltrombopag 10 months post-transplant. Currently without cytopenia and without complications, he completed the post-transplant year without additional complications.
Conclusions: WAS represents a great diagnostic challenge in pediatric clinical practice. Despite the therapeutic option of transplantation of hematopoietic stem cells, patients may persist with different complications, within these; autoimmune cytopenias will require additional therapies. Eltrombopag, in addition to being used as a pretransplant transient measure, is useful for the management of post-transplant persistent thrombocytopenia in these cases.
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**(149) Submission ID#426867**

**Report of 9 years of Primary Immunodeficiencies in two centers in Argentina for the Latin American Society of Immunodeficiencies, registry model**

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Introduction/Background: In 2009 the Latin American Society of Immunodeficiencies, (LASID) created the Latin-American (LA) registry of Primary Immunodeficiencies (PID), including 124 centers from different countries in our region. Today LASID registry model has 7355 patients(p) with PID.
Objectives: Present the prevalence of PID, into a center of immunology in Argentina.
Methods: Retrospective study of medical records in two centers of Immunology
Results: In our center we have 1205 p with PID, with made retrospective study of medical records, up today we have registered 1155p (96%).
According to this record, these diseases are distributed in the following way: Predominantly antibody disorders: 890p (77%), Predominantly T cell deficiencies:88p (7,6%), Phagocytic disorders:41p (3.5%), Complement deficiencies:26p(2.2), Other well PID:53p(4.5), Autoimmune and immune

dysregulation syndromes:8p(0.6%), Unclassified immunodeficiencies:57p(4.9%). Predominantly antibody deficiencies are the most common PID, which comprise more than half of our all p. These group is represented by: Specific IgA deficiency (SAD):332p, Specific IgG deficiency:164p, Transient hipogammaglobulinemia:206p, Common Variable Immunodeficiency (CVID):96p, Agammaglobulinemia linked X:33p, Agammaglobulinemia unknown causes:8p, Secondary hipogamma globulinemia:23p, Selective IgM deficiency:1p, subclasses deficiency:10p, CD40L deficiency:7p, Hyper IgM unknown causes:32p, Other hyogammaglobulinemia:3p. Among them, Selective IgA is the most common PID.In our cohort of unclassified immunodeficiency, we have 25p with auto inflammatory syndrome, such as Family mediterranean fever, Hyper IgD syndrome and Candle like- syndrome and 6 p with NK deficiency. In all our PID 244p, are under replacement gammaglobulin (gg)treatment, 171p use intravenous gg and 71p use subcutaneous gg

Conclusions: The LASID registry model represents a powerful tool to improve health policies, showing that are under diagnosed and should receive more attention. More data are needed to define the exact prevalence of PID to avoid underestimation of these diseases due to under reporting.

As different reports in different countries, in our centers predominantly antibody deficiencies are the most prevalent. Although the number of patient diagnosed with PID, is growing. Many physicians still know little about these disorders.

**(150) Submission ID#428644**

**Reversible Symptomatic Panhypogammaglobulinemia Secondary to Lamotrigine**

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Introduction/Background: A number of anticonvulsant medications have been shown to cause hypogammaglobulinemia. Lamotrigine is a phenyltriazine anticonvulsant medication that is approved to treat seizure disorders and bipolar disorder.

Objectives: We describe a patient who developed hypogammaglobulinemia secondary to lamotrigine use.

Methods: We performed a chart review and case-based literature review.

Results: A-27-year old female presented to immunology clinic for evaluation of panhypogammglobulinemia. She was initially evaluated by general internal medicine for lightheadedness and fatigue and was found to have serum IgG of 382 mg/dL (767-1590 mg/dL), IgM 26 mg/dL (37-286 mg/dL) and IgA 33 mg/dL (61-356 mg/dL). The patient reported a history of recurrent sinus infections occurring around twice per year. She reported resolution with oral antibiotics. She denied any history of pneumonia and was never hospitalized for treatment of infection. She did report increased number of recurrent upper respiratory infections; around 4-5 per year for the last several months. She denied any family history of primary immune deficiency. She was never prescribed corticosteroids or immuno-modulators. Her past medical history was significant for bipolar disorder type 2 for which she was prescribed lamotrigine 100 mg oral twice daily seven months prior to her initial visit. The medication was prescribed prior to the onset of recurrent sinus infections. She had protective post-vaccination titers against tetanus, diphtheria, and acellular pertussis and non-protective pre-vaccination pneumococcal titers. Post vaccination pneumococcal titers were not obtained due to the cost of the pneumonia vaccine. The patient was started on prophylactic azithromycin three times weekly. Lamotrigine was discontinued and the patient switched to lurasidone due to concern for anticonvulsant-induced panhypogammaglobulinemia. Her serum immunoglobulin levels increased after 2.5 months off of lamotrigine (IgG 416 mg/dL, IgM 46 mg/dL, IgA 34 mg/dL), and she is currently asymptomatic without further infections.

Conclusions: Lamotrigine is likely responsible for the reversible hypogammaglobulinemia in this patient. Serial immunoglobulin levels should be checked in all patients who experience recurrent sinopulmonary infections while on lamotrigine.

In two separate reports, lamotrigine induced hypogammaglobulinemia began within 7 months and 13 months of starting therapy respectively. In another study, hypogammoglubinemia was reported in 28% of 74 patients taking lamotrigine. Further studies are needed to accurately describe onset and frequency of hypogammgeobulinemia in these patients. Currently, it is unclear whether post vaccine titers are protective in patients with lamotrigine induced hypogammaglobulinemia.

**(151) Submission ID#419508**

**Rubella Virus Associated Cutaneous Granulomatous Disease: A Unique Complication in Patients with DNA Repair Disorders**

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Introduction/Background: The association of vaccine strain rubella virus with cutaneous and sometimes visceral granulomatous disease has been reported previously in 19 patients with various primary immunodeficiency disorders (PIDs). The majority (14/19) of these PID patients with rubella positive granulomas had DNA repair disorders, namely ataxia telangiectasia (AT) (n=9) or Nijmegen breakage syndrome (NBS) (n=3) or RAG1 (n=1) and RAG2 (n=1) deficiency.

Objectives: To support this line of inquiry, we provide additional descriptive data on the previously reported NBS patients as well as additional previously unreported patients with rubella virus induced cutaneous granulomas and DNA repair disorders as well as additional previously unreported PID patients with rubella virus induced cutaneous granulomas.

Methods: We provide in-depth descriptive data on the previously reported NBS patients as well as 5 additional previously unreported patients with rubella virus induced cutaneous granulomas and DNA repair disorders including AT (n=4) and DNA Ligase 4 deficiency (n=1). We also provide

in-depth descriptive data on 4 additional previously unreported PID patients with rubella virus induced cutaneous granulomas, including cartilage-hair hypoplasia (n=1), MHC Class II deficiency (n=1), WHIM syndrome (n=1), and Coronin-1A deficiency (n=1).
Results: The median age of the patients is 10.5 years (range 3-33). The majority are females (83%). Cutaneous granulomas have been documented in all cases while visceral granulomas (spleen and liver) were observed in 3 cases. T cell and B cell lymphopenia as well as hypogammaglobulinemia or impaired antibody formation were present in most patients. All patients had received rubella virus vaccine. The median age at presentation of cutaneous granulomas was 84 months (range 14-377). The median duration of time elapsed from vaccination to the development of cutaneous granulomas was 19 months (range 12-152). The diagnosis of rubella was made by PCR in 83% of patients and by immunohistochemistry in the remainder. One patient was confirmed to have vaccine strain rubella virus. Hematopoietic cell transplantation was reported in three patients. Rubella associated complications did not contribute to death among those patients who died (16%).
Conclusions: Of the now 28 cases, 19 (67%) share the diagnosis of a DNA repair disorder and confirm that chronic rubella virus infection is associated with cutaneous granuloma formation. Analysis of patients with DNA repair disorders and other PIDs with this complication will help clarify determinants of rubella pathogenesis, identify specific immune defects resulting in chronic infection and may lead to defect-specific therapies.

**(152) Submission ID#403469**

**Safety and Efficacy of Hizentra® in Pediatric Hematopoietic Stem Cell Recipients**

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Introduction/Background: Introduction/Background: Hizentra® is a 20% liquid IgG product approved for subcutaneous administration in adults and children greater than two years of age who have primary immunodeficiency disease (PIDD). Limited information on use of Hizentra® is available for children who have received hematopoietic stem cell transplantation (HSCT).
Objectives: Objectives: The aims of this study are to determine the safety and efficacy of Hizentra® in pediatric patients post HSCT, and to characterize reasons for switch from intravenous IgG (IVIG) to subcutaneous (SCIG) delivery following HSCT.
Methods: Methods: A retrospective chart review involved 13 PIDD infants and children (mean age 6.3 [range 3.1 to 14.5] months) status post HSCT who received Hizentra®. Ten patients received Hizentra® by pump administration, and 3 patients by manual push.
Results: Results: Hizentra® administered weekly to 13 children included an average of 144 infusions (range 14-416). The mean dose was 722 mg/kg/4 weeks. The mean IgG level was 889 mg/dL while on Hizentra® in 11 patients compared to a mean trough IgG level of 563 mg/dL in 6 patients during immunoglobulin administration prior to Hizentra® of most children. Four patients naïve to IgG therapy were started on Hizentra®. Average infusion time was 4.7 (range 3-15) minutes for manual entry and 71 (range 60-90) minutes for pump entry, and the average number of infusion sites was 1.9 (range 1 to 2). Local reactions were mild and observed in 3/13 (23.1%) children. Ten patients had no local reactions. No serious adverse events were reported. The rate of serious bacterial infections (SBI) was 0.12 per patient-year while receiving Hizentra®, similar to reported efficacy studies. The reasons for switch from IVIG to SCIG in 13 patients (some patients had multiple reasons) were improved IgG serum levels and physician desire for steady state serum IgG levels (n=6), loss/lack of venous access (n=2), patient/caregiver preference (n=7), home site of care preference (n=4), and physician preference for SCIG (n=5).
Conclusions: Conclusions: Hizentra® is a safe and effective option in children who have received HSCT. Reasons for switch from IVIG to SCIG included improved serum IgG levels, desire for steady state serum IgG levels, and patient/caregiver preference.

**(153) Submission ID#388079**

**Seasonal Influenza Vaccination Among Persons with Primary Immunodeficiency**

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Introduction/Background: It is currently unknown how many persons with primary immunodeficiency (PI) receive the seasonal influenza vaccination, and what proportion becomes ill. Additionally, the effect of immunoglobulin (Ig) replacement therapy on the frequency of influenza diagnosis and severity of symptoms is not known.
Objectives: The current study sought to measure the prevalence of seasonal influenza vaccination and diagnosis among persons with PI, specifically those with antibody deficiencies: X-linked agammaglobulinemia (XLA), Common Variable Immunodeficiency (CVID) and Hypogammaglobulinemia.
Methods: 11,533 email invitations were delivered to members of the Immune Deficiency Foundation database requesting participation in an online survey regarding their zoster, influenza and varicella vaccination experiences. Data from 1009 persons with XLA (Total N=41; Children age<18; n=19; Adults age>17, n=22; 100% Male; 81% White non-Hispanic), CVID (Total N=824; Children age<18, n=57; Adults age>17, n=767; 79% Female; 92% White non-Hispanic), and Hypogammaglobulinemia (Total N=144; Children age<18, n=23; Adults age>17, n=121; 77% Female; 92% White non-Hispanic) were analyzed for the 2016-2017 influenza season.
Results: Overall, 74% (n=749) of the sample received a seasonal influenza vaccination during the 2016-2017 influenza season. Persons with XLA were less likely to receive an influenza vaccination (54%, n=22), than persons with CVID (76%, n=628) and Hypogammaglobulinemia (69%, n=99). A fifth of respondents (21% overall, 34% of children and 19% of adults) who attend school and/or work stayed home at some time to avoid seasonal influenza. Though the majority (81%, n=821) were not diagnosed with influenza, when stratified by age, children were twice as likely to be diagnosed than adults. Of the 99 children sampled, 30% (n=30) were diagnosed with seasonal flu compared to 15% (n=139) of the 910 adults that were sampled. The role of replacement Ig therapy in protection against flu can best be examined in the 41 patients with XLA since these patients cannot make specific antibodies to vaccine and only 54% (n=22) received the vaccine. Although 59% (n=24) of the individuals with XLA were exposed to flu, only 10% (n=4) were diagnosed with influenza; two of whom were not receiving Ig therapy at time of diagnosis. Concerning influenza severity, individuals receiving Ig therapy at the time of flu diagnosis tended to have modestly milder symptoms than those not

receiving Ig treatment (e.g., less likely to report sore throat (78% versus 92%, P<.05)), but the sample size is too low to draw firm conclusions.
Conclusions: A high proportion of antibody deficient persons received a seasonal influenza vaccination for the 2016-2017 influenza season. In addition to vaccination, many individuals attempted to avoid influenza infection by remaining home from school and/or work. There was a suggestion that Ig replacement therapy may partially protect XLA patients from symptomatic flu, although the role of cell mediated immunity in protection against flu is not clear. A prospective study could determine if Ig replacement therapy partially protects against clinical flu symptoms in XLA and CVID patients using two groups those that receive the current flu vaccine vs. those that do not receive the flu vaccine.

**(154) Submission ID#414570**

**Single center expierence: Immune reconstitution in Fanconi Anemia patients after Hematopoietic Stem Cell Transplant**

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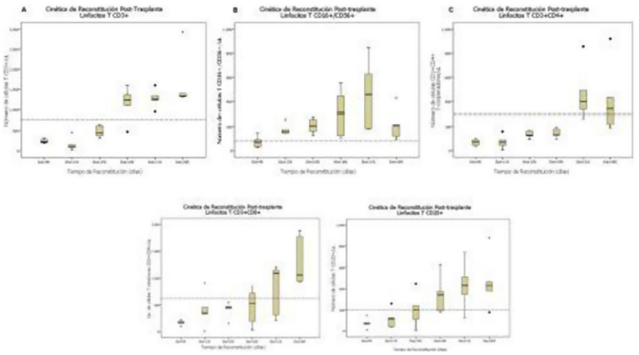
Introduction/Background: Fanconi anemia (FA) is an autosomal recesive or X- linked genetic disorder, characterized by cytopenia or bone marrow failure, this will lead to a severe anemia, neutropenia and thrombocytopenia, requiring frequent interventions with diversified therapies, including Hematopoietic Stem Cell Transplant (HSCT). A complete immune reconstitution is requiere for a success transplant. One of the main upcoming events asociated after the HSCT, is the secundary immunodeficiency, which is asociated with a significant morbility and mortality in the patients. To rich a successful alogenenic-HSCT, is impending a complete reconstitution of the T cells immunity, for this it is crucial the presence of factors like; thymic activity of the HSCT recipient, biological features of the allograft (eg, degree of histocompatibility, number and type of infused donor T cells) and preparative regimens.
Objectives: Describe the kinetics of the Immune reconstitution in FA patients after alogenic-HSCT, as well as the infections associated during this process and other comorbilities.
Methods: We decribed the lympocyte population (T, B and NK) in PB measured by Flow cytometry in FA patients on days +90, +120, +150, +180, +210 and +360 post alogenic HSCT from a match related donor. The conditioning was base on fludarabine 150mg/m2, cyclophosphamide 10mg/kg and antithymocyte globulin rabbit 20mg/kg. Infectious-disease survillance for virus was determined by the quantification by DNA PCR-RT for CMV, EBV, Adenovirus, as well as the presence of galactomannan and Candida sp antibodies, or isolation of fungus or bacterial culturing in the cases of infectious disease of known or uknown aetiology.
Results: The Lymphocyte population mesurement was performed in a total of five FA patients who undergo to an allogenic HSCT, all of them received stem cells from a match related donor and the source was bone marrow. Successful engraftment was observed in all 5 patients, there were no deaths reported. After the HSCT was performed, the kinetics of recovering for the distinct lymphocytes subsets was the the following: NK cells (CD16+CD56+) were the first to recover, followed by CD+8 T cells, B Lymphocyte and finally CD+4 T cells (Figure 1) all of them rich normal values and remain stable. Three out of five patients presented infectious disease: two of them were CMV positive, one patient has a concurrent detection of Adenovirus, and in the other was detected Aspergillus, in both of them it was presented at day +90. The third one developed acute GVHD which progressed to a chronic GVHD, at day +153 was diagnosed on him Listeria monocytogenes meningitis, at that momento he has just CD+16 +CD56 and CD8+ reconstituded. There is no new infectous disease detected in any of the patients after they reconstituted CD4+ T cells.



Conclusions: Complete immune reconstitution is the decisive for the presence of several morbilities and mortalities, mainly because of oportunistic infecctions and GVHD. we show that the kinetics of recovery of the different populations of lymphocytes follows those patterns also described for patients with other hematological malignancies: early recovery of NK cells, followed by effector cytotoxic T cells and B cells, and finally, CD4+ T-helper cells. The utility of post-transplant monitoring of PB-lymphocyte subsets for improved follow-up of patients undergoing BMT and prevent opportunistic infections.

**(155) Submission ID#393782**

**Skin Manifestations in Children with Primary Immunodeficiency Diseases in Qatar**

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Introduction/Background: Early detection of primary immunodeficiency diseases (PIDs) before serious infection is of utmost importance and a question of patient survival. One of the main organs involved in PIDs is skin with mucocutaneous manifestations being one of the common complaints in PIDs. The presenting skin symptoms may serve as an essential element in early diagnosis of PIDs.
Objectives: This study aims to determine characteristics, frequency, nature and incidence of skin manifestation in PID patients seen in Qatar.
Methods: This retrospective study was conducted at Hamad Medical Corporation the only tertiary hospital in Qatar from January 2009 to July 2017.The subjects included were PID patients < 14 years old, who had dermatological complaints. Information collected included dermatological diagnosis, gender, age at onset of signs and symptoms, age at definite diagnosis, family history of related disease, any lab results such as pathology or viral studies obtained from the clinical medical record. Patients were diagnosed and classified according to clinical and laboratory criteria by the International Union of Immunological Societies Primary Immunodeficiency Committee.
Results: A total of 109 patients were studied and skin/mucocutaneous manifestations were found in 65 patients (59%) with male to female ratio of 1.5:1. Age at onset of skin manifestations ranged from 0 to 12 years. Skin manifestations were divided in two categories according to presentation before PID diagnosis (Primary manifestations N=32, 47%) and during the disease period (Secondary manifestations N=35, 53%). The

type of PIDs predominantly having primary manifestation were SCID (N=5, 15%) CVID and CGD (N=4, 12%, each), XLA, AT and Griscelli syndrome (N=3, 9%, each).Various manifestations which were atypical in their presentation and persistently found in patients who did not respond to any effective treatment (N=13) led to the basis of clinical immunological study and diagnosis including rare diagnosis such as IPEX. Whereas secondary manifestations were primarily reported in SCID (N=9, 25%) CGD, AT and DiGeorge syndrome (N=5, 14%, each).
The nature of skin manifestations varied in both groups, primary manifestations notably had 41% cutaneous infections comprising of thrush (30%) stomatitis (17%) viral rashes (26%) bacterial rashes (13%) impetigo (8%) and fungal rash (4%). Eczema/atopic dermatitis were 31% and other minor miscellaneous skin alteration cases found were apthous mouth ulcer (5) erythroderma (4) GVHD like rash (1) alopecia (1) psoriasis (1) and scleroderma (1). Secondary manifestations were identified as 53% cutaneous infections 20% eczema and infantile seborrheic dermatitis (1) pruritus (1) GVHD and alopecia (1) each.
The causative microorganisms were confirmed in 44% of the cases via lab cultures whereas common infections such as chicken pox and herpes were validated through clinical symptoms. None of the cases were biopsied. Overall, highest skin infection was seen in Ataxia Telangiectasia (11). Other PIDs with prominent cutaneous manifestations included CGD (9), DiGeorge syndrome (5), Hyper IgE syndrome (4) and SCID (Rag 1 or 2, 4 cases). More than two types of skin manifestations were found in 47% patients over the due course of their illness.
Conclusions: An awareness of various cutaneous and skin disorders associated with PID which are persistent and unresponsive to treatments among dermatologist and family physicians is crucial to raise suspicion for early detection, timely management and prevention of complications.

**(156) Submission ID#393775**

**Skin Ulcers leading to residual hypo pigmented lesions with failure to thrive in Ataxia Telangiectasia case: A CASE REPORT**

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Introduction/Background: Ataxic Telangiectasia (AT) is a rare neurodegenerative autosomal recessive disease associated with immunodeficiency, poor coordination and disability. Ataxia Telangiectasia has a diverse clinical heterogeneity which may often lead to an incorrect diagnosis or late detection of the disorder resulting in exposing the patient to unnecessary radiation from various sources.
Objectives: We present a female child from the Asian subcontinent that was seen for the first time with ulcerative skin lesions, failure to thrive and marked lymphopenia.
Methods: A search of the PubMed database was carried out, using different combination of the terms "Ataxia", "Telangiectasia", typical , atypical and "presentation"
Results: Four-year-old Pakistani girl, product of first degree nuptial presented with generalized vesicular rashes mainly on abdomen and scalp which turned into ulcers with residual hypo pigmented lesions and diarrhea. She had short stature, failure to thrive (Weight and height < 5th percentile) and no previous infection or family history of primary immunodeficiency diseases (PID). Mild delay in milestones was observed especially in speech. Immunizations were up-to-date including BCG. On examination she had mild ocular telangiectasia but no cutaneous involvement. Investigations revealed lymphopenia (1.6) and eosinophilia. Workup for diarrhea including duodenoscopy revealing subtotal villous atrophy however the biomarkers for celiac disease were negative. Immune system workup showed high IgG levels, normal IgA, IgE and IgM level. IgG Subclasses: low IgG 2, and 3, antibody titers to H.Influenza and Pneumococcus vaccine were relatively low. Lymphocyte subsets showed low CD4,CD19 and high NK cells (40%), low naïve T cells (2%) and inverted CD4/CD8 ratio.T cell function with post PHA was 18.7% but had normal response to CD3. Alfa Feto Protein was requested due to continued low CD4 count and the level was found to be high: 260.9 IU/ml. Next-generation sequencing (NGS) showed homozygous mutation for Trp1750fs in chromosome 11 of ATM gene confirmed by whole exome sequencing. Immediate treatment with IVIG was started leading to mark improvement of skin lesions, diarrhea and weight gain.
Conclusions: The index of suspicion of AT should be highlighted when deciphering Lymphocyte subset. Currently there is no neonatal screening for PID diseases and next generation sequences in Qatar. Once implemented it may prove beneficial in discovering cases from early infancy and reaching upon a definite diagnosis.

**(157) Submission ID#418515**

**Sons Disease Gives Answers for Mom: Highly Skewed Lyonization Pattern in X-Linked Chronic Granulomatous Disease**

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Introduction/Background: Chronic granulomatous disease (CGD) is a genetic disorder of the NADPH oxidase complex in phagocytes which results in impaired production of microbicidal reactive oxygen species that can lead to recurrent life-threatening bacterial and fungal infections. The majority of affected patients in the United States have a X-linked defect in the CYBB that encodes gp91phox protein followed by an autosomal recessive defect in the NCF1 gene that encodes p47phox . X-linked female carriers show 2 populations of neutrophils and following lyonization, and severe skewing of X-chromosome inactivation can get CGD type infections. A recent study has shown that the lower dihydrorhodamine (DHR) percent in female carriers predicts a higher infection risk but carrier state on its own predicts autoimmunity
Results: A 39-year-old female presented for an evaluation of recurrent fevers. She had a history of several lobar pneumonias in childhood. At age 32, she developed erythematous bumps on her abdomen that were initially non-bothersome but later became painful. She then developed recurrent fevers, chills, and rapid weight gain. Biopsy of the skin lesions showed subcutaneous panniculitis-like T cell lymphoma. She was treated with chemotherapy and found to be in complete remission. She had no previous family history of recurrent infections. At the completion of her chemotherapy, her 1-year-old son became septic in the hospital and was then diagnosed with CGD from mutated CYBB gene. Post chemotherapy, the patient had a prolonged course with an atypical lung infection that required bronchoscopy and video thorascopic surgery, however no bacteria was ever identified. She did improve after a long course of IV antifungals. Evaluation for immunodeficiency was done after she had a recurrence of low grade fevers, cough, and fatigue. DHR flow cytometry with Phorbol Myristate Acetate (PMA) was done which showed 3.6% neutrophil oxidative burst activity after stimulation, consistent with a highly skewed lyonization pattern in X-linked CGD.
Conclusions: This case demonstrates an interesting lesson in a woman with recurrent infections since childhood whose clinical picture was confounded by her history of malignancy. The abnormal lung infections prior to malignancy were alarming and gave cause to do additional immunology testing. However, it begs the questions, if she did not have a son, would she have been diagnosed with severe X-lined carrier disease.

**(158) Submission ID#394894**

**Stratification of primary immunodeficiency patients using vaccine response and IgG subclass measurements reveals different frequencies of infection**

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Introduction/Background: Measurement of the specific antibody response following vaccine challenge provides clinicians with a better understanding of the adaptive immune response in individuals undergoing immunological evaluation.
Objectives: We hypothesised that after classification of primary immunodeficiency (PID) patients based on vaccine response (VR), further division using IgG subclass (IgGSc) 1-3 measurements may identify additional patients with abnormal B cell function and patients with different frequencies of infection at presentation.
Methods: VRs and serum IgGSc concentrations were quantified using the VaccZyme anti-Pneumococcal Polysaccharide (PCP) IgG ELISA and human IgGSc liquid reagent kits (The Binding Site Group Limited, UK) in 23 PID patients (1:1.3 M:F, median age 41.5 years, range 20-78). All patients were immunised with Pneumovax®23 (Sanofi Pasteur MSD). The lower limits of published normal IgGSc ranges were used as cut-off values (IgG1 3.2 g/L, IgG2 1.2g/L and IgG3 0.2g/L). PCP concentrations equal to or less than 70 mg/L post-vaccination was considered an abnormal response.
Results: Agreement between VR and IgGSc measurements was 48% (p=1.00). The frequency of respiratory tract infections at presentation among PID patients with a normal PCP IgG VR was 45% and 55% among patients with an abnormal VR. Subsequently, four separate groups could be identified by including IgGSc measurements. The frequencies of infections at presentation were for the VR+/IgGSc+ group (n=4) 36% vs. 48% for the VR+/IgGSc- group (n=10); and 33% for the VR-/IgGSc+ group (n=2) vs. 63% for VR-/IgGSc- individuals (n=7).
Conclusions: These results confirm that VR and IgGSc measurements are independent serum biomarkers of humoral immunity. Taken together the VR and IgGSc results provide more detailed information about the immune status that may influence diagnosis, treatment and monitoring decisions.

**(159) Submission ID#418990**

**Subcutaneous Immunoglobulin Therapy with Hizentra® in Patients with Stiff Person Syndrome**

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Introduction/Background: Hizentra® is a 20% liquid IgG licensed for subcutaneous administration in adults and children greater than two years of age who have primary immunodeficiency disease (PIDD). Subcutaneous immunoglobulin (SCIg) use in autoimmune conditions is reported. Stiff Person Syndrome (SPS), a rare neurologic disorder characterized by fluctuating muscle spasms and rigidity, is mediated by autoantibodies to glutamic acid decarboxylase (GAD). Symptoms of SPS have been shown to improve after administration of intravenous immunoglobulin (IVIG) however, there is a paucity of information regarding use of SCIg in SPS.
Objectives: The aim of this study is to describe the use of Hizentra® in patients with SPS, including indications for SCIg, clinical characteristics of patients, and clinical and laboratory response to SCIg.
Methods: A multicenter retrospective chart review examined 2 patients with Stiff Person Syndrome treated with Hizentra®.
Results: SPS was diagnosed in 2 patients at 12 and 25 years. Both patients were started on IVIG, steroids, and rituximab prior to initiation of weekly Hizentra® (ages 15 and 27 years). The average dose of Hizentra was 125mg/kg weekly. The average IgG level while receiving IVIG was 1,164.5 mg/dL, similar to the average IgG level while on Hizentra was 1,250 mg/dL. The number of administration sites ranged from 2-4, and duration of infusions ranged from 60-120 minutes. Serum anti-GAD antibody levels prior to Hizentra® were 845 U/mL and 134 U/mL respectively. Anti-GAD antibody level during treatment with Hizentra (available for one patient) was >5,000 U/mL, and 132,240 U/mL following discontinuation of Hizentra®. One of the reasons for switching to Hizentra® was lack of response while on IVIG. The average number of Hizentra infusions were 138. Patients reported improvement in spasticity related to SPS while on Hizentra®, and one patient had improvement in seizures. One patient discontinued Hizentra® in favor of intravenous immunoglobulin (IVIG) due to physician preference. The most common side effects were local reactions including pain, pruritus, and redness. No serious adverse events were reported.
Conclusions: Hizentra® was associated with improved symptoms in SPS in both patients including decreased spasticity, and improved seizure frequency in one patient. Serum anti-GAD levels did not decrease following administration of Hizentra. Hizentra® was well tolerated in patients with SPS, with most side effects reported as mild. Hizentra® may be considered as an alternative to IVIG treatment in patients with SPS.

**(160) Submission ID#421179**

**Successful use of abatacept to treat severe autoimmune enteropathy in patients with combined immune deficiency.**

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Introduction/Background: Combined immunodeficiency (CID) has been associated with a spectrum of secondary gastrointestinal manifestations, including infectious and non-infectious causes. Abatacept, a soluble CTLA4-IgG1 fusion protein targeting T cell activation, has demonstrated benefit in the treatment of autoinflammatory manifestations in patients with CID due to underlying heterozygous germline mutations in CTLA4 and LRBA.
Objectives: Herein, we report two patients with CID characterized by low immunoglobulin levels, low class-switched memory B cell counts, and low peripheral naïve CD4+ T cell counts. Both patients suffered from severe and persistent diarrhea complicated by protein-wasting and malnutrition, ultimately diagnosed as biopsy-consistent autoimmune enteropathy. The clinical history of patient 1 was also notable for granulomatous lung disease and autoimmune cytopenias.
Methods: A T-regulatory cell disorder was considered in both cases, however, CTLA4 and LRBA were found to be unaffected by whole exome sequencing (patient 1) and/or flow cytometry (patient 2). Due to progressive worsening of the autoimmune enteropathy despite management with chronic steroids (both patients), combination rituximab/mycophenolate mofetil (patient 1), and total parenteral nutrition (TPN) complicated by recurrent infections (patient 2), abatacept was trialed at 125 mg SC weekly.
Results: In both patients, the start of abatacept therapy produced a dramatic improvement as measured by decreased stool frequency, improved weight gain, and decreased protein-wasting. Patient 1 has been

maintained on this monotherapy for over one year, with improvement in the gastrointestinal as well as pulmonary autoinflammatory complications. The only documented adverse event has been hepatitis B reactivation, managed with tenofovir and abatacept continuation. Patient 2 has required azathioprine/abatacept combination therapy for clinical stabilization and is without a significant adverse event to date.
Conclusions: We conclude that abatacept may be a relatively safe and effective therapeutic in the management of severe autoimmune enteropathy in the background of CID, even when used outside of the classical clinical context of CTLA4 or LRBA haploinsufficiency.

**(161) Submission ID#425318**

**Suppressive effects of SG-SP1 on mast cell-mediated allergic inflammation via regulation of FcRI signalling**

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Introduction/Background: Mast cells play important roles on allergy thorough the secretion of allergic mediators.
Objectives: As increasing the outbreak of allergic diseases, study for treatments comes to the force. In this research, we aimed to assess the effects of SG-SP1, a derivative of gallic acid, on mast cell-mediated allergic inflammation using various animal and in vitro models.
Methods: Ovalbumin-induced systemic anaphylaxis and immunoglobulin E (IgE)-induced passive cutaneous anaphylaxis are standard animal models for immediate-type hypersensitivity. Oral administration of SG-SP1 hindered the allergic symptoms in both animal models. These inhibitions were deeply related to the reductions of histamine and interleukin-4.
Results: SG-SP1 reduced degranulation of mast cells and expression of inflammatory cytokines in a dose dependent manner. SG-SP1 showed better anti-allergic effects compared to gallic acid and dexamethasone. Down regulations of intracellular calcium level and nuclear factor-B activation by SG-SP1 were causative of the reduction of allergic mediators. To anticipate the exact target of SG-SP1, phosphorylation of proteins involved in mast cell signalling was assessed. SG-SP1 suppressed the activations from Lyn and was aggregated with high affinity IgE receptor (FcRI).
Conclusions: From these results, we assured that SG-SP1 directly interact with FcRI. All together, we propose that SG-SP1 might be a therapeutic candidate for allergic disorders.

**(162) Submission ID#425361**

**Systematic assessment of pain in patients with primary immune deficiency using validated pain questionnaires: A prospective study.**

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Introduction/Background: The number of primary immunodeficiency (PID) patients is rising dramatically because of good medical care as well as increased awareness. Around 1 in 1200 live births are affected. More than 300 disorders have been discovered so far and this number is expected to rise in the coming years. As with any other chronic illness, PID patients are also prone to acute and chronic pains. Chronic pain is a big challenge and lack of understanding of the etiology and underlying mechanisms limit our ability to diagnose or treat it effectively.
Objectives: To systematically assess chronic pain in patients with PID using validated questionnaires and to try to understand the underlying mechanisms of neuropathic versus non neuropathic pain.
Methods: Short-form McGill pain questionnaire (SF-MPQ) is a recognized way to ascertain different pain characteristics as well as severity. A validated Arabic version of the SF-MPQ was used to prospectively assess chronic pain in patients with PID. Furthermore, a validated Arabic version of the Neuropathic Pain Questionnaire-Short Form (NPQ-SF) was also used to assess neuropathic pain and to differentiate it from non-neuropathic pain. A total of 27 patients with PID were included.
Results: Males: females were 33.3%: 66.6% respectively. Mean age was 29.73 years. Commonest diagnosis was Combined Immune deficiency in 37% of the patients followed by Common Variable Immune deficiency which was 25.9 %. Chronic pain was found in 64% of the patients that participated in the study. 50% of the patients who complained of pain found it to be tiring and exhausting. 35% each had aching or heavy or sharp pain. 28% had cramping pain, 25% had tender pain and 21% characterized their pain as throbbing or burning. 18% felt shooting pain, 14% had splitting pain and 7% had gnawing pain.
28% found it to be frightening and 17% described it as being sickening in itself. The commonest pain complaint was abdominal pain in 14% of the patients followed by headache 11 % and chest pain 11%.
Pain attributed to neuropathy was present in about 17.8% of the study population. Most patients described that they had been experiencing pain for at least 2-3 years.
Conclusions: This is the first international study to understand the prevalence, duration and severity of chronic pain among PID patients. A significant number of patients reported ongoing pain. This is the first time any kind of pain has been studied systematically in the patients with Primary Immune deficiency. Treating pain should have a major impact on improving the patients quality of life.

**(163) Submission ID#420314**

**Tap Water - a nosocomial source of rapid growing nontuberculous mycobacteria**

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Introduction/Background: Primary Immunodeficiency (PIDD) patients, particularly those with severe T cell defects, are at increased risk of infections. Bone marrow transplant also contributes to significant T cell lymphocytopenia, which can be associated with similar risks. Several prophylactic measures, which vary per institution, are placed on these patients in order to prevent infections. We report on a likely outbreak of rapid growing nontuberculous mycobacteria (NTM) at our institution, with a suspected association to tap water
Objectives: To recognize nontuberculous mycobacteria as a threat to patients with immunodeficiency disorders
Methods: Case series of consecutive patients admitted to one institution with similar infections
Results: In a period of 16 months, 4/2016 to 8/2017, 5 patients admitted to our institution were found to have rapid growing NTM species on central line culture or bronchoalveolar fluid. These cultures were obtained due to fever and symptoms of systemic infection. Per institutional practices no

peripheral blood samples were obtained at the time of initial culture. All had significant T cell dysfunction: Wiskott-Aldrich syndrome (1), an undefined combined immunodeficiency (1), and three patients post bone marrow transplantation (BMT), one 6 months (173 days) post an unrelated cord blood transplant for Farber syndrome, one 120 days out of a matched related transplant for Epstein Barr Virus (EBV)-associated lymphoproliferative disorder, and one patient with high risk neuroblastoma 106 days out of his second tandem autologous transplant. Both allogeneic transplant recipients had received serotherapy. All species were rapid growing, identified as Mycobacterium mucogenicum (n=3), immunogenum (n=1), or abscessus-chelonae complex (n=1).
The patients had not shared rooms, caregivers, invasive procedures, or medications from the same batch. Three (60%) cases met CDC criteria for hospital acquired infections (HAI). All patients were in general ward rooms, and were on standard precautions given diagnosis had not been established (n=2) or they were considered outside the typical window of strict BMT precautions (n=3). NTM species were subsequently isolated from hospital tap water. This has resulted in a significant increase in infection precautions, including the use of sterile water only for cares, as well as bottled water for drinking (with no use of ice machines), for all patients being evaluated for PIDD or with significant lymphocytopenia.
Conclusions: NTM are a threat to patients with PIDD. Tap water is a potential source of mycobacterial infections in PIDD patients. Minimizing exposure risk to water sources containing NTM is very important in this population. Patients with concern for PIDD or significant T cell lymphocytopenia should take steps to avoid NTM exposures, including the use of sterile water for cares, and bottled water for drinking.

**(164) Submission ID#398872**

**Ten Year Old Girl with Purine Nucleoside Phosphorylase Deficiency**

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Introduction/Background: Purine nucleoside phosphorylase (PNP) deficiency is a rare autosomal recessive condition leading to severe combined immunodeficiency and neurologic impairment, typically presenting in early childhood. The condition is progressive and typically fatal in the first or second decade of life without hematopoietic stem cell transplantation (HSCT).
Objectives: To illustrate the clinical and laboratory presentation of PNP deficiency with a novel mutation in the PNP gene via a case study.
Results: Case: A four year old female of Hispanic descent, with a past medical history of spastic diplegia, initially presented with chronic nasal congestion, recurrent sinusitis, and cough. Laboratory studies were significant for an ALC 500 cells/mcL (2000-8000 cells/mcL). She was lost to follow-up for two years, before returning to medical attention for pneumonia. Evaluation of lymphocyte subsets at age 6 years revealed CD3+ 145 cells/mcL (1200-2600 cells/mcL), CD4+ 119 cells/mcL (650-1500 cells/mcL), CD8+ 28 cells/mcL (370-1100 cells/mcL), and CD19+ 44 cells/mcL (270-860 cells/mcL). T cell mitogen stimulation to PHA measured by flow cytometry was 30% of control. PNP activity was nearly absent and urine guanosine and inosine were significantly elevated. Gene sequencing revealed a homozygous c.655A>G mutation in the PNP gene. Prophylactic antimicrobials were started. Despite strong recommendation for HSCT, the patient was again lost to follow up until age 10 years, at which time she was found to have progressive lymphopenia, bronchiectasis, and developmental delay. T cell mitogen stimulation to PHA measured by thymidine uptake assay was 9% of control and PNP functional activity was undetectable. The patient underwent a 9/10 matched unrelated HSCT six years after her initial presentation. One year post-transplant, the patient continues on immunoglobulin replacement therapy. Her course was complicated by cutaneous GVHD, treated successfully with topical corticosteroids.
Conclusions: This case study illustrates the progressive nature of PNP deficiency in the first decade of life. Our patient is notable in that she survived without significant medical intervention to the age of 10 years. Her presentation at age 4 years was not unlike those previously reported in the literature, with muscle spasticity, ataxia, and recurring bacterial infections. To the authors knowledge, this case reports a novel mutation in the PNP gene.

**(165) Submission ID#419999**

**The Clinical, Laboratory, Molecular Characteristics and Remission Status in Children with Severe Congenital Neutropenia and Severe Idiopathic Neutropenia**

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Introduction/Background: Severe congenital neutropenia (SCN) is a primary immunodeficiency disease characterized by early onset recurrent infections, persistent severe neutropenia and congenital genetic defect. Severe idiopathic neutropenia (SIN) is a rare disease defined by persistent severe neutropenia, in the absence of an identifiable etiology.
Objectives: Here, we aim to find out clinical, laboratory, genetic characteristic and remission status in children with SCN and SIN in Chinese population.
Methods: In this study, we enrolled 39 Chinese children who experienced severe neutropenia longer than 6 months without any virus infection or auto-immune antibodies from June 2008 to July 2017 in hospitals affiliated to Shanghai Jiao Tong University School of Medicine. Their clinical, laboratory and molecular characteristics were analyzed and the patients were followed up to observe their remission status. Targeted gene capture combined with next-generation sequencing technology was used to find out related gene mutation.
Results: Patients in this study had a mean age of 29.21±27.94 months. Molecular analysis revealed that 7 patients had associated mutations of SCN, including ELANE and G6PC3. Among 26 patients with continuous follow-up, one died for unknown reason. Ten patients have recovered from SIN (R-SIN) with mean neutropenia duration of 19.40±10.91 months. SCN patients had more frequent infection (5.86±1.57 times per year) than SIN (3.95±1.05 times per year, P=0.008) and R-SIN patients (P=0.005, 3.89 ±1.27 times per year). SCN patients had significantly higher count of ANC and monocytes than SIN (P=0.015) and R-SIN patients (P=0.029). However, there was no difference in ANC and monocytes counts between SIN and R-SIN patients. Bone marrow examinations demonstrated a myeloid maturation arrest at the myelocyte-metamyelocyte stage in SCN patients, while most of SIN and R-SIN patients were normal.
Conclusions: Our study indicated that, patients with mild infection, lower ANC, monocytes count and normal bone marrow are likely to be SIN. Whereas others with relatively more severe infection, higher ANC, monocytes count and maturation arrest in bone marrow are inclined to be SCN.

**(166) Submission ID#428516**

**The Effects of BMI and Route of Administration on Efficacy of Immunoglobulin G Replacement Therapy**

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Introduction/Background: Patients with qualitative and/or quantitative defects of humoral immunity often require immunoglobulin (IgG) replacement therapy (IGRT). Usual starting doses range between 400-600 mg/kg and the dose is adjusted as needed depending on the patient. There is a paucity of information about whether and how extreme BMI (obese or underweight) and route of administration may affect a patients rate of infections.
Objectives: The objective of the study is to determine whether rate of infection is associated with BMI, dose or route of administration in patients receiving IGRT.
Methods: This is a retrospective chart review from December2000-October 2017. We included patients between the age of 1 month and 100 years old who were evaluated initially and had at least one follow up evaluation. Data reviewed included the route of administration, dose, infection history, IgG serum levels, height and weight to calculate BMI, gender, age and diagnosis requiring immunoglobulin replacement therapy. Participants were excluded if the type of immunodeficiency is unknown or if the participant had incomplete data for the requested data fields. The number of infections between visits was modeled using Poisson regression as a function of dose, route of administration and the BMI with the log of the follow up interval as an exposure offset.
Results: Eighty-five patients were eligible, and preliminary results for 50 patients are presented here. The mean infection rate per 100 weeks of follow up was 1.9 in obese patients, 3.0 in overweight patients and 1.5 in underweight/normal weight patients adjusted for route of administration; however these rates do not differ significantly from one another. The mean infection rate per 100 weeks of follow up differed by administration route; 1.4 infections for IVIG versus 3.1 infections for SCIG when adjusting for BMI ($P<0.0131$). The mean infection rate per 100 weeks of follow up was not associated with dosage.
Conclusions: Overweight patients may experience more infections than obese or underweight patients, regardless of administration route. IVIG patients may have a lower rate of infections compared to SCIG patients regardless of BMI. Work is ongoing to complete analyses for the remainder of the eligible patient population.

**(167) Submission ID#421931**

**The evaluation of malignancies in Turkish PID patients; A multicenter study**

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Introduction/Background: Primary immunodeficiencies (PIDs) are a rare group of genetic disorders associated with a tendency to infectious diseases and an increased incidence of cancer. There is no data regarding the prevalence of malignancies in patients with PID in Turkey.
Objectives: In this study, we aimed to evaluate patients who diagnosed as PIDs and developed malignancies, and calculate the estimated frequency of malignancies associated with PIDs in Turkey.
Methods: Forty five patients who were diagnosed with malignancy in the follow-up period of PID at the four tertiary immunology clinics between 1992 and 2017 years were included in the study. The data were obtained retrospectively from the hospital records and the database of ESID online patient registry.
Results: The prevalence of malignancies in patients with PID was found as 1.05% (45/4285). The male to female ratio of the patients was 29/16, the median age was 13.5 years (minimum: 1.5, maximum: 57) and the median age at which patients get diagnosed with malignancy was 9 years (minimum: 1.5, maximum: 51). There was no cancer history in their family members. The most common type of PID which associated with malignancy was ataxia telangiectasia (n=15, 33.3%). Non-hodgkin lymphoma was the most common malignancy (n = 25, 55.5%) in our group (Table 1). EBV quantitative PCR was positive in 6 lymphoma cases (17.6%). The median number of x-rays and CT scans in patients with AT and Bloom syndrome before malignancy developed were 5 (minimum: 1, maximum: 24) and 1 (minimum: 0, maximum: 3), respectively. Two cases had dual malignancies (papillary thyroid cancer and anal adenocancer). Twenty cases were treated with chemotherapy, 7 cases with hematopoietic stem cell transplantation, 5 cases with radiotherapy, and 5 cases with surgical treatment, treatment information of 17 patients was not reached. Remission was detected in 16 cases, while resistance to therapy in 2 cases and recurrences in two patients were observed. Four patients are still on chemotherapy. Twenty cases died.

Table 1. Primary immunodeficiencies associated with malignancies

| PID | Malignancy | Subgroup of malignancy | n |
|---|---|---|---|
| Ataxia telengectasia | Non HL | B cell | 7 |
| | | T cell | 2 |
| | | Anaplastic large cell | 1 |
| | | unknown | 1 |
| | HL | | 3 |
| | Thyroid papillary cancer | | 1 |
| Bloom syndrome | Non HL | B cell | 1 |
| CD27 deficiency | Non HL | Burtkitt lymphoma | 1 |
| | HL | | 1 |
| CVID | Non HL | B cell | 2 |
| | | Unknown | 1 |
| | HL | | 5 |
| | Glioblastoma, and secondary anal adenocancer | | 1 |
| | AML | | 1 |
| DOCK8 deficiency | Non HL | B cell | 2 |
| | Skin squamouse cell carsinoma | | 1 |
| | Small bowel sarcoma | | 1 |
| | Small bowel plasmacytoma | | 1 |
| WAS | Non HL | B cell | 1 |
| | Hemangioperiostoma | | 1 |
| HIGM | Non HL | Burkitt lymphoma | 1 |
| | | Unknown | 1 |
| | AML | | 1 |
| | Glial tumor | | 1 |
| | Schwannoma | | 1 |
| Lymphoproliferative disease (XLP n = 2, ALPS n = 1) | Non HL | B cell | 2 |
| | | Burkitt lymphoma | 1 |
| ZNF341 | Malignant small round cell tumor in nasal cavity, and secondary thyroid papillary cancer | | 1 |
| PNP Deficiency | Non HL | T cell | 1 |
| | | | Total=45 |

CVID: Common variable immun deficiency, DOCK8: Dedicator of cytokinesis 8: HIGM: Hyperimmunoglobulin M, ZNF: zinc finger transcription factor, XLP: X linked lymphoproliferative disease, ALPS: Autoimmune lymphoproliferative syndrome, HL: Hodgkin lymphoma, AML: Acute myeloid leukemia

Conclusions: The tendency of malignancy in patients with PIDs is due to the deficiency in the immune response that lead to failed surveillance against oncogenic viruses, premalignant/malignant cells, or both. Lymphoid malignancies are the most common malignancies associated with PIDs. PIDs-associated malignancy incidence has increased in recent years because of that improved survival of the patients. This study is the largest cohort investigating the association of malignancy in patients with PID in Turkey. Additionally, we first reported tendency to malignancy in a patient with ZNF 341 deficiency.

**(168) Submission ID#409693**

**The Evolving Phenotypes of Forkhead Box P3 Mutations**

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Introduction/Background: Next-generation sequencing (NGS) has become an integral tool in the evaluation of primary immunodeficiency disorders (PID). We describe a patient with a previously described pathogenic FOXP3 variant who met clinical and laboratory criteria for CVID.
Objectives: Describe a patient with a previously described pathogenic FOXP3 variant who met clinical and laboratory criteria for CVID.
Results: Case description: An 8-year-old male born premature at 32 weeks presented with a history of recurrent infections. Family history was negative for immunodeficiency. The patient developed recurrent acute otitis media beginning at 1 year of age, three episodes of pneumonia beginning at 3 years of age, and recurrent sinus infections requiring treatment on average four times a year beginning at 5 years of age. Initial immunologic evaluation at age 8 was notable for: IgG 305 mg/dL (reference 673-1734 mg/dL), IgM 15 mg/dL (reference 47-311 mg/dL), IgA 173 mg/dL (reference 41-368 mg/dL), IgE 166 IU/mL (reference 0-90 IU/mL). Lymphocyte subpopulations were normal. Specific responses to vaccines showed: protective antibody titers to Diphtheria, but not to Tetanus or Pneumococcal antigens. He did not respond to booster vaccination and was started on IVIG with significantly reduced frequency of infections.
At age 10, while on IVIG, he developed oral ulcers (biopsy consistent with ulcerative eosinophilic granuloma), abdominal pain, and recurrent arthralgias involving ankles, elbows, hips and the sacroiliac joint. Magnetic resonance imaging (MRI) was consistent with sacroilitis. Subsequent imaging was consistent with chronic relapsing osteomyelitis (CRMO). Gastrointestinal biopsies showed severe active chronic pangastritis with antralized oxyntic gastric mucosa with enterochromaffin cell hyperplasia; suggestive of autoimmune gastritis. Plasma cells were present throughout the gastrointestinal tract. NGS (BCM-NGS, Baylor Miraca Genetics Laboratory, Houston TX) identified a hemizygous pathogenic missense variant, c.1190G>A (p.R397Q) in the X-linked FOXP3 gene, that has been reported previously in patients with immunodysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome.
Flow cytometry studies showed a decreased percentage of Treg cells of total CD4 expressing cells, 2.6% (reference 4.2-9.9%), but normal FOXP3 expression within these cells, 68% of Treg cells with FOXP3 expression (reference 55-81%). Interestingly, Treg cell subset phenotyping obtained at the same time showed a normal percentage of natural Tregs, 3.2% CD4 T cells (reference 0.7-4.0%), as well as normal percentage of naive Tregs, 5.6% CD4 T cells (reference 0.6-9.0%).
Up to this point, he had not had any signs of diabetes, thyroid disease or frank enteropathy involving the small or large intestine.
Conclusions: We report a pathogenic FOXP3 variant, occurring in a patient with a CVID-like phenotype, autoimmune gastritis, and an association with CRMO. This case demonstrates the increasing utility of NGS, which can profoundly impact prognostic and therapeutic considerations.

**(169) Submission ID#413429**

**The natural history of patients with profound combined Immunodeficiency (PCID): Interims analysis of an international prospective multicenter study.**

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Introduction/Background: We present our data on behalf of the PCID study consortium of the Inborn Errors Working Party (IEWP):
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Profound combined immunodeficiencies (P-CID) are inherited diseases with impaired T-cell function leading to infections, immune dysregulation or malignancies. Genetic, immunologic and clinical heterogeneity make patient specific decisions on indication and timing of hematopoietic stem cell transplantation (HSCT) difficult.
Objectives: Since 2011 the PCID study recruits non-transplanted P-CID patients aged 1-16 years to prospectively compare natural histories of age and severity-matched patients with or without subsequent transplantation and to determine whether immunological and/or clinical parameters may be predictive for outcome. We report on the clinical, immunological and treatment data from so far recruited patients (>130).
Methods: Our prospective/retrospective international observational multicenter study recruits pediatric P-CID patients to identify biomarkers and clinical parameters that are predictive of outcome.
Results: So far >130 patients have been recruited. About 1/3 of patients have a genetic diagnosis of atypical SCID and 1/3 of CID. In another 1/3 of patients the molecular diagnosis is not yet identified. The majority of analyzed patients had < 10% naïve T-cells, reduced/absent T-cell proliferation and at least one significant clinical event per year, demonstrating the profound

immunodeficiency in this patient group. So far, 48 patients were transplanted after enrolment, overall 19 patients died (9 in the HSCT group, 10 in the non-HSCT group). Analysis of the HSCT decisions revealed the divergent decisions in patients with similar disease burden, favoring an ongoing prospective matched pair analysis of patients with similar disease severity with or without transplantation. So far, neither the genetic diagnosis nor simple measurements of T-cell immunity emerged as good predictors of disease evolution.
Conclusions: The P-CID study for the first time defines and characterizes a group of patients with non-SCID T-cell deficiencies from a therapeutic perspective. Since genetic and simple T-cell parameters provide limited guidance, prospective data from this study will be an important resource for guiding the difficult HSCT decisions in P-CID patients.

**(170) Submission ID#419587**

**The plasma contact system and its role in common variable immunodeficiency: an explorative study.**

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Introduction/Background: A growing body of evidence suggests that the contact system is involved in the activation of various vascular and immunological pathways and acts as an interface to help regulate allergic reactions, coagulation, complement, innate immunity and inflammation. As demonstrated in mice experiments, contact activation and high molecular weight kininogen (HK)-derived peptides increased homing of T and B lymphocytes into lymph nodes, which suggests an important area of research for understanding the contact systems role, specifically FXII, in immune-mediated inflammation and immune dysregulation. This novel mechanism prompted further inquiry into its role of various human disease states characterized by inflammation. Plasma HK cleavage has been proposed as a useful and minimally invasive biomarker in various inflammatory disease states. This pathway has not been explored in CVID, in which inflammatory complications are found in one-third of patients with an unidentified genetic cause. Characterizing the contact system biomarkers in CVID patients could elucidate a role in pathogenesis.
Objectives: Assess the presence of contact activation at baseline in sera from CVID patients with and without inflammatory complications compared to healthy controls.
Methods: CVID patients were recruited in the outpatient setting and the measurement of cleaved plasma HK (cHK) levels was determined by Western blot analysis, under reducing conditions, with quantitation of total and cHK bands using an Odyssey imaging system (Licor). A one-way ANOVA test for differences among the 3 studied groups will be applied. C1 inhibitor levels, C3 and C4 levels and high-sensitivity CRP were also measured as comparable biomarkers for inflammation.
Results: To date, 9 CVID patients were studied, 7 with and 2 without inflammatory complications. Repeated determinations of cleaved HK% (cHK%) revealed an average of 1.20% (Range: 0.46-2.66%) in CVID patients with inflammatory complications and those without complications averaged 1.07% (Range: 0.79-1.35%). Healthy controls had an average cHK of 1.15% (N=10, Range: 0.60-2.10%).

| Study ID | Age (years) | Gender | Avg. Cleaved HK % | C1 inhibitor (mg/dL) [Ref: 21-30] | C3 (mg/dL) [Ref: 88-201] | C4 (mg/dL) [Ref: 10-40] | hs-CRP (mg/L) [Ref: <3.0] |
|---|---|---|---|---|---|---|---|
| *CVID with inflammatory complications* | | | | | | | |
| 201-1 | 54 | M | 0.46% | 32 | 124 | 32 | 0.7 |
| 202-1 | 48 | M | 0.93% | 35 | 135 | 25 | 2.5 |
| 203-1 | 55 | F | 0.77% | 34 | 143 | 60 | 27.0 |
| 204-1 | 29 | M | 2.66% | 27 | 90 | 21 | 4.5 |
| 205-1 | 52 | F | 0.76% | 30 | 145 | 38 | 0.9 |
| 206-1 | 45 | F | 0.79% | 22 | 111 | 37 | 5.9 |
| 207-1 | 68 | F | 2.03% | 27 | 131 | 44 | 3.1 |
| *CVID without inflammatory complications* | | | | | | | |
| 101-1 | 47 | F | 0.79% | 26 | 156 | 34 | 13.5 |
| 102-1 | 51 | F | 1.35% | 24 | 89 | 33 | 0.5 |
| *Controls* | | | | | | | |
| 301-1 | 35 | F | 0.90% | N | N | N | N |
| 302-1 | 40 | F | 0.69% | N | N | N | N |
| 601-01 | 28 | M | 0.94% | N | N | N | N |
| 602-01 | 43 | F | 0.82% | N | N | N | N |
| 603-01 | 50 | M | 0.85% | N | N | N | N |
| 604-01 | 55 | M | 1.30% | N | N | N | N |
| 605-01 | 43 | F | 2.10% | N | N | N | N |
| 606-01 | 60 | F | 0.60% | N | N | N | N |
| 608-01 | 32 | F | 2.15% | N | N | N | N |
| 609-01 | 34 | M | 1.25% | N | N | N | N |
| 610-01 | 22 | M | 1.10% | N | N | N | N |

N= within normal limits

Conclusions: Cleaved kininogen detected in the sera of CVID patients was found at similar levels compared to healthy controls (cHK<5%). Findings suggest that systemic activation of the contact system might be absent in CVID, however, future considerations include developing detection methods for local tissue activation.

**(171) Submission ID#428729**

**The underlying primary immunodeficiencies and lung diseases, and low CD3 and CD4 counts are associated with recurrent pneumonia in HIV negative lymphopenia patients.**

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Introduction/Background: Lymphopenia can be considered as primary or acquired immunodeficiency. Lymphopenia is associated with a considerable increase in susceptibility to infections and the treatment focus for lymphopenia is mainly consists of prophylaxis and treatment of opportunistic infections. AIDS is the most well known cause of acquired lymphopenia and the CD4 count serves as an effective surrogate marker for disease progression and guideline for prophylaxis for HIV positive lymphopenia patients (HPLP). HIV negative lymphopenia patients (HNLP) have been following the same prophylaxis guideline for HPLP patients. However, it is unclear whether same prophylaxis guideline will be appropriate for both groups since the underlying immune mechanisms are different between these two groups.
Objectives: We aimed to define the optimal treatment and prophylaxis guideline for HNLP. In this study, we compared the clinical phenotypes and absolute counts of lymphocyte subsets between HNLP with recurrent pneumonia (PNA), recurrent upper respiratory infection (URI) and no pulmonary infection.
Methods: Electronic medical records of HNLP (n=29) seen at an academic immunology clinic between the year of 2012 and 2017 were reviewed retrospectively. Lymphopenia was defined as absolute CD3 count less than 1000/μl. The age, absolute counts of CD3, CD4, CD8, CD19, NKT and NK cell counts, history of antibiotic or antiviral prophylaxis use, autoimmune disease, lung disease, immunosuppressive therapy use, hypogammaglobinemia and IVIG therapy use were compared between patients with recurrent PNA (n=8), recurrent URI (n=13) and no pulmonary infection (n=8).

Results: This study showed that patients with recurrent PNA had significantly lower absolute CD3, CD4, NK cell counts and age compared to the patients with recurrent URI (Table 1). None of the clinical phenotype was significantly different between these two groups. When we compared the patients with recurrent PNA vs no infection, all lymphocyte subset counts and age were similar between these two groups except the frequency of underlying lung disease which was significantly higher in the recurrent PNA group (Table 1). Lastly, we grouped the recurrent PNA and URI groups together as the pulmonary infection group. When we compared the pulmonary infection group with the no infection group, lymphocyte subset counts were not significantly different, however, infection group showed significantly higher incidence of PID and the trend of lower rate of IS therapy use than the no infection group (Table 1).

| | PNA (n=8) | URI (n=13) | No infection (n=8) | P- value |
|---|---|---|---|---|
| age | 43.3 ± 14.7 | 64.2 ± 6.8 | 47.5 ± 15 | 0.004*, 0.017** |
| CD3 | 419.5 ± 100.1 | 650.7 ± 148.4 | 564.3 ± 217.1 | < 0.001* |
| CD4 | 230.5 ± 108.4 | 451.3 ± 203.1 | 353.9 ± 177.1 | 0.007* |
| NK | 108.8 ± 72.1 | 215.7 ± 136.1 | 118.8 ± 90.3 | 0.037* |
| Lung disease | 5: asthma/ BRR (3), ILD/ BRR (1), CF (1) | 4: asthma (2), asthma/BRR (1), ILD/BRR (1) | 1 (ILD) | 0.032*** |
| PID | 4: (ICL (2), CVID on IVIG (2) | 10: ICL (1), Goods syndrome on IVIG (1), CVID on IVIG (8) | 1 (CVID on IVIG) | 0.009# |
| Autoimmune | 5: SLE (1), GPA (1), sarcoidosis (1), UC (1), ITP (1) | 7: SLE (2), RA (1), SS (1), Crohn's (1), MC (1), MS (1) | 4: GPA (1), SS (1), Crohn's (2) | |
| Malignancy | None | 2: Ovarian CA (1), Breast CA (1) | 2: breast CA/ lymphoma (1), lymphoma (1) | |
| IS therapy | 5: Chronic steroid (2), steroid/Tacrolimus (1), Steroid/Tacrolimus/MMF(1), CTX/AZA/steroids/RTX (1) | 5: Chronic steroid (2), MTX/steroid (1), Mepolizumab (1), HCQ (1) | 7: RTX/steroid (3), CTX/AZA/steroid/ RTX (1), 6MP/antiTNF/steroid (1), CTX/lenalidomide/steroid (1), cyclosporine (1) | 0.051## |

**Table 1.** Absolute counts of CD3, CD4 and NK cells are shown as mean ± standard deviation. Statistically significant by student t test between PNA and URI groups (*), URI and no infection groups (**), and PNA and no infection groups (***), by chi square test between PNA and no infection groups (#).*P*-value between pulmonary infection (PNA and URI) and no infection (##). Abbreviations: Granulomatosis with polyangiitis (GPA), Bronchiectasis (BRR), Interstitial lung disease (ILD), Cystic Fibrosis (CF), Systemic Lupus Erythematosus (SLE), Ulcerative Colitis (UC), Idiopathic thrombocytopenia (ITP), Rheumatoid arthritis (RA), Sjogren's syndrome (SS), Microscopic colitis (MC), Multiple Sclerosis (MS), hydroxychloroquine (HCQ), rituximab (RTX), cyclophosphamide (CTX), azathioprine (AZA), mycophenolate mofetil (MMF).

Conclusions: The initial study has several limitations. This was a retrospective study from a single clinic and patient population was limited to 29 patients. Despite these limitations, we believe that this study provides valuable messages regarding prophylaxis guideline for pulmonary infection in NHLP; the underlying PIDs including ICL and CVID, lung diseases particularly bronchiectasis and the absolute CD3 and CD4 counts less than 500/μl and 300/μl, respectively, are associated with recurrent PNA and the patients with these risk factors likely will benefit from antibiotic prophylaxis. In addition, patients with acquired lymphopenia due to chronic use of IS therapies without underlying PID or lung disease less likely develop pulmonary infection despite of low CD3 and CD4 counts. Further investigations are crucial to elucidate the clinical significance of our initial observation by increasing the patient population and analysis of detailed immunologic and genetic profile of these patients which will likely reveal immunological markers and genes that are involved in the pathogenesis of both primary and HIV negative acquired lymphopenia.

**(172) Submission ID#404472**

**Title: If You Do Not Think of It, You Will Not Look for It, GATA2 Mutation Diagnosis Triggered by Immunohematological Profile**

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Introduction/Background: GATA2 is a hematopoietic transcription factor, required for the development and maintenance of a healthy stem cell pool. Heterozygous mutation of GATA2 has been associated with different but overlapping syndromes affecting both myeloid and lymphoid cell lines including aplastic anemia, myelodysplastic syndrome, acute myeloid leukemia, pulmonary alveolar proteinosis and lymphedema. It is also associated with immunodeficiency and susceptibility to mycobacterial, fungal, and viral infections. Hematopoietic stem cell transplantation (HSCT) is the only available cure which should ideally occur before patients develop neoplasia, severe infections or lung disease.
Objectives
- To describe the clinical presentation of a genetically confirmed case of GATA2 mutation
- To describe the characteristic hematological and immunological profile of patients with GATA2 mutation
- To emphasize the importance of high index of suspicion and early diagnosis in improving outcome with HSCT
Methods: Case: A 17-year-old female presented with prolonged fever, fatigue, nonspecific rash, and unremarkable clinical exam. Laboratory evaluation was significant for mild pancytopenia with profound monocytopenia. Bone marrow analysis was remarkable for hypocellularity without evidence of dysplasia or malignancy. Peripheral blood flow cytometry showed decreased T- and B- cells and absent NK cells.
Results: The constellation of pancytopenia, marked monocytopenia and absent NK cells, were suggestive of GATA2 deficiency. Sanger gene sequencing of GATA2 revealed a heterozygous nonsense mutation (p.Arg337*).
Conclusions: Mutation of GATA2 is the underlying defect in overlapping clinical syndromes and is associated with immunodeficiency and malignant predisposition. Incidence of organ dysfunction, infections and neoplasia increases with age. Confirming the diagnosis during the phase of marrow hypocellularity/monocytopenia and pursuing HSCT prior to malignant transformation may improve patient outcome.

**(173) Submission ID#428228**

**Uncovering Primary Immune Deficiency among children in North Kerala A bumpy ride**

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Introduction/Background: Reduction in child mortality has kept pace with improved immunization and nutritional status. However, there are children with severe infections who have no identifiable reason. The search for children with PID was initiated in a tertiary care referral hospital from a region with no documentation of the prevalence of this disorder, but with a high rate of consanguinity. Financial constraints, poor availability of laboratory facilities or therapeutic options locally, limited awareness among pediatricians and vast distances between premier centres were major obstacles.
Objectives
To identify children with primary immune deficiency and study the spectrum of PID in North Kerala
To prevent infectious and other complications in these children
To provide curative therapy whenever feasible

Methods
1. Acquisition of knowledge and skills to diagnose and manage PIDs
2. Establishment of an immune deficiency clinic
3. Liaison with centres across the country offering diagnostic tests and stem cell transplant
4. Formation of project team and submission of a project to the Central Government
5. Improvement of diagnostic facilities at home institution
Results: 180 children with recurrent, severe or persistent infections or with two or more ESID warning signs were screened for PID. Severe Combined Immune Deficiency was diagnosed in 3 children, Wiskott - Aldrich syndrome in 5 children, Chronic Granulomatous Disease in 6 children and X linked Agammaglobulinemia in 11 children and Leucocyte Adhesion Deficiency in 3 children. Prophylaxis with IVIG was initiated in 22 children and stem cell transplant was done for 8 children.
Conclusions: In a country with resource constraints, limited awareness among health care providers and vast distances, it is possible to make a difference to the lives of families of children with PID by networking across centers with expertise in immunological and molecular genetic diagnostic methods and life - saving therapeutic modalities like stem cell transplantation.

**(174) Submission ID#420941**

**Unmanipulated Matched Sibling Donor Hematopoietic Cell Transplantation (HCT) in an Infant with SCID caused by TBX1 mutation as a life saving therapy A Clinical Update**

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Introduction/Background: Complete thymic aplasia is a rare cause of SCID, and requires thymic transplantation for curative treatment. Because thymic transplantation is not widely available, there can be significant delay between diagnosis and curative therapy placing the infant at risk of invasive life threatening infections.
Objectives: Describe modalities of therapy for adenoviremia in a patient with SCID due to thymic aplasia.
Methods: Chart review was performed. Treatment including HLA-partially matched third party cytotoxic T cell therapy and matched related hematopoietic cell transplant were assessed for treatment of life threatening adenoviremia in a SCID patient with thymic aplasia.
Results: Case Presentation
We identified an infant with a mutation in Tbox transcription factor 1 (TBX1) (c.1176_1195dup20) by way of state newborn screening for SCID. She had severe T cell lymphopenia and abnormal T cell function. At 7 months age, she developed adenoviremia with associated fulminant hepatitis, and an initial viral load of 5 million copies/mL. Despite prolonged therapy with cidofovir, viral load increased to as high as 264 million copies/mL. Treatment with two infusions of partially HLA-matched third-party cytotoxic T cells specific to adenovirus (5/10 and 4/10 HLA matched, with HLA class II-mediated antiviral restrictions) led to partial clinical improvement without viral clearance. Due to continued severe adenovirus-related hepatic dysfunction, unmanipulated bone marrow from an HLA-identical sibling was infused without conditioning (10 million CD34+ cells/kg and 5.2x107 CD3+ cells/kg), at 9 months of age. After an initial surge in adenoviral loads attributed to massive viral lysis, the degree of viremia progressively declined and was <1,000 copies/mL within 7 weeks of marrow infusion. Antiviral T-cell activity against adenovirus was detected at low level in peripheral blood via IFN ELISpot at 3 weeks post-marrow infusion. She subsequently developed acute cutaneous and hepatic GvHD responsive to tacrolimus and steroids without recrudescence of viral illness.
Conclusions: Delay in curative therapy in SCID substantially increases risk of invasive life threatening infections. One strategy of allogeneic HCT can be to eradicate severe infection in SCID by providing the necessary T cell directed therapy against infectious agents. Antigen-specific partial immune reconstitution can be achieved with HCT in patients with thymic aplasia but concern regarding the development of full immunologic T cell diversity in athymic patients remains.

**(175) Submission ID#419726**

**Unusual fungal infection in adults chronic granulomatous diseases**

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Introduction/Background: Chronic granulomatous disease (CGD), an inherited disorder of granulocyte function caused by a failure of intracellular superoxide production, normally presents as severe recurrent bacterial and fungal infections in the first years of life. The majority of affected individuals are diagnosed before the age of 2 years, although patients may remain undiagnosed until adulthood despite the early onset of the symptoms
Objectives: Investigation of fungal infection in adult CGD patients
Methods: NBT and DHR 123 for detection of CGD and molecular analysis for detection of type of mutation in CGD and fungal characterization.
Results: We report here the detection of causative fungal infections in 2 adult patients with CGD. In the first patients we found paecilomyces formosus infection in an adult patient (18 years old female) with undiagnosed CGD who was referred to the Shahid Beheshti University Hospital (Tehran, Iran) complaining of cough, dyspnea and fever for 5 weeks prior to admission. Microscopic analysis revealed branching solitary phialide with ellipsoidal conidia with long chain arrangement and many chlamydospores which mostly resemble Botryotrichum species but during subcultures on potato dextrose agar (PDA) and Sabouraud Dextrose Agar (SDA), phialides typical of Paecilomyces species appeared. Typically, Paecilomyces spp. rarely cause infections in humans and if these fungi are detected in blood urine or cerebrospinal fluid cultures they are considered as contaminants. The second patient was a 26-year-old man was referred to the hospital with weight loss,

fever, hepatosplenomegaly and coughing. He had previously been diagnosed with lymphoadenopathy in the neck at age 8 and prescribed anti-tuberculosis treatment. BAL and serum galactomannan tests were negative. Low, subnormal levels of ROS were produced following stimulation of purified neutrophils with the phorbol ester PMA. Genomic DNA was extracted and Gene scan was used to determine the ratio between the number of exon 2 sequences of neutrophil cytosolic factor 1 (NCF1) gene, which encodes p47phox, and the number of -NCF1 exon 2 sequences. In addition, the fungal culture was disrupted with glass beads and DNA was extracted. The DNA sequence results were blasted using the NCBI Genebank database, which showed 99% similarity to an Aspergillus terreus isolate in the Gene Bank fungal library with accession no 1168.
Conclusions: Thus, despite its current relative rarity in older patients, the presence of fungal infection is changing our understanding of the diagnosis, management and outcome of CGD. Greater appreciation of the potential of fungal infection in older CGD patients is important in regions, such as Iran, where tuberculosis is endemic and that sarcoidosis and CGD are considered as differential diagnosis. The demonstration of the successful patient-orientated treatment after using sequencing to confirm CGD and to identify the presence of the specific infectious agent emphasises the importance of adopting this approach across the region.

**(176) Submission ID#411573**

**Use of the SPIRIT Analyzer to Detect Patients at Risk for Primary Immunodeficiency from Within a large pediatric health plan.**

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Introduction/Background: Primary immunodeficiencies (PI) represent a heterogeneous group of over 350 genetically distinct disorders which interrupt normal host-defense mechanisms and predispose to significant morbidity and mortality. Presently, we are only able to screen for severe T-cell deficiencies at birth; however, the most common forms of PI often go undetected for years leading to adverse patient outcomes and excessive healthcare spending. Given the relatively high incidence of PI in the general population, informatic measures could be useful for determining individual risk for PI and facilitating earlier, correct diagnosis and appropriate treatment across the spectrum of PI.

| Ethnicity | Main Cohort (no) | % | MHR Cohort (no) | % |
|---|---|---|---|---|
| Hispanic | 111413 | 59.9 | 636 | 59.5 |
| African-American | 27671 | 14.9 | 111 | 10.4 |
| Caucasian | 25091 | 13.5 | 143 | 13.4 |
| No Ethnicity Noted | 16227 | 8.7 | 146 | 13.7 |
| Asian/Pacific | 4948 | 2.6 | 32 | 2.9 |
| Alaskan/American Indian | 542 | 0.29 | 0 | 0 |
| **Gender** | | | | |
| Female | 95157 | 51.2 | 449 | 42 |
| Male | 90718 | 48.8 | 619 | 57.9 |
| **Age** | | | | |
| 3 to 5 | 35192 | 18.9 | 439 | 41 |
| 6 to 12 | 89948 | 48.3 | 526 | 49 |
| 13 to 18 | 42220 | 22.7 | 77 | 7.2 |
| 19 to 21 | 13749 | 7.4 | 25 | 2.3 |
| 22 to 64 | 4783 | 2.6 | 1 | 0.09 |
| **Total** | 185892 | | 1068 | |

Objectives: The purpose of this study was to test the Jeffrey Modell Foundations SPIRIT (Software for Primary Immunodeficiency Recognition Intervention and Tracking) Analyzer on the Texas Childrens Health Plan. Major aims were to identify individuals with medium-high risk for PI, assess the clinical characteristics of at risk patients and determine if risk identification led to diagnosis of PI over a 12 month period.
Methods: After removing all known PI diagnosed patients from the database, 185,892 individual Texas Childrens Health Plan enrollees were screened for risk of PI with the SPIRIT Analyzer using relevant, weighted ICD9 and ICD10 codes. Patient characteristics are shown in Table 1. Following identification of Medium-High Risk (MHR) individuals, letters were sent to their primary care physicians to alert them of patient risk. A second analysis of the MHR individuals was performed 12 months later. Detailed chart reviews were conducted on 769 MHR individuals to further assess clinical features of this group. The study was approved by the Baylor College of Medicine Institutional Review Board (Study H-38501).
Results: Of the original cohort, 2188 (1.2%) were identified as MHR for PI. From that group, 1068 (0.6%) were accessible for analysis and 769 (0.4%) had electronic health records for review. In the 12 months following the first analysis, 43 (0.02%) were diagnosed with a PI. (Figure 1) Another 61 patients had concerning diagnoses coded warranting further investigation. (Figure 2) Concerning diagnoses included: cellulitis(18), abscess(14), recurrent otitis media(11), recurrent sinusitis(5), osteomyelitis(2), and mastoiditis (1) among others. In total 104 patients had a PI diagnosis or a history concerning for PI (0.06% of Main Cohort; 13.5% of MHR Cohort).
Conclusions: The SPIRIT Analyzer is effective at identifying persons at risk for PI and facilitates diagnosis. Potential MHR yield by the Analyzer is over 10%. These patients are treatable and will benefit from targeted intervention once identified. The Analyzer can also highlight concerning conditions worthy of additional assessment. Future work should focus on longitudinal healthcare outcomes for patients diagnosed with PI via SPIRT and physician perspectives on the utility of the tool.

**(177) Submission ID#422140**

**Using multiplexed genome editing to distinguish between loss- and gain-of-function variants in CARD11**

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Introduction/Background: Genetic variants in CARD11 contribute to several diseases caused by dysregulation of the adaptive immune system. Dominant-negative, loss-of-function and gain-of-function variants in CARD11 variants lead to primary immunodeficiency disease. However, primary immunodeficiency disease caused by CARD11 gain-of-function variants often progresses to B-cell malignancy. The clinical course and treatment options depend on the type of CARD11 mutation. Unfortunately lymphocyte immunophenotyping and traditional proliferation assays can't distinguish the variant effect or predict the likelihood of malignancy.
Objectives: To use multiplexed functional assays for determining variant effect to distinguish between dominant-negative, loss- and gain-of-function effects in CARD11. Our ultimate goal is to generate a variant

function map that can be used to guide diagnoses and treatment of immune dysregulation caused by mutations in CARD11.
Methods: We co-delivered CRISPR/Cas9 ribonucleoprotein complexes with libraries of single-stranded oligonucleotide repair templates thus generating lymphoma cell populations containing all possible single-nucleotide variants (~2400 different protein coding changes) in the N-terminal 140 amino acids of CARD11. Following culture with and without BCR pathway inhibitors, we used next generation sequencing to quantify variant abundance before and after culture, both with and without BCR pathway inhibitors.
Results: Due a requirement for CARD11 in these lymphoma cells, those with dominant-negative and loss-of-function CARD11 variants grow more slowly, whereas those with gain-of-function variants grow faster in the presence B cell receptor pathway inhibitors. By tracking the relative abundance of each variant in the population by next generation sequencing over multiple conditions, we determined the functional effect of each. We assessed the functional effects of thousands of CARD11 variants in parallel. This enabled us to confirm several previously reported gain-of-function and dominant-negative variants in CARD11, as well as identify several additional novel variants. Finally, we evaluated previously undescribed dominant-negative, loss-of-function and gain-of-function variants during differentiation of primary human B cells and during NF-B signaling.
Conclusions: The results of our experiments demonstrate the utility of multiplexed functional assays for determining variant effect in proteins where distinguishing between dominant-negative, loss- and gain-of-function effects are required to guide diagnoses and treatment.

**(178) Submission ID#420719**

**Using the whole exome sequencing (WES) approach for better diagnosis and clinical management in primary Immuno-deficiencies (PIDs) in Israel**

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Introduction/Background: Primary immune-deficiencies (PIDs) are a heterogeneous group of nearly 300 monogenic inborn errors of immunity. In recent years, whole exome sequencing (WES) became a valuable diagnostic approach for the identification of molecular defects in patients with clinical manifestations suggestive of PID. This approach provides a definitive diagnosis and may help in genetic counseling, prenatal diagnosis and pre-symptomatic identification of patients with a potentially lethal disease. The diagnostic yield using WES was found to be ~25% in rare Mendelian disorders and ~40% in PIDs. We present here a markedly higher yield, of 65%, in PIDs with a high percentage of consanguinity (65% in this study).
Objectives: Using the whole exome sequencing (WES) approach in 55 Israeli PID patients with high percentage of consanguinity to identify the genetic causes underlying their diseases for better diagnosis and clinical management.
Methods: WES was performed on genomic DNA obtained from WBC of 55 immune deficient patients with potential genetic causes. The sequencing data was analyzed bioinformatically. Each of the discovered mutations was validated and the familial segregation was confirmed, using Sanger sequencing.
Results: The 55 probands (32 males and 23 females) ranged in age from 2 weeks to 26 years with a mean of 4.4 years. Of them, 20 patients are Jewish (36%), 34 Palestinians (62%) and 1 (2%) is from a Greek ethnicity (Cyprus). Based on their clinical and immunologic phenotype at the time of initial evaluation, patients were assigned to one of seven PID groups: (I) humoral immunodeficiency 5 patients (9%); (II) combined immunodeficiency (CID) 16 (29%); (III) CID with syndromic features 10 (18%); (IV) SCID 9 (16%); (V) congenital defects of phagocytes 6 (11%); (VI) immune dysregulation 7 (13%) and (VII) phenocopy of PID 2 (4%).
We identified 67 mutated alleles, all in coding regions, that are highly likely to be causative in 36 of the 55 patients, achieving a 65% molecular diagnostic yield. Among the 36 patients, the mode of inheritance in 28 patients (78%) is autosomal recessive and in 3 is compound heterozygote (8%). Four patients (11%) harbor a non-inherited mutation on one allele, either de novo or somatic. The inheritance of the mutation in one patient (3%) is X-linked. The high rate of bi-allelic inheritance (93% of the alleles) is mainly due to the high frequency (65%) of consanguinity among the studied cohort. Twenty eight mutated genes were identified in this study. Of them, 6 were found to be novel in causing an inherited disease in man. Interestingly, some genetic defects in known genes were found in patients with atypical phenotypes.
In 23 patients (42% of the total number of patients and 64% of the WES diagnosed) the discovery of the genetic cause led to a change of therapy, towards a more targeted and personalized one. The revised treatments included bone marrow transplantation, conditioning protocols, reduced intensity of immune suppression, and prevention of unnecessary treatments due to their possible deleterious outcome.
Conclusions: Except of being a useful tool for diagnosing and deciphering novel or atypical forms of PIDs, our WES study demonstrates an immediate and powerful impact on patient therapy in PIDs.

**(179) Submission ID#399719**

**Utility of a Second T-cell Receptor Excision Circle (TREC) Screen to Detect T-cell Lymphopenia and Severe Combined Immunodeficiency (SCID)**

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Introduction/Background: Severe combined immunodeficiency (SCID) represents a group of immunologic disorders characterized by absent T-lymphocyte number or function. Most children born with SCID die from life-threatening infections in the first years of life. Early intervention with bone marrow transplant or gene therapy is critical and best when the infant in uninfected. Newborn screening for SCID and T-cell lymphopenia has been implemented in 45 states. The screening test is performed from dried blood spots collected at birth and involves PCR quantification of circular DNA byproducts of T-cell receptor gene rearrangement, T-cell receptor excision circles (TREC). TRECs are generated during T-cell maturation in the thymus and are indicative of naïve T-cell output. Assays and protocols to measure TRECs vary by state and there is no standardized guideline at this time. Washington State is unique in that it is one of few states where all newborns undergo two or more independent newborn screens, the first

around 24 hours of life and the second after one week. There is no data on the utility of one versus two screens for SCID screening. Here we present our data evaluating whether patients with SCID or T-cell lymphopenia were identified on the first or second TREC screen.
Objectives
1. Determine the benefit of a second TREC screen in identifying SCID and T-cell lymphopenia at birth.
2. Examine outcomes of TREC screening in Washington state since implementation.
Methods: Results of SCID newborn screening performed in Washington State between January 2014 and June 2017 were reviewed retrospectively with the staff of the Washington Department of Health Laboratory where the TREC assay is performed. TREC thresholds (copies/μL) were defined as follows: Absent (20), Low (21-60), Borderline (61-80) and normal (>80). All TREC assays were run with a beta-actin control to assure sample adequacy. A screen is considered abnormal if there is one low/absent TREC or two borderline TREC. Newborns with abnormal TREC screening have follow-up diagnostic testing consisting of lymphocyte flow cytometry to evaluate numbers of naïve and mature T cells, B cells, and NK cells, performed at Seattle Childrens Hospital.
Results: A total of 68 positive TREC screens were found in Washington State between January 2014 and June 2017. Five patients who did not have diagnostic flow cytometry testing were excluded from the analysis (One protocol deviation, one lost to follow-up and three who died before testing could be performed). The first screen was abnormal in forty three patients, while the second screen was abnormal in 16 patients. Five patients had an abnormal third or fourth TREC drawn for other newborn screen follow-up. Three patients with SCID were identified, all with abnormal values on first screen. Forty-five patients with T-cell lymphopenia were identified; 30 from the first screen and 11 from the second screen. There was one patient with MHC II deficiency was missed by both first and second screens because she did not have T-cell lymphopenia. The false positive rate with the first screen was 21% versus 28% with subsequent screens. The false positive rate dropped to 3% with two abnormal TREC. The positive predictive value of SCID or T-cell lymphopenia with the first abnormal TREC was 82% versus 96% with two abnormal TREC. Average age of collection among infants with a positive screen was 30.5 hours for the 1st NBS and 11.5 days for the 2nd NBS. Live viral vaccines were postponed in three patients who had an abnormal secondary screen (one with idiopathic T-cell lymphopenia, one with ectrodactyly-ectodermal dysplasia-clefting (EEC) syndrome and one with 22q11.2 deletion). One of these was started on PJP prophylaxis.
Conclusions: The practice of obtaining a second NBS from all newborns in Washington State has led to increased identification of patients with T-cell lymphopenia but did not result in identification of additional patients with SCID. The false positive rate of the first and subsequent newborn screens was similar and decreased in patients with two abnormal TREC. Interventions including delaying live viral vaccines and PJP prophylaxis were instituted in patients who had a normal initial TREC but abnormal secondary screen and documented T cell lymphopenia. Two TREC screens in all newborns can result in identification of additional patients with T-cell lymphopenia who may require intervention and additional follow-up. It is not yet clear whether the cost of a second mandatory SCID newborn screen is balanced by the additional sensitivity gained by this approach.

**(180) Submission ID#421424**

**Utility of Immunoglobulin Replacement Therapy in a Patient with Mannose-Binding Lectin Deficiency**

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Introduction/Background: Mannose-binding lectin (MBL) is a multimeric lectin that recognizes a wide array of pathogens independently of specific antibody, initiates the lectin pathway of the complement system, and acts as a proinflammatory mediator. MBL deficiency is reported to increase the frequency of infections in patients with impaired immune systems or Cystic Fibrosis (CF) patients. MBL replacement is experimental and unavailable. Immunoglobulin replacement therapy (IGRT) is controversial in the treatment of MBL deficiency; however, there are no reports of its efficacy or role in this condition. We describe a CF carrier patient with MBL deficiency, ciliary dyskinesia and mildly low IgG who did not respond to IGRT.
Objectives
1. Understand when MBL deficiency can be symptomatic.
2. Define the relationship between MBL deficiency and CF.
3. Describe the treatment options of MBL deficiency.
4. Define the efficacy of IGRT and MBL deficiency.
Methods: A single case report.
Results: 11 year old girl with MBL deficiency, CF carrier state with polymorphisms (2752-26 A>G CF variant with 7T/7T and m470V polymorphism) and ciliary dyskinesia (diagnosed by ciliary biopsy) who initially presented at eight years of age with recurrent sinusitis, recurrent otitis media status post tympanostomy tube placement, reactive airway disease and tracheomalacia. She had nine episodes of recurrent sinusitis with six negative sinus cultures and one positive for Pseudomonas aeruginosa. She required a total of nine courses of antibiotics. Labs showed several MBL levels <50 ng/mL on three occasions, normal CH50, AH50, IgG 577-590 mg/dL (low normal for age), normal IgA, IgM, T cells, B cells, NK cells, and robust specific antibody titers. Despite adequate pulmonary hygiene including nebulized levalbuterol, budesonide, dornase alfa, ipratropium, hypertonic saline and compression vest twice daily, she continued to have recurrent bronchitis and cough. In addition, even with sinus rinses and intranasal corticosteroid, she continued to have 6-8 episodes of sinusitis yearly. For this reason, she underwent bilateral total ethmoidectomies and maxillary antrostomies with modest reduction in frequency of sinus infections and symptoms. However after two years, her bacterial sinusitis recurred. Four episodes were positive for methicillin Staphylococcus aureus or Pseudomonas aeruginosa. This did not improve significantly with a trial of intranasal mupirocin. Due to increased frequency of bacterial sinusitis refractory to traditional therapy, she was started on subcutaneous IGRT dosed approximately 350 mg/kg/month dosing maintaining IgG troughs around 700 mg/dL. After a six month trial, she did not have improvement in the frequency of sinusitis, bronchitis and otitis media. She required 3-4 courses of antibiotics for bacterial upper respiratory tract infections and IGRT was stopped. Prophylactic antibiotics and repeat sinus surgery were instituted.
Conclusions: Majority of the patients with low/deficient MBL levels do not manifest significant symptomology due to the redundancy of the innate immunity. The increased susceptibility to infections is thought to be due to additional factors that compromise other components of the immune system. Specifically in CF patients, MBL deficiency is associated with earlier colonization with Pseudomonas, more rapid decline in lung function and earlier death secondary to end-stage lung disease. There are no reports of combined MBL and CF carrier symptomatic patients similar to this patient. There are no validated age-corrected values for MBL levels in pediatric patients. The clinical relevance of these levels to infection frequency, severity, or treatment of MBL deficiency remains to be proven. It has been proposed that <100 ng/mL is considered deficient in children. MBL therapy is still experimental and not commercially available. Management when provided for severe or frequent infections includes prompt treatment with antibiotics, prophylactic antibiotics, appropriate vaccinations, and a trial of IGRT. There are no reported cases describing the efficacy of IGRT in MBL deficiency, and the mechanism

by which IGRT treatment helps in these patients is unreported. Here we present the first case of an MBL deficient patient with other complicating conditions (low IgG and multiple CF polymorphisms, ciliary dyskinesia) who did not show improvement on IGRT, thus refuting current literature.

**(181) Submission ID#425060**

**Warts as a Predominant Manifestation of ADA2 Deficiency**

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Introduction/Background: Adenosine Deaminase 2 (ADA2) deficiency is an autosomal recessive autoinflammatory disease caused by a loss-of-function mutation in Cat Eye Syndrome Chromosome Region Candidate 1 (CECR1) gene encoding for ADA2 protein. Affected individuals may present with immune deficiency, vasculopathy (manifested by recurrent stroke, intracranial hemorrhages, livedo reticularis, or polyarteritis nodosa), pure red cell aplasia, antibody deficiency, and bone marrow failure.
Objectives: To present the clinical phenotype of a patient evaluated at the National Institutes of Health with ADA2 deficiency.
Methods: Clinical and laboratory features of a patient with ADA2 deficiency, confirmed with Sanger sequencing, evaluated at the National Institutes of Health.
Results: A 28-year-old Caucasian male patient presented due to recalcitrant warts of the hands and feet. He had childhood otitis media status post tympanoplasty, mild molluscum contagiosum, and eczema. Also, history of migraines with a negative brain MRI. A trial of weekly pegylated interferon alpha 80mcg for his warts was discontinued due to significant neutropenia and depression. Physical exam was notable for bulky skin colored warts on the lateral and dorsal fingers and dorsal hands including periungual skin. Clusters of verrucae were noted on the feet. Initial laboratory testing was notable for mild hypogammaglobulinemia with protective specific antibodies. Lymphocyte subset analysis revealed a predominantly T cell lymphopenia with decreased naïve and memory CD4 and CD8 subsets, normal absolute numbers of B cells but with low memory subsets, and normal numbers and function of NK cells. Whole exome sequencing ultimately revealed homozygous R169Q mutations in the CECR1, a mutation previously described as causing ADA2 deficiency. ADA2 activity was about 40%.
Conclusions: ADA2 deficiency is a relatively newly defined genetic defect, with a clinical phenotype that continues to evolve with newly diagnosed cases. Our patient had not had evidence of vasculitis or stroke, but had recalcitrant warts with lymphopenia as his primary presentation. Approach to therapy for those without vasculitis or significant cytopenias remains unknown.

**(182) Submission ID#403479**

**Whole exome sequencing: Digging deeper to uncover a diagnosis**

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Introduction/Background: Whole exome sequencing has added greatly to our library of primary immune deficiencies. However, interpretation of findings is not always straightforward. Clinicians need to understand the limitations of this diagnostic tool and be familiar with the next steps in order to achieve a diagnosis.
Objectives: A 7 year old male presents for evaluation of bronchiectasis of bilateral lower lobes and poor growth. Past medical history was significant for GERD with poor weight gain during infancy and childhood, oral thrush, recurrent respiratory infections and bilateral partial hearing loss. The BAL showed abundant neutrophils and was positive for Strep pneumoniae and Haemophilus influenza.
Methods: Serum immunoglobulins were normal with an elevated IgA (369) and mildly elevated IgE (361). Tetanus IgG was protective (0.4 IU/mL) but post-vaccine responses to the pneumococcal polysaccharide vaccine were poor with protective titers achieved to 3/14 serotypes. Lymphocyte phenotyping was remarkable for a complete absence of B and NK cells. CD4:CD8 ratio was inverted (0.25) and the majority of the CD45+ T cells were memory phenotype. Mitogen proliferation was essentially normal. Due to the absence of B cells and despite normal total serum immunoglobulins, the patient was started on SC IG weekly and antibiotic prophylaxis with trimethoprim-sulfamethoxazole and fluconazole. Clinically he demonstrated good weight gain and a reduction in cough and respiratory symptoms.
Results: Whole exome sequencing was performed. A single base mutation in ADA1 (c.320 T>C) was identified in one allele. This mutation (p.L107P) is known to be deleterious and when homozygous is associated with typical SCID. However, this mutation was not present on the other allele. Secondary analysis looking for large deletions and duplications failed to identify any abnormality in sequence in the normal allele. But the expression of this allele was markedly diminished causing us to suspect that its function might be impaired.
Functional testing was performed and demonstrated that there was no ADA activity in the patients red blood cells, thus confirming a diagnosis of combined immune deficiency due to ADA deficiency.
Conclusions: Null mutations in ADA result in an absence of ADA function with profound CD3 cell lymphopenia and dysfunction. Clinically affected infants have typical SCID. Hypomorphic mutations may lead to partial function of ADA with more variable immune defects. Interestingly, most patients with ADA deficiency have neurologic complications, frequently hearing loss. We believe that most likely this patient has had some degree of spontaneous reversion of the mutation in the normal allele leading to a less severe phenotype. As reported previously in a case involving a mutation in IL2RG chain, normal function was not restored and the patient remains with a combined immune deficiency. This case highlights an important limitation of whole exome sequencing and the need for confirming the impact of a genetic defect with a functional assay.

**(183) Submission ID#424350**

**Will early allogeneic hematopoietic stem cell transplantation yield better outcomes in STAT3 deficient HIES patients?**

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Introduction/Background: The autosomal dominant Hyper-IgE-Syndrome (AD-HIES) is a rare primary immunodeficiency and multisystem disorder resulting from heterozygous loss-of-function mutations in the STAT3 gene. AD-HIES is characterized by skeletal dysplasia, recurrent pulmonary and skin infections (e.g. staphylococcal abscesses, eczematoid dermatitis) due to an increased susceptibility to bacteria and fungi. Since many patients do rather well on anti-infective prophylaxis and supportive care, and early case reports suggested no benefit of allogeneic hematopoetic stem cell transplantation (HSCT), AD-HIES patients are rarely referred for HSCT. The literature still contains only a handful of patients who underwent HSCT. All these patients had experienced severe disease related complications before HSCT and consequently the benefit of HSCT was reported to be variable.
Objectives: Currently, the general consensus is to only consider patients with severe pulmonary disease for HSCT. It could however be postulated that transplanting patients earlier in their disease course and correcting their immunodeficiency before permanent organ damage due to infectious complications has occurred may extend life-expectancy and improve the quality of life of AD-HIES patients.
Methods: We report on a 15 year old female AD-HIES-patient who presented with bronchiectases following recurrent pneumonias, one pneumatocele, and normal pulmonary function tests. Her past medical history also included recurrent skin infections, serious haemoptysis, pathological fractures and atopic eczema. Considering that lung infections are the major life-limiting complication in AD-HIES and are potentially positively influenced by HSCT, our patient had enquired about a transplant. After extensive discussion of the pros and cons and obtaining full informed consent from her and her family, she underwent an elective HSCT. She received bone marrow from an HLA-matched sibling donor after a reduced-intensity conditioning consisting of alemtuzumab (2x0,2mg/kg), treosulfan (3x14g/m2), fludarabine (5x30mg/m2) and thiotepa (2x5mg/kg), and graft-versus-host disease (GVHD) prophylaxis with cyclosporine A (CSA) and mycophenolate mofetil (MMF).
Results: The peri-transplant course was complicated by acute lower gastrointestinal tract bleeding and renal failure of unknown origin. Continued kidney function impairment led to early tapering and discontinuation of CSA on day +132 after HSCT in the absence of any acute GvHD. Neutrophils and platelets engrafted on days +15 and +26 respectively. She is currently on day +150, free of GvHD or infection, exhibiting full donor chimerism and recovered kidney function. In view of the pre-existing pulmonary damage she currently remains on antibiotic prophylaxis and inhalation therapy.
Conclusions: This AD-HIES patient who underwent HSCT with few pre-HSCT disease complications and relatively little permanent organ damage may add to our understanding of whether early HSCT will lead to improvement of quality of life and possibly increased life-expectancy in AD-HIES patients. It remains to be elucidated whether her rather uncommon peri-HSCT complications are connected to her underlying disease. Future research should be directed at identifying AD-HIES patients at high risk of severe pulmonary complications early, so these could be referred for timely HSCT.

**(184) Submission ID#404475**

**XIAP Deficiency causing Recalcitrant Inflammatory Bowel Disease with Immune Dysregulation**

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Introduction/Background: Monogenic defects are more frequent in patients with early onset inflammatory bowel disease (IBD). In XIAP deficiency, IBD can be severe and refractory to standard medications used for Crohns and Ulcerative Colitis. MEFV variants can similarly cause intractable IBD as well.
Objectives: This is a case study of a patient who had refractory IBD symptoms and recurrent infections who was found to have XIAP deficiency and MEFV variant mutations.
Methods: A 37 year old male presented at age 16 with recalcitrant IBD unresponsive to multiple medications including steroids, mesalamine, azathioprine, infliximab, adalimumab, methotrexate, and vedolibumab. In addition, he developed severe infections from a combination of immune dysregulation and immunosuppressant medications. Currently he is on ustekinumab but still has severe abdominal symptoms. Patient does not have a history of hemophagocytic lymphohistiocytosis (HLH). Family history was significant for IBD in both his mother and sister.
He has had persistent lymphopenia which ranged between 210 to 838 cells/mm3. T/B/NK panel showed decreased CD3 T cells (596 cells/mm3) with normal CD4, CD8, and CD4/CD8 ratios. B and NK cells were normal in quantity. T cell antigen/mitogen assays showed normal response to all mitogens (PHA, PWM, Con A) and most antigens (Tetanus, Candida, HSV, VZV, ADV) but low response to CMV antigen. IgG was elevated at 2,750, but IgA, IgM, and IgE were normal. His EBV DNA PCR is negative.
Results: Exome sequencing revealed a novel XIAP hemizygous variant at position c.693G>A (p.=?) of unknown significance and a MEFV heterozygous variant. Functional testing performed at Medical College of Wisconsin showed no expression of XIAP on lymphocytes and a defect in NOD2 pathway. Given the XIAP deficiency, bone marrow transplant was discussed as an option for refractory IBD and prevention of HLH. Rituximab was also offered to decrease the possibility of HLH. Currently the patient is in the decision making process of both treatment options.
Conclusions: Genetic evaluation with clinical exome in early onset refractory IBD, family history of IBD, and recurrent infections demonstrated a novel mutation in the XIAP gene with possible contribution of MEFV variant as well. The absence of XIAP protein expression and abnormal functional assay of the NOD2 pathway confirm the pathogenicity of the mutation. Identification of this genetic variant will help guide future therapeutic options and prognosis for this patient.

**(185) Submission ID#427740**

**XLA Immune Deficiency Complicated by Flexispira infection**

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Introduction/Background: X-linked agammaglobulinemia (XLA) is a primary immunodeficiency disease caused by mutations of Bruton tyrosine

kinase (Btk), which is essential in B cell maturation. XLA is typically associated with bacterial infections of upper and lower respiratory systems, enteritis and increased risk for malignancies.
Objectives: To present the evolution and treatment of a complicated flexispira (Helicobacter bilis) infection with delayed diagnosis in an XLA patient.
Methods: Clinical and laboratory features of a patient with XLA evaluated at the National Institutes of Health.
Results: A 29-year-old male patient with XLA presented for initial evaluation of indurated lower extremity and torso lesions. He was diagnosed with XLA at age 2 years secondary to bacterial sepsis pneumonia and empyema. After starting IVIG at age 2, he was well without significant infections except for recurrent otitis media. At 12 years of age, he developed right leg edema below the knee, which progressed to patchy skin thickening and discoloration. A tissue biopsy at 15 years of age revealed marked fibrous thickening of the subcutaneous septum with diffuse infiltrate of eosinophils with negative cultures for bacterial, fungal, and mycobacterial infections and thought to be consistent with eosinophilic fasciitis. MRI demonstrated infiltration in the superficial and deep muscle compartments with fibrosis. Symptoms persisted despite empirical treatment with IV antibiotics and steroids. At 16 years of age his left leg became involved with similar findings, while under treatment including multiple immunosuppressants (dapsone, methotrexate, tacrolimus, hydroxychloroquine, IV cyclophosphamide, Remicade, Cytoxan, and Enbrel) targeting eosinophilic fasciitis. At age 28, he developed ulcerations over the left shin and ankle and was diagnosed with chronic multifocal osteomyelitis based on MRI findings. Physical exam was notable for bilateral leg swelling below the knees with woody appearance and induration, hyperpigmentation, and tenderness. Indurated and nodular lesions without discoloration were noted above the waist line, on right forearm and above right nipple. Skin biopsies of right lower extremity and right forearm were positive for numerous spirochetal-like organisms with Warthin-Starry stain. Treatment was initiated with Meropenem and Gentamicin. Gentamicin was discontinued due to vestibular ototoxicity six weeks later and doxycycline was added. Initial response was followed by worsening of symptoms and intolerance to treatment, which led to the addition of tigecycline and azithromycin. With continued progression, seven months into initial evaluation, chloramphenicol and nitazoxanide were added to the regimen. Due to the persistence of flexispira organisms on skin biopsy Warthin-Starry stains, fresh frozen plasma (FFP) was introduced four months later (11 months after initial evaluation) as an adjunctive treatment. Cultures performed at the CDC were negative; however, PCR and sequencing resulted in identification of Helicobacter bilis. 6 units of FFP was infused weekly for over three years, then reduced to every two weeks, with goal IgM levels > 40mg/dL. Subsequently, labs including cytopenias, inflammatory markers, and immunoglobulins improved as well as a negative Warthin-Starry stain. Symptoms have improved with almost complete resolution of findings with only residual small areas of discoloration over both lower extremities.
Conclusions: XLA immune deficiency is associated with flexispira (Helicobacter bilis) infections, with typical appearance of discoloration and induration, which may evolve to osteomyelitis due to delayed treatment. Although typically observed over the lower extremities, immunosuppressive treatment may lead to further expansion above the waist line. Approach to therapy with weekly to bimonthly FFP infusions in addition to antibacterial treatment has proven to be beneficial in controlling the infection. Higher IgM levels resulting from the FFP may also provide antibacterial effects.

**(186) Submission ID#426165**

**X-linked Severe Combined Immunodeficiency with Permissive Lymphocyte Nitogen Proliferation: A Diagnostic Dilemma**

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Introduction/Background: X-linked severe combined immunodeficiency (SCID) is a well described primary immunodeficiency associated with mutations in the common gamma chain. Patients with X-linked SCID classically present with profoundly low or absent T cells and NK cells with a variable number of B cells. The lymphocytes that are present typically have a proliferation index <10% control when stimulated with mitogens and antigens. Patients must undergo corrective therapy with bone marrow transplant (BMT) or gene therapy to avoid the life-threatening infections that are associated with the nearly absent adaptive immune system.
Objectives: A 2-week-old boy presented to Childrens National Immunology Clinic for initial evaluation of critical result on newborn screen.
Methods: Targeted partial exome sequencing was performed on a 7-day-old patient who was picked up via TREC assay on the Maryland Newborn Screen. Flow cytometry was completed at Childrens National and proliferation studies completed at Cincinnati Childrens Hospital Diagnostic Immunology Lab.
Results: Flow cytometry revealed markedly decreased lymphocytes with nearly absent CD8+ T cells and low CD4+ T cells with a relative increase in CD4+CD45RO T cells (Ratio CD45RA:CD45RO: 18%:12%). The B and NK cells were within the reference range for age. Mitogen proliferation studies showed a mild decrease to PHA and normal responses to pokeweed and ConA (35587 cpm, 40924 cpm, and 82651 cpm, respectively). Partial exome sequencing revealed a hemizygous nonsense substitution in IL2RG (c.982C>T, p.R328) . Maternal engraftment accounted for 3% of the T cells. The patient was started on prophylaxis with IVIG, Bactrim, and fluconazole with the plan to proceed with bone marrow transplant. As patient approached BMT maternal engraftment became absent in whole blood and repeat proliferation studies revealed normalization of the response to PHA (stim index) with continued normal responses to ConA and pokeweed. The patients flow cytometry values and ratios remained unchanged. Patient completed a reduced intensity preparative regimen of busulfan, fludarabine and alemtuzumab prior to receiving his 8/8 matched unrelated donor bone marrow transplant.
Conclusions: It remains to be determined why initial proliferation studies showed >10% function with improvement over time in a patient with a well-described genetic mutation causing SCID

**(187) Submission ID#428348**

**Promise and pitfalls of next-generation sequencing**

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Introduction/Background: The advent of next-generation sequencing (NGS) has led to a proliferation of newly discovered genetic

diseases and expanded phenotypes of known immunodeficiencies. Availability of specific gene panels or whole exome sequencing (WES) with targeted analysis based on broad phenotypes, coupled with clinicians increasing awareness has led to higher utilization of NGS. Published reports from high throughput sequencing labs indicate exome analysis identifies causative mutations in only 20-30% of probands.
Results: We have seen several patients who underwent high quality NGS in whom causative mutations were not identified. Two separate families with multigenerational histories of leukemia, aplastic anemia, myelodysplastic syndrome, and cytopenias suggestive of GATA2 deficiency had myeloid gene panel screens at commercial labs without causative mutations identified. Targeted GATA2 sequencing in the first family identified a novel change in GATA2, c.1017G>T, p.L339L. cDNA analysis demonstrated this synonymous variant resulted in aberrant splicing leading to a frameshift and premature termination. The WES bioinformatics pipeline failed to recognize the splice mutation. In the second family, PCR amplification spanning the 2 terminal exons revealed a shortened PCR product with a 426 base deletion fully encompassing the penultimate exon and leading to a 42 amino acid in-frame deletion. The deletion spanned all capture probes for the exon resulting in only the wild-type allele being captured and sequenced.
Additionally, capture kits targeting only coding regions of genes fail to capture deep intronic mutations such as those seen in GATA2 (Hsu, 2013) or in the 5 untranslated region of IKBKG, encoding NEMO, (Mooster, 2014; Hsu, submitted). Lastly, even with good capture and sequencing, the presence of pseudogenes may confound downstream sequence alignment as seen in NCF1, encoding p47phox preventing recognition of disease causing mutations.
Conclusions: With NGS becoming more widely available as a clinical diagnostic tool, it is important to remember that WES results, unlike many laboratory tests, are not binary. Inadequate bioinformatics pipelines, deletions, intronic or untranslated mutations, and pseudogenes can all mask the presence of causative mutations. Targeted panel captures or analysis will miss novel genes. Astute clinicians need to recognize the limitations of the current technology and pursue alternate assays when suspicions warrant.

**Submission ID#407701**

**A Cell Based Assay for the Detection of Autoantibodies to IL-17 in Human Serum.**

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Introduction/Background: Patients with chronic candida infections are typically deficient in some aspect of IL-17 signaling. One mechanism, which has recently come to light, is through the production of IL-17 autoantibodies, particularly in patients who already suffer from specific autoimmune diseases.
Objectives: Our objectives are to develop a diagnostic assay to accurately and easily detect IL-17 autoantibodies in patient serum.
Methods: We developed a cell-based reporter assay using HEK Blue IL-17 cells (InvivoGen) to detect the ability of patient serum to block IL-17 receptor signaling. Once stimulated with IL-17, the cells secrete Alkaline Phosphatase (AP) into the surrounding media which is detected by InvivoGen HEK Blue media and an absorbance reader. Addition of serum containing blocking antibodies inhibits secretion of AP.
Results: We were able to demonstrate, using a single patients serum with known IL-17 autoantibodies, the inhibition of IL-17 signaling in HEK Blue IL-17 reporter cells. We further characterized the sensitivity of our assay with a commercially available anti-IL-17 monoclonal and found it to be sensitive to between 1.4 x 10-6 M and 6.7 x 10-7 M.
Conclusions: Loss of IL-17 signaling can lead to problematic immune deficiencies including difficulty in clearing extracellular pathogens such as candida. Some people who have an immune deficiency in the IL-17 pathway may have developed autoantibodies to IL-17 and thus have difficulty generating an appropriate immune response. We have developed a relatively low maintenance, cost effective, and simple test for detecting IL-17 autoantibodies in human serum.

**Submission ID#428121**

**Alternations in repertoire of T and B cell subsets in patients with partial recombination activating gene (RAG) deficiency with autoimmunity and history of viral infections**

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Introduction/Background: Patients with partial deficiency of recombination-activating genes 1 or 2 (RAG1/2) can present with a wide spectrum of primary immunodeficiencies including combined immunodeficiency with granuloma and/or autoimmunity (CID-G/AI). Prior case reports have highlighted alterations in B and T cell compartments; however comprehensive characterization of T and B cell receptor repertoires of lymphocyte subsets regarding diversity and autoreactivity has not been reported.
Objectives: Defects in V(D)J recombination due to RAG deficiency results in a skewed T and B cell repertoire that may be further modified by viral infections and promote inflammatory or autoimmune phenotype.
Methods: Peripheral T and B cell compartments were sorted from two patients with combined immunodeficiency secondary to hypomorphic RAG1 and RAG2 mutations. B cells were stimulated with CD40L, CpG and IL-21 to transition to antibody secreting cells (ASCs), mimicking viral infection. Repertoire analysis and single cell cloning of BCR heavy and light chain variable regions from sorted B cell populations has been performed. Repertoire of T cell subsets (Treg and follicular helper) were also examined
Results: We noted skewing towards proximal J usage in all B cell compartments (mature naïve, marginal zone, CD21-/low and memory) of two RAG deficient patients compared to healthy controls. B cell clones with V4-34 V genes with low rate of somatic hypermutation expanded during B cell development. After in vitro stimulation mature naïve B cells from RAG deficient patient were capable of transitioning to antibody secreting cells

and enriched for polyreactivity to dsDNA, insulin, LPS and IFN cytokine (25 to 36%) compared to healthy control (5 to 14%). In connection to altered B cell compartments, restricted repertoire of regulatory T cells and an expanded and skewed follicular helper T subset were detected.
Conclusions: Our data indicate that patients with partial RAG deficiency have skewed T and B cell subsets that can further be altered towards antibody secretion and polyreactivity after stimulation such as viral infections.

**Submission ID#419696**

**Chromosome 22q11.2 Deletion Size Affects Peripheral Lymphocyte Subset Counts**

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Introduction/Background: The clinical features of 22q11.2 deletion syndrome include virtually every organ of the body. T cell lymphopenia, as a consequence of thymic hypoplasia, is the most commonly described immunologic feature and is most prominent in childhood. Later in life, T cell exhaustion may be seen and secondary deficiencies of antibody function have been described in patients with 22q11.2 deletion syndrome.
Objectives: The role of deletion breakpoints in determining 22q11.2 deletion syndrome immunophenotype is unknown. In this study, we examined the effect of 22q11.2 deletions with and without TBX1 on lymphocyte counts.
Methods: Lymphocyte counts were compared between 52 total 22q11.2 patients with TBX1-containing deletion (A-B, A-C, A-D deletions), and a total of 8 patients with TBX1-noncontaining deletion (B-D, C-D, D-E, D-F deletions). Lymphocyte counts of patients with 22q11.2 deletions were compared to a set of 6 patients with a 22q11.2 duplication including the TBX1 locus. Lymphocyte subset counts for each group were analyzed by t-test.
Results: CD3 counts were significantly lower in the TBX1-deleted cohort compared to the other two cohorts (mean 2169 cells/mm3 in TBX1 deleted cohort, 3709 CD3 cells/mm3 in the non-TBX1 deleted cohort, and 3657 in the duplication cohort, $p<0.01$ for all). Similarly, CD4 counts were lower in the TBX1-deleted patients compared to the other two cohorts. There were no significant differences in CD8, CD19, and NK cell counts between the three cohorts.
Conclusions: These represent the first data to examine T cell counts in 22q11.2 deletion syndrome patients with different breakpoints. Our data highlights an important role for TBX1 or other genes in the A-B region in regulating T cell production.

**Submission ID#405415**

**Paracoccidioidomycosis associated with a heterozygous STAT4 mutation and impaired IFN- immunity**

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Introduction/Background: Mutations in genes affecting IFN- immunity have contributed to understand the essential role of this cytokine in the protection against intracellular bacteria and fungi. However, inborn errors in STAT4, which controls IL-12 responses, have not yet been reported.
Objectives: To determine the underlying genetic defect in a family with a history of paracoccidioidomycosis (PCM) disease.
Methods: Genetic analysis was performed by whole-exome sequencing and Sanger sequencing. STAT4 phosphorylation and translocation from the cytosol to the nucleus, as well as IFN- release by patient lymphocytes were assessed. The effect on STAT4 function was evaluated by site-directed mutagenesis using a lymphoblastoid B cell line (B-LCL) and U3A cells. Microbicidal activity of patient monocytes/macrophages was also analyzed.
Results: A heterozygous missense mutation, c.1952 A>T (p.E651V) in STAT4 was identified in the index patient and her father. Patients and fathers lymphocytes showed reduced STAT4 phosphorylation and nuclear translocation as well as impaired IFN- production. In accordance, B-LCL and U3A cells carrying the STAT4 mutant displayed reduced STAT4 phosphorylation. Patient's and father's PBMCs and macrophages (alone or in the presence of T cells) displayed impaired fungicidal activity compared with those from healthy controls that improved in the presence of recombinant human (rh) IFN-, but not rhIL-12.
Conclusions: Our data suggest autosomal dominant STAT4 deficiency as a novel inborn error of IL-12-dependent IFN- immunity associated with susceptibility to PCM disease.

**Submission ID#428320**

**Profound B cell lymphopenia in GOF-STAT1 that improves post Ruxolitinib**

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Introduction/Background: Subjects with Gain of function Signal Transducer and activator of Transcription (GOF-STAT1) mutations have a variable clinical phenotype including combined immunodeficiency (CID). Ruxolitinib, a janus kinase 1/2 inhibitor has been successful at treating immune dysregulation in subjects with GOF-STAT1. Two subjects with profound B cell and/or T cell lymphopenia as a major manifestation are described, one of which was successfully treated with ruxolitinib.
Objectives: To discuss GOF-STAT1 mutations, their effect on the immune system, and the potential benefit of ruxolitinib in these subjects.
Methods: Retrospective chart review was performed.
Results: Subject 1 (c.494A>G) is a 21 year old male with a history of recurrent shingles, chronic mucocutaneous candidiasis (CMC), pneumocysitis jiroveccii pneumonia, varicella zoster meningitis, severe enteropathy, cerebral aneurysm and lymphoproliferation, and autoimmune hypothyroidism. He has profound lymphopenia predominantly affecting T and B cells (CD3+ 456 cells/uL, CD4+ 104 cells/uL, CD8+ 296 cells/uL, CD56+ 42 cells/uL, CD19+ 0 cells/uL). Subject 2 (c. 1053G>T) is a 12 year old male with a history of severe CMC, recurrent pneumonia, enteropathy, and autoimmune thyroiditis. He had severe B cell lymphopenia (CD3+ 2168 cells/uL, CD4+ 1461 cells/uL, CD8+ 589 cells/uL, CD56+ 47 cells/uL, CD19+ 24 cells/uL). Treatment with ruxolitinib 12.5mg BID led to clinical improvement of enteropathy and increased B cell counts in subject 2 (CD19+ 124 cells/uL). Ruxolitinib has not yet been initiated in subject 1. Both subjects were treated with anti-microbial prophylaxis and immunoglobulin supplementation.

| | Patient 1 | Patient 2 |
|---|---|---|
| Age/Sex | 21 year old male | 12 year old male |
| Mutation in STAT1 | c.494A>G | c.1053 G>T<br>p.L351F |
| Infections | • Shingles<br>• Thrush<br>• *Pneumocystis jirovecii* pneumonia<br>• Varicella zoster meningitis and encephalitis | • Severe chronic mucocutaneous candidiasis<br>• Recurrent pneumonia |
| Autoimmunity | • Autoimmune hypothyroidism<br>• Basal ganglia lesion – movement disorder | • Autoimmune thyroiditis<br>• Enteropathy |
| Immune Phenotype | | |
| CD3 (cells/μl) | 456 | 2168/1238 |
| CD4 (cells/μl) | 104 | 1461/806 |
| CD8 (cells/μl) | 296 | 589/358 |
| CD19 (cells/μl) | 0 | 24/164 |
| CD56 (cells/μl) | 42 | 47/60 |
| Lymph proliferation | • Low proliferation in response to phytohemagglutinin and pokeweed-mitogen<br>• Normal proliferation in response to *Candida* and tetanus | Not Performed |
| IgG (mg/dL) | 733 (on IVIG) | Not performed |
| IgA (mg/dL) | <7 | |
| IgM (mg/dL) | 7 | |

Second set of values for Patient 2 are post ruxolitinib.

Conclusions: Combined immunodeficiency with variable degrees of B and T cell lymphopenia and hypogammaglobulinemia can be profound in subjects with GOF-STAT1 mutation. Despite proper anti-microbial prophylaxis, this immunodeficiency can lead to severe infections. In addition to treating the autoimmune and immune dysregulatory features, treatment with ruxolitinib can improve the CID present and potentially reduce infectious susceptibility.

**Submission ID#427968**

**IgG4-Related Disease (IgG4-RD), its common mimickers and response to anti-IL5-(Reslizumab) treatment**

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Introduction/Background: We describe a complicated case of IgG4 Related Disease (IgG4-RD) both in its presentation and novel treatment
Objectives: To review the common mimickers of IgG4- Related diseases which often lead to delayed diagnosis and treatment. To discuss novel therapeutic treatments for IgG4-Related Disease
Results: A 70-year-old woman with a history of thyroid disease, sicca symptoms, lipodystrophy, relapsing parotid enlargement, asthma and Erdheim Chester syndrome initially presented with recurrent bacterial and fungal sinusitis despite multiple sinus surgeries. Immunologic work-up was notable for lymphopenia of 300/ml, CD4 count of 132 (677-1401 cells/uL normal range) and elevated IgG4 of 511 (4.0-86.0 normal range). Imaging was notable for nasal septal perforations and hypoplastic maxillary sinuses. There was high suspicion for IgG4 disease however the patient was lost to follow up during which time she developed cachexia, eosinophilic pleural effusions (80% eosinophils), lung mass and a parotid mass with predominant T cell infiltrate misdiagnosed as follicular lymphoma. Features consistent with IgG4-RD included >50% ratio of IgG4/IgG and a predominant T cell infiltrate A biopsied lung mass showed IgG4 plasma cell >50/hpf also consistent with IgG4-RD. Bone marrow biopsy was within normal limits. The patient was treated with Rituximab, an effective treatment for IgG4-RD. On treatment her IgG4 levels normalized, however she developed recurrent large eosinophilic lung effusions requiring repeat drainage. Fractional exhaled NO (FeNO) was elevated to 86ppb. She was started on Reslizumab at a dose of 5mg/kg resulting in marked improvement in her respiratory status along with normalization of peripheral eosinophilia and reduction of FeNO to the normal level of 12ppb.
Conclusions: IgG4-RD is a fibro-inflammatory condition which can affect any organ system and is diagnosed via tissue histology showing IgG4 positive plasma cells and a typical morphologic pattern. This case outlines the common mimickers of IgG4-RD often leading to a delayed diagnosis. Before the final diagnosis of IG4-RD was made by us, the patient carried multiple diagnoses including: thyroid disease, recurrent parotid enlargement and seronegative Sjogrens. These diagnoses in hindsight may have been Mikuliczs syndrome, Kuttners tumor and/or Riedels thyroiditis which are common manifestations of IgG4-RD. Chronic sinusitis, atopic diseases, peripheral eosinophilia and destructive osseous lesions as noted in our patient are seen in up to 40% of patients with IgG4-RD. Destructive bony lesions and eosinophilia can mimic granulomatous polyangiitis, which was ruled out in our patient. Her cachectic appearance and diagnosis of lipodystrophy can be explained by destruction of osseous tissue in the craniofacial skeleton which was later confirmed on imaging. Lymphoid inflammatory infiltrates are commonly seen in IgG4-RD and are can be misdiagnosed as a follicular lymphoma as in our case. A novelty in this case is the successful treatment with Reslizumab targeted at the eosinophilic component of the disease. On Reslizumab our patients asthma was for the first time controlled, pleuritis improved, fractional exhaled NO (FeNO) normalized and her cachexia is improving. Treatment with Reslizumab should be considered in patients with IgG4-RD who manifest with eosinophilic respiratory disease.

**Submission ID#421049**

**IKBKB Severe Combined Immunodeficiency: Clinical and Immunologic Phenotype and Stem Cell Transplant Outcomes**

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Introduction/Background: IKBKB deficiency (c.1292dupG in exon 13) is a rare autosomal recessive form of severe combined immune deficiency (SCID) originally described in Canadian infants of Northern Cree descent. IKBKB SCID is characterized by normal lymphocyte development, but impaired T-cell activation, along with innate immune defects.
Objectives: To report the clinical presentation, immunologic phenotype, and outcomes for patients with confirmed or suspected IKBKB SCID due to this founder mutation.
Methods: We retrospectively reviewed hospital records dating back to 1973 of patients with confirmed homozygous IKBKB mutations, as well as patients suspected to be affected due to their clinical presentation and family relations to molecularly confirmed cases.
Results: Fifteen patients were included. They presented early in life (average age 2 months) with invasive and disseminated viral, bacterial, mycobacterial, and fungal infections. Patients had concurrent and multiple infectious organisms, with a notable predilection for Candida, gram negative organisms, and mycobacteria. Four patients in our cohort received BCG vaccination at birth, resulting in fatal disseminated M. Bovis infection in all, and 2 additional patients succumbed to atypical mycobacterial infection following hematopoietic stem cell transplant (HSCT). One newborn was identified in 2016 through new initiatives for targeted newborn screening for the mutation. Immunologic features at presentation included normal to elevated lymphocyte counts with normal to elevated CD3, CD4, and CD8 T cells. When tested, response to PHA varied from absent (1/10), to low (4/10), to normal (5/10). Most patients had hypogammaglobulinemia, most often of the IgG isotype (10/14). Six had assessment of TREC levels, and all had values above thresholds for screening programs. Eight patients died before they could undergo HSCT, and 6 received transplants in the setting of ongoing severe, life-threatening infections. Only 1 patient underwent HSCT prior to the onset of infection. In our cohort, there are only 2 long-term survivors.
Conclusions: IKBKB deficiency is a severe form of SCID with early onset of invasive and disseminated multi-organism infection. The immunologic phenotype is characterized by normal to elevated lymphocyte numbers which do not meet PIDTC criteria for SCID, variable (and sometimes normal) mitogen response, normal TREC levels, and low IgG levels. The disease is universally fatal without HSCT, however, conclusions regarding efficacy and long-term outcomes of HSCT are uncertain given the small sample size.

**Submission ID#428736**

**Immune -dysregulation mimicking systemic lupus erythematosus in a patient with lysinuric protein intolerance**

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Introduction/Background: Lysinuric protein intolerance (LPI) is an inherited aminoaciduria caused by defective amino acid transport in epithelial cells of the intestine and kidney due to bi-allelic, pathogenic variants in SLC7A7. The clinical phenotype of LPI includes failure to thrive and multi-system disease including hematologic, neurologic, pulmonary and renal manifestations. Individual presentations are extremely variable, often leading to misdiagnosis or delayed diagnosis. Here we describe a patient that presented with suspected immunodeficiency in the setting of early-onset systemic lupus erythematosus (SLE), including renal involvement, who was subsequently diagnosed with LPI post-mortem.
Objectives: Describe a clinical a patient with Lysinuric Protein Intolerance that presented as early-onset systemic lupus erythematosus (SLE), including renal involvement and Primary Immunodeficiency.
Methods: After informed consent was obtained, DNA samples were obtained from the proband and his parents. Trio whole exome sequencing was performed to identify a cause of early onset autoinflammation resembling systemic lupus erythematosus.
Results: The male proband had a history of failure to thrive starting at 12 months of age, recurrent bacterial otitis media, and one episode of severe bacterial pneumonia requiring hospitalization. He presented at 30 months of age with multifocal pneumonia, anemia (Hgb 8.2 mg/dL) and mild thrombocytopenia. Initial laboratory studies revealed low albumin (2.8 mg/dL), elevated LDH (718), and mild hepatomegaly. Renal and liver function testing was initially normal. Immunologic evaluation for suspected primary immune deficiency showed normal immunoglobulin titers, low C3 (60) and low C4 (6.3). Lymphocyte phenotyping revealed low B cell counts (0.6% of total lymphocytes) with T cells and NK cells within the normal range. Despite antibiotic therapy, the patient worsened, developing fevers, a generalized erythematous rash, edema and nephrotic syndrome with oliguria. Renal biopsy uncovered glomeruli with accentuated, global thickening and diffuse, peripheral capillary loops, as well as focal spiculated defects, there was endothelial swelling and other signs of acute damage including epithelial flattening, adluminal irregularity and extensive intraluminal proteinaceous detritus. Endocapillary proliferative lesions, extracapillary crecents or tubular atrophy was not observed. Immunofluorescence studies were positive for C3, IgG and C1q granular deposits, mainly at the mesangium, interpreted as lupus nephropathy. Endothelial swelling and massive, subepithelial electron-dense deposits with spike formation from the basement membrane were noted on electron microscopy, mimicking a Stage II membranous pattern of injury. Autoantibodies included ANA (1:320), anti-dsDNA, Smith, SSA and RNP were positive in agreement with the diagnosis of SLE. Immunosuppressive therapy with high dose IV corticosteroids and cyclophosphamide was initiated. Despite this, the patient developed pancytopenia, elevated ferritin levels, increased triglycerides, and low fibrinogen. Bone marrow biopsy displayed erythrocyte phagocytosis by macrophages, confirming a diagnosis of hemophagocytic lymphohistiocytosis (HLH). The patient subsequently died despite aggressive immunosuppression with high dose methylprednisolone and high dose IV immunoglobulin and dialysis.
Samples were collected from the deceased patient and his parents for research whole exome sequencing. Trio analysis identified compound heterozygous missense variants in SLC7A7. Ammonia levels were not evaluated during the patients hospitalization.
Conclusions: Lysinuric protein intolerance is a severe metabolic disorder that can present with protean systemic features including primary immunodeficiency. Impaired lymphocyte function, hypocomplementemia, immune-mediated glomerulonephritis, autoantibodies, and HLH are known complications of LPI. Exactly how ineffective amino acid transport triggers these systemic inflammatory features is not yet understood. LPI should be considered in the differential diagnosis of early-onset SLE, particularly in the absence of response to immunosuppressive therapy.

**Submission ID#420852**

**Molecular Characterisation of Chronic Granulomatous Disease (CGD) Patients in a Cohort of India**

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Introduction/Background: Chronic Granulomatous Disease (CGD) is a primary immunodeciency disorder with recurrent pyogenic infections and granulomatous inflammation resulting from loss of phagocyte superoxide production. Mutations in any one of the five structural genes of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex viz. CYBB and CYBA encoding for membrane bound gp91phox and p22phox; and NCF1, NCF2, and NCF4 encoding for cytosolic components p47phox, p67phox, and p40phox respectively, have been found to cause CGD. The relative incidence of these gene defects varies significantly depending on the ethnic background of the population. Identification of molecular defect is important for patient management as well as for prenatal diagnosis in the affected families. The present study was aimed at studying the pattern of underlying genetic defects in a cohort of Indian patients affected with CGD.
Objectives
1.To identify the underlying genetic defect in patients with chronic granulomatous disease in India
2. Clinical,Immunological and molecular characterisation
3. To utilise this information for genetic counselling and prenatal diagnosis of the affected families
Methods: Eighty- seven (n=87) patients with abnormal NBT and DHR were included in this study. In case of male patients, mothers were first screened for carrier status to rule out X- linked CGD (XL-CGD). Those patients where mother is not showing mosaic pattern were suspected for autosomal recessive CGD (AR-CGD) and were screened for the ratio of NCF1 gene to pseudo NCF1 gene by Genescan analysis. Additionally, evaluation of NADPH oxidase components expression by flow cytometry also helped us to determine the underlined genetic defect and it is validated by DNA sequencing of respective genes.
Results: Eighty-seven patients were molecularly characterized to identify disease causing mutation which includes: 12 novel mutations in CYBB, 1 in CYBA, 2 in NCF2 gene in our cohort.
29.8% (n=26) of the patients belonged to XL-CGD. 58.6% (n=51) of the patients are suspected to have NCF1 gene defect among which; homozygous DelGT mutation was identified in 39 patients. 6.8% and 4.5% patients showed abnormal p22phox and p67phox expression suggesting defect in CYBA gene and NCF2 gene respectively. Spectrum of mutations involve: 41% of DelGT mutations, 15% nonsense, 14% missense, 10% deletion, 2% insertion, 14% other than homozygous DelGT mutations. Male to female ratio is 1.87:1. Consanguinity is noted in 30% of the patients.
Conclusions: Despite the male predominance AR-CGD is more common (70%) as compare to XL-CGD (30%) in this cohort of Indian patients, which is distinct from the western data. 17% are the novel mutations suggesting, a wide heterogeneity in the nature of mutations in Indian CGD patients. Flow cytometric evaluation of NADPH oxidase component is used as a secondary screening test to identify CGD sub-group. Molecular characterisation of CGD genes was not only used in the confirmation of diagnosis but also in genetic counselling and pre-natal diagnosis in affected families.

**Submission ID#428351**

**Novel NLRC4 Gain-of-function mutation presenting with neonatal enterocolitis and autoinflammation, with positive clinical response to Rapamycin and Anakinra.**

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| Suspected CGD subtype | % out of 87 patients | Molecularly Characterized patients |
|---|---|---|
| $gp91^{phox}$ (CYBB:X linked) | 29.8% | 26 |
| $p22^{phox}$ (CYBA: AR) | 6.8% | 06 |
| $p47^{phox}$ (NCF1: AR) | 58.6% | 51 |
| $p67^{phox}$ (NCF2: AR) | 4.5% | 04 |

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Introduction/Background: The patient is a 6-month-old boy, born at 35+4 weeks gestation to non-consanguineous parents of Italian origin. He was admitted to the Intensive Care Unit at 12 days of age with profuse bloody diarrhoea, weight loss, severe metabolic acidosis and acute renal failure. He had a rapid respiratory deterioration necessitating intubation, ventilation and inotropic support.
The patient developed features of macrophage activation syndrome with: (i) prolonged fever > 38.5° C, (ii) hepatosplenomegaly, (iii) bicytopenia (anaemia and thrombocytopaenia), (iv) hypertriglyceridemia, (v) high ferritin (14,7000) and (vi) haemophagocytosis on bone marrow smear. He also presented with three interesting features (i) a macular erythematous rash that slowly resolved and was replaced by reticulo-livedoid rash, (ii) a marked hypereosinophilia and (iii) no significant elevation of HLADR/CD8+ T cells on lymphocyte immunophenotyping (7-15%). The patient underwent rectal biopsy which confirmed the presence of eosinophils, but without significant inflammation or architectural changes. Stool microscopy showed presence of partially necrotic intestinal epithelial cells.
Immune work up demonstrated global T lymphopaenia without balanced subpopulation and eliminated a familial hemophagocytic lymphohistiocytosis (normal perforin and CD107a expression on CD8+ T-cell and NK cells). Circulating FOXP3+ CD25+CD127lowCD4+ T cells were within normal range. A Dihydrorhodamine Reduction Assay was normal.

Subsequent genetic analysis identified the presence of a de-novo heterozygous mutation in the nucleotide binding domain of NLRC4 (c.1021G>C, p.Val341Leu). A mutation involving this amino-acid position has already been described but with a different substitution pattern in a boy and his father who presented with MAS (p.Val341Ala) (1) . In order to prove the causality of this mutation, our team generated THP-1 cell lines expressing the two different mutations through gene-editing with the CRISPR system. In this system the mutation p.Val341Leu, as well as the p.Val341Ala mutation, were responsible for spontaneous activation of Caspase1 , as evidenced by FLICA assay.

The patient was treated with IV Methylprednisone 2mg/kg/day. He continued to have progression of his inflammatory state, and was therefore commenced on Anakinra. Following confirmation of mutation, he was started on rapamycin, reasoning that (i) through autophagy induction, Rapamycin could potentiate the action of Anakinra (2,3) and (ii) through mTOR inhibition counteract the effect of IL-18 on T-cells (4).

With a combinatory therapy of Anakinra up to 15mg/kg/day and Ramapycin (with trough levels of 10-15 ng/L), the patient showed a marked clinical improvement, allowing weaning of steroids and establishment of enteral feeds. Ferritin levels reduced to 800-1000 ng/mL. We observed a significant decrease in IL-18 plasmatic level following treatment initiation (pre- vs post-treatment levels of 82844 pg/ml and 10055 pg/ml, respectively).

We report a novel NLRC4 gain-of-function mutation, presenting with neonatal enterocolitis and autoinflammation with improvement under combinatory therapy of Anakinra and Rapamycin. To our knowledge this is the first case to report the use of Rapamycin in this disease, with what appears to be encouraging results. Further studies are required to elucidate the potential role of Rapamycin in the management other inflammasome disorders.

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**Submission ID#427863**

**Aberrant T cell activation and exhaustion develops in poorly reconstituted SCID survivors after transplant and correlates with the absence of conditioning regimen: a Primary Immune Deficiency Treatment Consortium (PIDTC) study.**

Berthe C*, Bourbonnais S*, Parrott RE, St-Denis A, Dela Cruz M, Chan W, De Waele D, Joly JA, Vallée A, Griffith LM, Kohn DB, Notarangelo L, Shenoy S, Craddock J, Pai SY, Chaudhury S, Chandra S, O'Reilly R, Cowan M, Haddad E, Puck J, Buckley R, Le Deist F, Decaluwe H (presenting author).

Introduction/Background: Allogeneic hematopoietic stem cell transplantation (HCT) is currently standard treatment for patients with severe combined immunodeficiency (SCID), with 2-year overall survival >90% for typical SCID (Heimall Blood 2017). Previous studies revealed that poor clinical outcomes correlated with poor long-term T cell reconstitution, including low CD4 T cell counts and low naïve CD45+ T cell counts (Pai NEJM 2014). We hypothesized that T cells developing in a poorly reconstituted immunologic environment would show features of chronically activated T cells with increased expression of inhibitory co-receptors. We further hypothesized that the intensity of the conditioning regimen would correlate with the expression of inhibitory co-receptors.

Objectives: To characterize the T cell phenotype of SCID patients at >2 years post-HCT and to investigate the impact of conditioning regimen on the quality of T cell reconstitution and the expression of inhibitory co-receptors

Methods: We analyzed 34 SCID patients 3-28 yrs (median 14 yrs) after HCT. We excluded from the analysis patients with chronic graft-versus-host disease (GVHD), chronic DNA viral infections or patients who had received donor lymphocyte infusion or boost in the 6 months prior to study. SCID genotypes (n) included IL2RG/JAK3 (20), Rag1/Rag2/DCLRE1C (5), ADA (2), IL7R (1) and other/unidentified (4). Nine patients had received a reduced intensity (RIC) or myeloablative (MAC) conditioning regimen, while 25 had received either no conditioning or immunosuppression only (None/IS). Poor T cell reconstitution was defined as CD4 T cell counts below 500 cells/mm3 (21 patients). T cell phenotype, including expression of inhibitory co-receptors, was assessed by flow cytometry.

Results: Compared to patients with CD4 counts above 500 cells/mm3, patients with low CD4 counts had low naïve CD45RA+CCR7+ T cells ($p=0.0004$) and high CD45RA-CCR7- T effector memory (TEM) cells ($p=0.02$), low numbers of naïve thymic CD45RA+ CD31+ T cells, low numbers of TRECs and a less diverse T cell repertoire ($p<0.0001$). Additionally, they had an increased frequency of CD8 T cells expressing PD1 (8% vs 3%), CTLA4 (5% vs 1.5%), CD160 (25% vs 5%, $p=0.01$), and 2B4 (63% vs 29%, $p=0.0001$) inhibitory co-receptors. Increased inhibitory receptor expression was associated with a differentiation profile skewed toward a TEM phenotype, reduced T cell diversity, increased markers of T cell activation, and the development of a highly exhausted CD39high PD-1high T cell population. More importantly, a fraction of CCR7+ CD45RA+ CD8 T cells expressed PD1 (6% vs 1%, $p=0.03$) and 2B4 (27% vs 4%, $p=0.01$) in patients with low CD4 counts, suggesting that some naïve T cells of poorly reconstituted patients were chronically activated. Inhibitory receptor expression did not increase with HLA disparities between the donor and recipient, a history of GVHD after transplant or the infection status of the patient prior to transplant. However, inhibitory co-receptor expression was correlated with conditioning regimen, with increased frequency of 2B4+ CD8 T cells in unconditioned patients (54% vs 28% in None/IS and RIC/MAC patients respectively, $p=0.01$). Conversely, RIC/MAC conditioning was associated with higher naïve CD8 T cell numbers ($p=0.01$), higher naïve thymic CD45RA+ CD31+ T cell numbers ($p=0.001$), a more diverse T cell repertoire ($p=0.001$) and low expression of inhibitory co-receptors. RIC/MAC conditioning was also associated with improved naïve T cell generation and limited expression of inhibitory receptors in IL2RG/JAK3 patients (23% vs 50% for 2B4, 1% vs 11% for CD160 in RIC/MAC versus None/IS IL2RG/JAK3 patients respectively), a genotype permissive to T cell engraftment.

Conclusions: Collectively, our results suggest that the expression of inhibitory co-receptors may be a biomarker of poor T cell reconstitution in transplanted SCID patients. Further, we propose that lack of conditioning limits T cell reconstitution, which correlates with increased expression of inhibitory receptors on circulating CD8 T cells. Antibodies targeting inhibitory receptors are now available in clinical trials to treat cancer and viral infections. It will be necessary to evaluate the relationship between inhibitory receptor expression, T cell function and clinical outcome, to see if a selected group of SCID patients could benefit from these immunotherapies.

Additional authors of this work are:

De Waele D, Dela Cruz M, Chan W, Joly JA, Vallée A, Griffith LM, Kohn DB, Notarangelo L, Shenoy S, Craddock J, Pai SY, Chaudhury S, Chandra S, OReilly R

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**Submission ID#419003**

**Clinical, imaging, and pathology features of cytotoxic T-lymphocyte antigen 4 haploinsufficiency associated neuroinflammation**

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Introduction/Background: Cytotoxic T-lymphocyte antigen-4 (CTLA-4) is an essential negative regulator of the immune response and its function is critical for immune homeostasis. Uni-allelic mutation in the CTLA4 gene leading to reduced function or expression of CTLA-4, termed CTLA-4 haploinsufficiency, can lead to systemic immune dysregulation with wide spread clinical disease, but with variable clinical penetrance. The neurological manifestations of CTLA-4 haploinsufficiency are not known.
Objectives: To perform detailed phenotyping of the neurological manifestations of CTLA-4 haploinsufficiency.
Methods: A retrospective review and prospective collection of clinical, imaging, cerebral spinal fluid, and pathological specimens was performed in a cohort of genetically confirmed patients (N=50) with CTLA4 mutations who are followed at the National Institutes of Health. Neurological symptoms and exams were collected on patient visits and from historical records. The data collected included 289 brain MRIs and 53 spinal cord MRIs that were visually inspected for evidence of inflammation. Cerebral spinal fluid values were obtained from 14 patients including flow cytometry in 10 patients. Pathological tissue from brain biopsies of inflammatory lesions was examined from 10 patients.
Results: Central nervous system (CNS) inflammation was found in 14/50 (28%) of the cohort. Common clinical manifestations from the 14 patients with CNS inflammation were headaches 13/14 and seizure 8/14. Focal deficits were rare. MRI findings included contrast enhancing neuroinflammatory lesions in the brain 14/14, brainstem/cerebellum, and spinal cord 8/12. Figure A-C show representative inflammatory lesions. Lesions were multifocal in 13/14 patients and 12/14 had recurrent inflammatory lesions on longitudinal follow up. Lesions were, at times, extremely large, 5/14 with a lesion > 25 cm^3. Leptomeningeal enhancement (LME) was seen in 10/13 patients and clearly preceded intraparenchymal lesion development in 3 patients. Figure D shows a site of LME (green chevron) that develops into an intraparenchymal lesion (yellow chevron), MRIs are separated by 11 days. Spinal fluid analysis showed a lymphocytic pleocytosis (mean 32 cells/mm^3) with the presence of oligoclonal bands in 6 patients. Pathological features included a mixed cellular infiltrate, predominantly lymphocytes or plasma cells, with little evidence of demyelination or necrosis (figure E).
Conclusions: The neurological manifestations of CTLA-4 haploinsufficiency include recurrent and, at times, severe neuroinflammation. However, even large lesions and lesions in eloquent anatomical locations had little to no focal clinical defects resulting in a striking clinical-radiological dissociation. Future studies into the mechanisms of CNS-related disease may reveal important information related to peripheral and central immune system functioning.

**Submission ID#420376**

**Conditioning with anti-CD45 immunotoxin in a mouse model of hypomorphic Rag1 deficiency allows complete reconstitution of the immune system with lack of toxicity**

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Introduction/Background: Hematopoietic stem cell transplantation (HSCT) has been used for the treatment of hematologic malignancies and primary immunodeficiencies (PID), for several decades with increasing efficacy. However, toxicity related to conditioning regimens based on the use of chemotherapy and/or irradiation to ensure engraftment of donor cells, remains a significant problem. Recently, an alternative, potentially low-toxicity, approach has been proposed, which makes use of an immunotoxin targeting CD45-expressing cells, which include HSC and more mature leukocytes. This approach is particularly attractive for leaky forms of Severe Combined Immune Deficiency (SCID), with residual production of dysfunctional T and/or B cells, such as atypical forms of RAG deficiency.
Objectives: We have developed a mouse model carrying a hypomorphic mutation in the Rag1 gene (p.F971L) resulting in a combined immunodeficiency with signs of autoimmunity, recapitulating the phenotype seen in patients.
Methods: Using this model, we have tested the efficacy of conditioning with an anti-CD45 immunotoxin (CD45-SAP) alone or in combination with low irradiation (200rads; CD45-SAP/200), and compared these regimens to a myeloablative dose of irradiation (800rads) [NIAID protocol LCIM 6E]. Following conditioning, the mice were transplanted with wild-type (WT) bone marrow (BM) lineage-negative cells and followed over time to evaluate immune reconstitution.
Results: Conditioning with CD45-SAP alone or with CD45-SAP/200 led to a consistent engraftment of donor T and B cells in the peripheral blood (PB) of F971L that increased overtime, reaching 90% donor chimerism at 16 weeks. Myeloid (CD11b+) and NK cells in PB of F971L mice also showed high level of donor engraftment that remained stable at around 70% in CD45-SAP

treated mice and 80% in CD45-SAP/200 treated mice. At sacrifice, in the BM we observed strong selective advantage for donor B cells at all developmental stages, but in particular in the most mature subsets, in both CD45-SAP and CD45-SAP/200 treated mice, rescuing the block in development at pre-B cell stage found in untreated F971L mice. Donor engraftment in BM HSC reached levels around 70% and 80% in CD45-SAP and CD45-SAP/200 treated mice, respectively. Donor chimerism in T and B cells in the spleen was also higher than 90% in both CD45-SAP and CD45-SAP/200 groups. In the thymus, full donor chimerism was achieved in both CD45-SAP and CD45-SAP/200 treated mice, starting at the DN4 stage and persisting at DP, SP4 and SP8. Importantly, in both treatment groups, T cell development was corrected both in terms of subset distribution and absolute numbers to levels comparable to those of WT mice. Finally, the thymic epithelial cell compartment was also fully reconstituted, with a normal number, distribution and maturation of both cortical and medullary thymic epithelial cells.
Conclusions: In conclusion, we show here that conditioning with CD45-SAP immunotoxin, alone or in combination with 200rads TBI, is safe and leads to full reconstitution of the immune system in Rag1 hypomorphic mice, suggesting that this conditioning regimen should be considered for testing in clinical setting.

**Submission ID#427120**

**FOXP3 Gene Transfer in T cells and FOXP3 Gene Editing in HSC as Novel Treatment Options for IPEX Syndrome**

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Introduction/Background: FOXP3 is a key transcription factor for the maintenance of immune tolerance. FOXP3 mutations result in dysfunction of FOXP3+ regulatory T cells (Tregs) causing Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX) syndrome, a severe early onset autoimmune disease, which can be fatal if not promptly diagnosed and treated. Our recent international study analyzing the long-term outcome in 96 I.E. patients of the two currently available treatments, pharmacological immune suppression and allogeneic hematopoietic stem cell (HSC) transplantation, showed poor long-term disease-free survival or overall survival limitations, respectively (Barzaghi F. et al, JACI, 2017). IPEX syndrome is a good candidate for gene therapy as it has been demonstrated that reconstitution of wild-type Treg cells can control the disease. However, FOXP3 expression is highly regulated, and its safe and physiological expression in Treg and Teffector (Teff) cells is challenging. Lentiviral-mediated (LV) FOXP3 gene transfer successfully converts IPEX patients-derived CD4+ T cells into Treg-like cells (CD4LV-FOXP3 T cells) with stable suppressive capacity (Passerini L. et al, Sci Transl Med, 2013). These ex vivo converted Tregs are ideal as a short term cell-based therapy for IPEX patients, but this approach does not re-establish regulated FOXP3 expression in Teff cells, that also likely contribute to the IPEX pathology. Thus, we are further characterizing CD4LV-FOXP3 T cells and, at the same time, developing gene editing strategies for IPEX, whereby autologous T cells or HSCs are genetically modified or corrected, respectively, and reinfused into the patients.
Objectives: To provide more effective treatments for IPEX patients, we are i) optimizing LV-FOXP3 gene transfer in T cells to be suitable for clinical use, and ii) establishing a novel FOXP3 gene editing in HSCs and testing both approaches in preclinical models.
Methods: LV-FOXP3 gene transfer can be obtained in CD4+ T cells activated polyclonally or in an antigen-specific manner. The vector construct is bidirectional, FOXP3 expression is under the EF1a-promoter and the truncated form of NGFR, used as marker gene, is under CMV promoter. FOXP3 gene editing is performed using a combination of CRISPR/Cas9, a chemically modified sgRNA targeting FOXP3, and an AAV6 packaged homologous donor DNA template and the efficacy and safety of the resulting construct is tested in different cell types in vitro and in humanized mice.
Results: We demonstrate that CD4LV-FOXP3 T cells can successfully be generated specific to different antigens. This result opens to new potential clinical benefit of CD4LV-FOXP3 T cells with more safe and specific regulatory effect than polyclonal CD4LV-FOXP3 T cells. We are currently adapting the protocol to optimal in vitro production for clinical use and assessing dose, survival and efficacy of the CD4LV-FOXP3 T cells using different in vivo models.
Due to the wide distribution of identified mutations throughout the FOXP3 gene, we have designed a gene editing strategy that uses homology directed repair to insert the coding sequence of the FOXP3 gene at the start codon of the endogenous mutated FOXP3 gene. This strategy permits regulated expression of the inserted wild-type, functional FOXP3 protein in patient cells independent of the location of the downstream mutation. Using this site-specific gene knock-in, we find that the system effectively targets expression of FOXP3 in different cell types, namely Tregs, Teff cells and primary human cord blood- or bone marrow-derived HSCs. Gene editing of normal donor and IPEX Tregs and Teff cells allowed us to test for regulated gene expression and for establishment of normal Treg suppressor function and T cell proliferation upon activation. Additionally, preliminary results demonstrate that gene edited HSCs can be transplanted into NSG mice for long-term reconstitution.
Conclusions: Our results show the feasibility of different gene therapy approaches for IPEX syndrome. In addition, they suggest that CD4LV-FOXP3 T cells, either polyclonal or antigen-specific, could be applied not only in IPEX but also in immune mediated diseases of different origins. The results from the FOXP3 gene editing support the use of CRISPR/Cas9 to treat IPEX syndrome patients with autologous edited HSCs. This gene editing approach may also be applied to treat other pediatric monogenic blood and immune disorders.

**Submission ID#421054**

**Human PI3Kgamma deficiency with humoral defects and lymphocytic infiltration of barrier tissues**

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Introduction/Background: The phosphatidylinositol 3-kinase (PI3K) signaling pathways play a key role in transducing signals from a diverse array of stimuli by producing the PIP3 second messenger. Class IB PI3K is primarily activated by G protein-coupled receptors (GPCRs), and this class is comprised of the p110gamma catalytic subunit in complex with the p84 or p101 regulatory subunit. In contrast to the Class IA PI3K subunits, inherited mutations in the genes encoding the Class IB subunits have not been described.
Objectives: Given the leukocyte-restricted expression pattern of p110gamma, we hypothesized that mutations affecting this kinase may be found in cohorts of patients with rare immunodeficiency disorders. Our objective was to identify such mutations and determine molecular, biochemical, and cellular derangements in patients with mutated p110gamma.
Methods: We used whole-exome sequencing of families to identify inherited gene mutations and determined the mechanistic basis of disease using biochemical assays to assess effects on protein function and cell-based assays to define functional defects with disease relevance.
Results: We identified a patient (here called A.1) harboring compound heterozygous mutations in PIK3CG, the gene encoding p110gamma, who presented in early life with autoimmune cytopenias and eczema and, at the age of 9 years, developed cryptogenic organizing pneumonia and prominent T cell infiltration of the lungs. She also has a history of skin infections, lymphadenopathy/splenomegaly, eosinophilia, defective antibody production, and more recently, lymphocytic colitis. She inherited a frameshift PIK3CG mutation from her mother and a missense mutation resulting in an R1021P amino acid substitution from her father. Expression of p110gamma protein was lost, and stability of its p101 binding partner was reduced. Despite defective T cell signaling responses to chemokines (i.e., GPCR stimulation), chemotaxis of patient T cell blasts in vitro was normal. Intriguingly, the frequency of peripheral blood Treg cells was low in patient A.1, and her CD4 T cells more frequently expressed the tissue-homing CXCR3 chemokine receptor. Consistently, serum levels of CXCR3 ligands were elevated in patient A.1. Moreover, we found augmented inflammatory cytokine production from M1-polarized macrophages differentiated from patient A.1 monocytes or from THP1 cells treated with p110gamma inhibitor or stably expressing PIK3CG shRNA.
Conclusions: We report the first human with loss of PI3Kgamma activity and present her clinical presentation with notable T cell infiltration of barrier tissues. Based on our analyses, we propose that loss of p110gamma activity in humans causes T cell-intrinsic effects of reduced Tregs and increased tissue-homing propensity and the T cell-extrinsic effect of augmented inflammatory responses in macrophages. Together, these consequences of p110gamma deficiency drive aberrant accumulation of T cells in lung and gut.

**Submission ID#428730**

**Impact of pulmonary complications on quality of life in the USIDNET registry**

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Introduction/Background: Pulmonary disease is a frequent complication across many primary immunodeficiencies (PIDDs), however its impact on the quality of life (QoL) in PIDDs is not well characterized.
Objectives: To ascertain the types of infectious and non-infectious pulmonary complications occurring in PIDDs and to determine how these complications affect QoL.
Methods: We analyzed the pulmonary complications, disability descriptions, and clinical status of 3610 subjects with PIDDs in the USIDNET registry using descriptive statistics. Karnofsky or Lansky performance indices (n=1267) and PROMIS29 QoL data (n=120) were also analyzed. The t- test/Mann-Whitney test and Chi square test were utilized to compare continuous and categorical variables, respectively.
Results: Infectious pulmonary disease was reported in a majority of subjects (52.2%), most commonly pneumonia (42.3%) and bronchitis (17.9%). Non-infectious pulmonary disease was reported in 30.8% of all subjects, most commonly asthma/reactive airway disease (21.7%), bronchiectasis (7.0%) and interstitial lung disease (4.2%). Pulmonary insufficiency was listed as a cause of disability in 4.3% of all subjects with PIDDs, with highest rates of this disability in subjects with immune dysregulation (15.6%). Lower Karnofsky/Lansky performance scores were observed in subjects with pneumonia, lung abscess, bronchiectasis, interstitial lung disease, and emphysema/COPD as compared to without these disorders ($p<0.001$). PROMIS29 QoL metrics were largely similar among subjects with and without pulmonary disease, although physical function scores were significantly worse in those with COPD/emphysema (mean= 37.5 +/- 5.1) as compared to without (mean= 43.6 +/- 8.4, $p=0.007$). PROMIS29 physical function scores were also worse in subjects with non-infectious pulmonary disease (mean =40.3 +/- 6.81) compared to those with infectious pulmonary disease only (mean =45.2 +/- 8.53, $p=0.046$). A significantly greater percentage of patients with a history of COPD/emphysema (24.0% vs. 8.95%) or interstitial lung disease (16.7% vs. 8.9%) were deceased as compared to those without a history of these disorders ($p<0.0001$).
Conclusions: Both infectious and non-infectious pulmonary disorders cause significant morbidity in PIDDs and are associated with higher mortality in this population. Infectious and non-infectious pulmonary complications were often associated with worse Karnofsky/Lansky scores while there was limited impact on PROMIS29 QoL measures.

**Submission ID#408977**

**Latin-American Consensus on the Management of Patients with Severe Combined Immunodeficiency, part 1: Supportive Measures During the Time from Diagnosis to Definitive Treatment.**

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Introduction/Background: Primary immunodeficiencies (PIDs) are more than 300 congenital diseases, caused by monogenic defects compromising the function or development of the immune system. Severe combined immunodeficiencies (SCID) represent the most lethal form of PIDs with mortality rates >95% within the first two years of life without treatment. Hematopoietic stem cell transplant (HSCT), gene therapy and thymus transplantation are the only curative treatments available. The best known prognostic factors for treatment success are the age at diagnosis, age at HSCT transplant and the comorbidities that develop in-between. In developed countries and under ideal conditions, curative treatment obtains 95% success rates. Socioeconomic and cultural conditions of Latin-American countries

result in a majority of patients with late diagnosis, more comorbidities and reduced access to curative treatments. The interventions during such period are vital to keeping optimum health status to improve the probability of success of curative therapies. Many interventions are not supported by clinical trials, are based mainly on clinical experience, and there are no clinical guidelines to standardize such treatments.
Objectives: To generate a consensus on the supportive care of patients with SCID, from the diagnosis until a curative treatment is given, under a Latin-American perspective taking into account particular challenges for our region.
Methods: In a first step, we gathered available information about SCID diagnostic and therapeutic guidelines from two sources: a) Literature search and b) Personal communications with PID experts from Europe and USA. Next, we developed an expert consensus through a modified Delphi technique (electronic and anonymous). We used Google® Forms® to gather the information and Microsoft Office Excel® for the analysis of agreement through kappa coefficient and rounds concordance through repeated measures analysis of variance (ANOVA).
Results: We gathered an expert panel of 34 subjects from 6 Latin-American countries (Argentina, Brazil, Chile, Costa Rica, Mexico, and Peru) including the primary centers caring for SCID patients. We generated a document with 123 agreed diagnostic and therapeutic interventions grouped in 8 topic-domains (i.e. protective and isolation methods to decrease the risk of infections, antimicrobial prophylaxis, immunoglobulin treatment, immunizations, nutritional aspects, antimicrobial treatment, blood derivatives use, routine laboratory workup, imaging and other studies, conventional multidisciplinary approach). We also included 38 non-agreed interventions, but where relevant arguments are shared, to allow for particular clinical scenario decisions.
Conclusions: This is the first document of its type, and it intends to standardize clinical care of Latin-American patients with SCID, reduce disease burden and ultimately improve health outcomes. We see this effort as a starting point for the continuous improvement of our professional care to such patients and is intended to help as a tool not only for immunologists but for primary care physicians and other specialists involved in SCID patient's care. This work will hopefully be published during 2018 as a LASID collaborative work, and it will help as a guide for clinicians caring for SCID patients not only in Latin America but in other world regions. Also in the future, this consensus may be improved by collaboration from immunologists worldwide.

**Submission ID#419361**

**Neurologic Complications of Common Variable Immunodeficiency: Findings From the USIDNET Registry**

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Introduction/Background: Though rare, neurologic manifestations have been described in a small number of CVID cases. Major categories of neurologic manifestations previously reported in the literature include infection, autoimmunity, neuroendocrine disorders and nutritional deficiency. (Nguyen, J Clin Immunol. 2016)
Objectives: Here, we report the prevalence of neurologic complications in CVID from the USIDNET Registry and investigate its associated factors.
Methods: Investigators obtained demographic, laboratory, and clinical data on CVID patients within the USIDNET Registry. Neurologic diagnoses were identified and subsequently categorized into major categories previously identified in the literature.
Results: Of 1227 CVID patients, 8% (93/1227) had neurologic infections (4.9%, 60/1227), neurologic autoimmune disease (1.4%, 17/1227) and neuroendocrine disorders (1.3%, 16/1227). Nutrient deficiencies causing neurologic symptoms were not observed. Neurologic infections were mostly cases of meningitis (70%, 45/60), with rare reports of opportunistic and viral infections. When comparing CVID patients with and without neurologic autoimmune disease, those with neurologic autoimmune disease had higher rates of other autoimmune diseases (64.7% versus 13%, $p < 0.001$), a lower mean age at symptom onset (12 versus 19 years, $p = 0.2$, and lower serum IgA (29 versus 68 mg/dL, $p = 0.001$) and IgM concentrations (33 versus 71 mg/dL, $p = 0.001$). Growth hormone deficiency comprised the majority of neuroendocrine cases (65%, 11/16). Other notable neurologic diagnoses included headache (17%, 211/1227), seizure (5%, 62/1227), cerebrovascular accident (2%, 25/1227), and neurologic tumors (0.4%, 5/1227). In addition to somatic neurologic conditions, many CVID patients (37.7%, 462/1227) had reported diagnoses of depression, anxiety, and post-traumatic stress disorder.
Conclusions: Our findings suggest that neurologic diagnoses are more common in CVID patients than previously recognized. Patients with neurologic autoimmune disease appear to have a more severe phenotype with earlier age at symptom onset. Many CVID patients had depression, anxiety, and post-traumatic stress disorder, highlighting the burden of disease in these patients.
**Submission ID#416443**

**Prophylactic antibiotics vs. immunoglobulin replacement in patients with specific antibody deficiency**

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Introduction/Background: Prophylactic antibiotics (abx) and immunoglobulin replacement (IGRT) are commonly used to treat Specific antibody deficiency (SAD), but the optimal therapy is not established.
Objectives: To compared outcomes (number of infections and hospitalizations) in SAD treated with IGRT vs. prophylactic antibiotics.
Methods: Two-center, retrospective chart review of SAD patients from Jan 2012-May 2017. We excluded patients with Hypogammaglobinemia and/or other immunodeficiency diagnosis. characteristics and treatment were reported, rates of infections/hospitalizations among treatment groups were compared using linear regression model.
Results: 78 SAD patients included. Mean age was 18 years, 54% were females. 22 (28.8%) received prophylactic antibiotics, 45 (57.6%) received IGRT, 11 (14.1%) did not receive any specific treatment. Number of infections decreased from 8.2 (year before treatment) to 2.0 (year after treatment) in prophylactic antibiotics group ($p=0.008$), and from 7.2 to 2.3 in IGRT group ($p<0.001$).

No significant difference in hospitalizations one year before vs. year after treatment for prophylactic antibiotics (p=0.85) or IGRT (p=0.07). Baseline IgG was higher in prophylactic antibiotics vs. IGRT (770.6 vs. 914.4 mg/dL, p=0.03). Sex, severity of SAD, IgG subclasses deficiency, and lymphocyte counts were not significantly different between treatment groups.
Conclusions: Prophylactic antibiotics are not inferior to IGRT in preventing infections in some SAD patients. While, clearly some patients with SAD will need IGRT, our date indicate that larger prospective studies are needed to identify patients who will benefit most from IGRT vs prophylactic antibiotics alone.

**Submission ID#420702**

**PTCRA mutations yield novel T cell immune deficiency**

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Introduction/Background: T cell thymic development is dependent on signals received via the pre-TCR complex and we here report the first case of pre-TCR alpha (PTCRA) autosomal recessive T cell immunodeficiency in an infant with a positive SCID newborn screen (NBS).
Objectives: We sought to uncover the mechanistic links between PTCRA mutations and immune dysfunction.
Methods: The patient was tracked clinically, with serial clinical immunophenotyping and T cell function testing. In addition, we performed deep immunophenotyping with mass cytometry and single cell RNA sequencing to delineate the molecular circuitry underlying her immune phenotype.
Results: The patient presented with T cell lymphopenia and impaired response to mitogen stimulation. HSCT was considered, but she did not meet clinical criteria and remained healthy, so she was watched closely on prophylaxis while awaiting genetic testing. Response to serial mitogen stimulation remained between ~20-100%, response to serial CD3/CD28 activation was normal and TCRV spectratyping was normal. Whole exome sequencing revealed two mutations in PTCRA. No prior human cases of PTCRA deficiency have been published, but a mouse model bears a striking resemblance to this case (Fehling et al, Nature, 1995), with elevated T cells and decreased T cells. Her mitogen stimulation responses became persistently normal around 2 years of age with stable T cell lymphopenia, elevated T cells and normal switched memory B cells. Anti-fungal prophylaxis was halted, and she remained on atovaquone alone with persistent T lymphopenia. She was able to mount an antibody response to rabies vaccine at 2.5 years and was weaned off scIg replacement and is planned to initiate vaccination.
Deep immunoprofiling with mass cytometry (CyTOF) demonstrated a unique immunophenotype. Single cell RNA sequencing confirmed normal CD4 and CD8 TCR clonotypic diversity but increased clonotype diversity in T cells and increased and transcript levels. In addition, CD4 naïve, CD4 memory and CD8 naive T cells demonstrated both increased numbers of expressed genes and transcriptomic diversity, with altered cytoskeletal and TCR proximal signaling pathways across T cell subsets versus control. This may reflect a peripheral role for PTCRA, a durable imprint of thymic signaling events mediated by PTCRA, evidence of homeostatic proliferation or a combination of the above.
Conclusions: SCID NBS led to identification of homozygous variants in PTCRA causing a novel T cell immunodeficiency characterized by T cell lymphopenia, altered proximal TCR and cytoskeletal signaling and increased number of altered T cells. We will continue to pursue the mechanism of these mutations by developing iPSC and studying their T cell differentiation capacity in vitro, as well as further defining her immunometabolic phenotype.

**Submission ID#421217**

**Rag1 hypomorphic mouse mutants show partial preservation of thymocyte development but peculiar abnormalities of thymic epithelial cell phenotype**

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Introduction/Background: The recombination-activating gene (RAG) 1 and RAG2 proteins are essential for V(D)J recombination. In the absence of these proteins, the development of B and T cells is blocked at early progenitor stages, resulting in severe combined immunodeficiency (SCID). Hypomorphic mutations in RAG1, allowing residual activity, result in delayed-onset combined immunodeficiency with residual development of T and B lymphocytes, associated with autoimmunity and/or granulomas (CID-G/AI).
Objectives: To study in details the effect of Rag1 hypomorphic mutations at the early stages of T cell development, we have generated 3 mouse models carrying mutations described in patients with CID-G/AI (R972Q, R972W, F971L) (NIAID animal protocol: LCIM 6E).
Methods: We performed an extensive evaluation of the thymic phenotype in the 3 mouse models.
Results: The number of total thymocytes was found to be drastically reduced in all three models. However, two of these mouse models (R972Q and F971L) retained a significant level of Rag1 activity, and resulted in the development of mature T cells in the thymus, while the mouse model carrying the R972W mutation had minimal Rag1 activity and presented a phenotype more similar to that of complete Rag1 knockout mice. In R972W mice, almost all thymocytes were blocked at the double negative (DN)3 stage and there were virtually no mature T cells, as found in Rag1 ko mice. On the other hand, R972Q and F971L mice presented double positive (DP) and single positive (SP)4 and SP8 cells. The cross talk between T cells and thymic epithelial cells (TEC) in the thymus is fundamental for the development and maturation of both types of cells. In Rag1-/- mice, and consequently in the absence of mature T cells, TECs cannot complete their maturation, and the mTEC subset is virtually absent. These results were also observed in the R972W mouse model. Instead, in R972Q and F971L mice, the residual Rag1 gene activity allows development of a reduced number of mature T cells. Although the number of TECs was markedly reduced in R972Q and F971L mice, cTECs and mTECs were both present, but with an excess of cTECs. Furthermore, mTECs were predominantly MHC-IIhigh

(mTEChi), and only a minority of mTECs were MHC-IIlow (mTEClo) cells, the opposite of what found in adult wt mice. Finally, mTEChi cells from Rag1 mutant mice were found to express AIRE to levels and frequencies comparable to those of wild-type (WT) mice.
Conclusions: Our results show that TEC in mouse models carrying Rag1 hypomorphic mutations are affected both in terms of absolute numbers and in terms of subset distribution and maturation state. To further investigate the functional consequences of impaired cross-talk between thymocytes and TECs in Rag1 mutant mice, we have performed RNAseq in SP4 and TECs sorted from R972Q, F971L and WT mice. Analysis of the gene expression profile in TEC may thus provide novel insights in the mechanisms that govern normal and pathologic thymic T cell development.

**Submission ID#422400**

**Vedolizumab for autoimmune enteropathy in primary immunodeficiency: a case series of outcomes**

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Introduction/Background: Gastrointestinal complications are common in patients with primary immunodeficiency. Infections are the leading cause, but autoimmune enteropathies including inflammatory bowel disease (IBD)-like colitis, sprue-like enteropathy, and nodular lymphoid hyperplasia (NLH) have been recognized in a subset of these patients. To date, there is no established treatment for these noninfectious disorders.
Vedolizumab is a humanized monoclonal antibody that binds to the alpha-4 beta-7 integrin, inhibiting the migration of memory T-lymphocytes across the endothelium into inflamed gastrointestinal parenchymal tissue. It is FDA approved as first-line therapy for inflammatory bowel disease. The safety and efficacy of treating autoimmune enteropathy with vedolizumab in patients with concurrent primary immunodeficiency (PID) has not previously been reviewed.
Objectives: To review the outcomes of a series of patients with hypogammaglobulinemia and autoimmune enteropathy following vedolizumab therapy
Methods: 7 patients (3 male, 4 female) at Mount Sinai with enteric biopsies demonstrating inflammatory enteropathy with T cell infiltrates have been treated with vedolizumab.
Results: Five of the seven patients completed induction therapy. One patient was recently started on therapy. Therapy was aborted in one patient who developed acute hepatitis during induction. Another developed severe cytomegalovirus enteropathy, prompting discontinuation. Two patients discontinued therapy due to response failure. At present, two patients remain on therapy at 12 months with symptomatic improvement.
Conclusions: Vedolizumab was effective in 2 cases, but had no benefit or deleterious side effects in 4 subjects. Its effectiveness in another patient is presently under investigation.

**Submission ID#427846**

**A Novel ATM Mutation Associated with Elevated Atypical Lymphocyte Populations, Hyper-IgM, and Cutaneous Granulomas**

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Introduction/Background: Ataxia Telangiectasia (AT) is an immunodeficiency most often associated with T cell abnormalities and abnormalities in serum immunoglobulin levels, primarily IgA. There is a subset of patients with a hyper-IgM phenotype, some with cutaneous granulomas, which may reflect a distinct clinical phenotype. A 5 year-old female presented for evaluation of concern for immunodeficiency because of frequent illnesses, presumed to be viral. She was found to have an ataxic gait, some speech delay, mild ocular and ear pinna telangiectasia, and an ulcerative rash on the left upper and right lower extremity. Initial blood work showed elevated -fetoprotein levels (50 ng/ml), elevated serum IgM (719 mg/dL), low IgG (<75 mg/dL), and IgA (0.9 mg/dL).
Objectives: To determine if the ATM mutations in this patient are associated with perturbations in the frequencies, distributions and functions of B and T cell subsets which account for the observed phenotype.
Methods: Next generation sequencing was used to identify the mutations in the ATM gene. B and T cells were purified from the patients peripheral blood by positive selection. Intracellular staining for FOXP3 and T-bet was performed. B cells were activated in the presence of polyclonal F(ab)2 rabbit anti-human IgM, multimeric, soluble, recombinant-human CD40L, gardiquimod (TLR7 agonist), or CpG (TLR9 agonist). The Treg suppression assay was carried out by co-culturing CD4+CD25hiCD127lo Tregs and CD4+CD25CD127+ responder T cells at a 1:1 ratio in the presence of beads loaded with anti-CD2, anti-CD3, and anti-CD28 for 4.5 days.
Results: Next generation sequencing revealed two pathogenic mutations in the ATM gene, a novel mutation creating a premature stop codon [c.237delA,(p.Lys79Asnfs370)], and a nonsense mutation [c.3372C>G, (p.Tyr124Ter)]. Proliferative responses of PBMC to mitogens (PHA, ConA, PWM) were reduced to roughly half of the control responses; the response to tetanus was normal whereas the response to C. albicans was absent. Serum cytokine analyses demonstrated elevations in levels of TNF (14.5 pg/mL) and IL-10 (7 pg/mL); levels of IFN, IL-2, IL-5, IL-6, and IL-12 were below the limits of detection. B cell abnormalities included markedly increased percentages of CD38loCD21lo cells (40%) expressing T-bet and Fas. Activation of these CD21/low B cells through the B cell receptor, TLR7 and TLR9, and CD40 was decreased in response to all of the stimuli as evidenced by a lower percentage of B cells expressing the activation markers

CD69 and CD86 relative to healthy control samples. The frequency of unswitched CD27+IgD+ memory B cells was also increased (54%). Among the naive B cells, the proportion of CD19+ CD27CD21CD10+IgMhi transitional B cells that newly emigrated from the bone marrow (BM) was found to be diminished to 1.1% of the naive B cell compartment. In the T cell compartment, there was a decreased frequency of total CD3+ cells but normal absolute numbers of CD3+CD4+ T cells. There was also a decreased proportion of naive CD3+CD4+CD45ROCD62L+ T cells and a striking increase in the CD3+CD4+CD45RO+ memory T cells (90%). This appeared to be largely attributed to the increased proportion of CD3+CD4+CD45RO+CD62L effector memory T cells (63%). The circulating T follicular receptor (cTfh) cell frequency in the patient was 5-fold higher (19%) than the average for healthy donors but ICOS expression levels were normal. Treg frequency was decreased but suppressive capacity was not impaired.
Conclusions: The mutations in ATM described here add to the growing understanding of the heterogeneity in degree and complex nature of the immunodeficiency seen in patients with AT. These mutations resulted in perturbations in frequencies and distributions of normal and atypical B and T cell subsets, which can explain some immunologic aspects of the clinical phenotype in this patient. The immunophenotype seen here may also differentiate AT patients with granulomas from those without cutaneous lesions.
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**Submission ID#428018**

**Constitutive Phosphorylation of DNA Repair Proteins in Specific Lymphocyte Subsets in a Patient with Myb-like, SWIRM, and MPN Domains 1 (MYSM1) Deficiency**

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Introduction/Background: A 10-month-old male presented with pancytopenia, B cell deficiency and developmental delay. He was born at 36 weeks with weight of 2.3kg. He was severely anemic with a Hb of 7.4, and transfused on day 2 of life. He received Hep B and BCG vaccines without complications. A month later he had a Hb of 3.6 with a febrile illness. A bone marrow aspiration performed at 45 days, showed dyserythropoiesis without hemophagocytosis, and normal numbers of precursors. T and B cells were decreased. Further evaluation with repeat bone marrow showed decrease in all 3 cell lineages. Exome sequencing of the family showed homozygous variant in MYSM1 (c.899_902delp. (Lys300Arg/s*11) OMIM: *612176) in the patient. Both parents and HLA-matched sister, were heterozygous for the same variant in MYSM1. Treatment consistent of replacement immunoglobulin, packed RBCs, and G-CSF. There was no history of recurrent viral or severe bacterial infections except for 2-3 episodes of urinary tract infections, which were treated with antibiotics.
Physical exam revealed low set ears, sunken and wide set eyes, depressed nasal bridge, mild micrognathia, frontal bossing, and 1 cm x 1 cm cafe-au-lait spot noted behind left knee. He was pancytopenic with a WBC count ranging 600/mcL to 6300/mcL, and ANC ranging from 10/mcL to 4500/mcL (on intermittent G-CSF). Hb = 7.7 gm/dl requiring transfusions every 3-4 weeks, and platelet count was 91,000/mcL. B cell deficiency was confirmed with total B cell count of 36 cells/mcL. B-cell maturation was essentially normal. T cell counts were normal with age-appropriate distribution of naïve and memory T cells, and T cell function. There was normal T cell receptor repertoire diversity.
Objectives: To assess defect in DNA repair using a flow cytometry-based assay in a patient with MYSM1 deficiency.
Methods: Patients with MYSM1 deficiency are reported to have increased genomic instability. DEB testing, and telomere length analysis revealed normal results. Defects in the DNA repair pathway were assessed using a flow cytometry-based assay measuring phosphorylation of ATM (pATM), SMC1 (pSMC1) and H2AX (gH2AX) without irradiation, or 1h or 24h after low-dose (2Gy) radiation using a Cs137 source. The analysis was performed in T, B and NK cells.
Results: The patient had higher pATM and pSMC1 in T cells compared to the experimental controls (HC) at 1h post-irradiation. Also, the amount of gH2AX in NK cells was significantly higher than HC at 1h post-irradiation. Interestingly, the patients B cells showed approximately 12% of B cells with constitutive gH2AX even without irradiation, and this subset increased slightly to 16% at 1h after irradiation. The MFI (amount) of gH2AX also increased at this time-point. At 24h post-irradiation, there was normal de-phosphorylation in healthy control lymphocyte subsets. However, the patients T cells did not de-phosphorylate completely and showed higher residual pATM, and pSMC1 in both T and B cells. Also, both T and B cells, at 24h, demonstrated a small subset of T cells (1%) with constitutive gH2AX without irradiation, which increased to 4% after irradiation. There was also an increase in gH2AX MFI in the irradiated sample. In B cells, 6% showed constitutive gH2AX without irradiation at 24h, and this increased to 37% after irradiation, with a corresponding increase in MFI.
Conclusions: In summary, this rapid flow analysis revealed defects in the DNA repair pathway, including higher pATM, pSMC1 and H2AX phosphorylation in T, B and NK cells at 1h post-irradiation. At 24h, only T cells showed a residual subset with pATM expression. But, pSMC1, a downstream target of ATM, revealed higher levels in T, B and NK cells at 24h post-irradiation. This assay, which allows lineage-specific analysis, permitted dissection of DNA repair defects, in individual lymphocyte subsets revealing heterogeneity within the cell subset to radiation susceptibility. The practical benefit of this rapid multi-parameter flow assay is selection of appropriate conditioning regimen for hematopoietic transplantation, as was the case with this patient. This has significant practical implications for treatment of patients with radiosensitive immunodeficiencies.

**Submission ID#427860**

**CTLA-4 haploinsufficiency-associated inflammation can occur independently of T-cell hyperproliferation**

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Introduction/Background: CD28 and CTLA4 provide opposing proliferative signals to T cells. We identified an 18-year-old female subject (S1) with heterozygous deletions of CD28 and CTLA4 and multi-organ inflammatory disease characterized by a lack of T cell infiltrates in affected organs. Inflammatory disease was remarkably responsive to S1 CTLA4-Ig therapy.
Objectives: Our goal was to characterize the immunologic consequences of combined deletion of CD28 and CTLA4, specifically assessing T cell proliferation and Treg function in comparison to patients with ALPS5-associated CTLA4 haploinsufficiency. We further sought to explain how this S1s inflammatory diseases could occur without a pathologic T cell infiltrate and why they were amenable to CTLA4-Ig therapy.
Methods: We performed phenotypic analyses of subject T cells and innate lymphoid cells (ILCs). We functionally characterized subject T cells. We created serum cytokine profiles. We stained and analyzed tissue biopsies.
Results: CD28 and CTLA4 expression on S1 T cells were half that of control T cells. S1 T cells were hypoproliferative. S1 Tregs were scarce and lacked suppressive function similarly to ALPS5 Tregs. S1 Tregs could suppress autologous T responder cells, likely due to their poor proliferative capacity. S1 colonic biopsies featured significantly fewer infiltrating intraepithelial lymphoid cells than biopsies from an ALPS5 patients. Unlike ALPS5 patients whose colonic gland infiltrates were overwhelmingly T cells, S1 intraepithelial lymphoid cells were neither T cells nor B cells, suggesting the presence of ILCs. Indeed, a greatly expanded population of type 3 innate lymphoid cells (ILC3) and prototypical ILC3 cytokines were identified in S1 peripheral blood. ILC3 frequency and cytokine levels decreased in response to treatment with CTLA4-Ig, corresponding with marked improvement in enterocolitis, hepatitis and pericarditis.
Conclusions: We report a novel genetic syndrome of combined CD28/CTLA4 deletion and describe the immunolopathologic correlates of this disease. Dual CTLA4- and CD28-haploinsufficiency results in a phenotype of multi-organ inflammatory disease characterized by ILC3 expansion in the setting of T-cell hypoproliferation and quantitative and qualitative Treg defects. Our patients clinical response to CTLA4-Ig parallels published mouse studies and suggests the existence of additional stimulatory B7 receptor(s) preferentially expressed on ILC3s over conventional T-cell populations.

**Submission ID#399101**

**Diagnosis of radiosensitivity and DNA repair defect in DNA Ligase IV deficiency with a rapid flow cytometry assay.**

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Introduction/Background: DNA Ligase 4 deficiency (LIG4-SCID) is one of several monogenic defects affecting DNA repair, and causing lymphopenia (T-B-NK+) and a radiosensitive SCID (RS-SCID) phenotype. The assignment of a timely diagnosis is vital in the management of patients with RS-SCID. Laboratory assessment of radiosensitivity is laborious, and utilizes fibroblasts (non-hematopoietic) or lymphoblastoid cell lines, and can take several weeks to months for results.
Objectives: We demonstrate for the first time, the application of a flow cytometric-based kinetic analysis of phosphorylated H2AX (H2AX) in lymphocyte subsets, especially NK cells, for the diagnostic assessment of LIG4-SCID.
Methods: Simultaneous measurement of multiple DNA repair markers phosphorylated (p) ATM, SMC1 and H2AX (H2AX) was performed by flow cytometry to assess DNA repair defects in a 3-year-old Korean female. The patient was evaluated for recurrent fevers, chronic respiratory tract infections, chronic diarrhea, and rash. Genetic testing revealed compound heterozygous variants (NM_001098268, c.1341G>T, p.Trp447Cys and NM_001098268, c.1103A>T, p.Asp368Val) in LIG4. Functional assessment (phosphorylation) was measured in T and NK cells (B cells were absent), before irradiation (background control), or after low-dose (2Gy) irradiation (1 and 24 hours).
Results: We observed maximal H2AX generation at 1 hour post-irradiation, with progressive dephosphorylation at 24 hours post-irradiation in healthy controls. The patient showed normal frequencies (%) of T cells and NK cells positive for H2AX (95.62% and 99.40% respectively); (controls (n=2) T cells = 99.29% and 99%; NK cells = 99.6% and 99.11%), but increased intracellular levels (mean fluorescence intensity, MFI) of H2AX (T cells = 50.67 and NK cells = 52.41) compared to controls (T cells = 24.86 and 19.58; NK cells = 41.33 and 35.03) at 1 hour post-irradiation. However, more importantly, at 24 hours post irradiation there was a lack of dephosphorylation in a substantial proportion of lymphocytes (64% of T cells and 99% of NK cells) compared to healthy controls (T cells= 1.75% and 1.51%; NK cells = 9.27% and 17.83%). Further, while there was dephosphorylation of H2AX at 24h in patient lymphocytes as compared to 1h, the amount, as measured by MFI, remained elevated at 24h (T cells = 8.97, NK cells =7.57) compared to controls (T cells =1.71 and 2.25; NK cells = 3.18 and 2.75). The data from pATM and pSMC1 were uninformative for the evaluation of LIG4-SCID.
Conclusions: Flow-based kinetic analysis of H2AX is a useful marker for the diagnosis of LIG4-SCID, and can be performed with a small amount (5cc) of blood, and provides a result in 3-4 days, facilitating rapid assessment of radiosensitivity in this condition.

**Submission ID#414819**

**Human PLCG2 Haploinsufficiency Results in NK Cell Immunodeficiency and Herpesvirus Susceptibility**

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Introduction/Background: Natural Killer (NK) cells are innate cytotoxic lymphocytes critical for the control of DNA viruses. NK cell deficiency (NKD) is a poorly understood disorder that results in severe or recurrent infections with Herpesviruses such as Herpes Simplex Virus 1 (HSV1) and Cytomegalovirus (CMV). However, the genetic causes of disease are unknown in most NKD patients.

Objectives: We aimed to investigate the cause of disease in three patients from two kindreds with recurrent or severe Herpesvirus infections and NK cell dysfunction.
Methods: We used exome sequencing and mass cytometry (CyTOF), as well as traditional immunologic techniques, to investigate the genetic causes, immune cell subpopulations/signaling, and NK cell function of these patients. We additionally used mouse models, CRISPR cell lines and in vitro assays to assess the role of PLCG2 haploinsufficiency in disease.
Results: Kindred A consisted of two patients presenting with HSV1 susceptibility and autoimmunity. Kindred B consisted of one patient with severe CMV myocarditis and adenoviral hepatitis. Both kindreds were evaluated for NK cell function and showed reductions in target killing in spite of normal cytotoxic granule degranulation against the same target. Microscopy analysis suggested that granule mobility was reduced in at least one kindred. CyTOF revealed reductions in PLCG2 phosphorylation after receptor crosslinking in the NK cells of both kindreds. Kindred A also presented with a reduction in naïve B cells without perturbations in immunoglobulin output, B cell memory formation or class switching. Trio whole exome sequencing was performed and revealed rare heterozygous PLCG2 mutations in both kindreds. Functional analysis, as well as mouse and CRISPR models, support a functional haploinsufficiency as a cause for NKD in these patients.
Conclusions: Heterozygous loss-of-function point mutations in PLCG2 have not been previously investigated as a cause of NK cell deficiency or recurrent Herpesvirus infection. Thus, these patients represent a novel immunodeficiency involving PLCG2 haploinsufficiency, NK cell dysfunction, and Herpesvirus susceptibility.

**Submission ID#417178**

**Hypomorphic Rag1 mutations alter the pre-immune repertoire at early stages of lymphoid development**

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Introduction/Background: Human RAG deficiency is associated with a spectrum of clinical phenotypes. While the most severe forms of RAG deficiency manifest with severe combined immune deficiency or Omenn syndrome since the first weeks of life, more recently patients have been identified who present to medical attention at a much older age predominantly with symptoms of autoimmunity and/or inflammation. Many of the mutations associated with this atypical syndrome are found in the C-terminal Domain (CTD) of the RAG1 gene and allow residual development of T and B cells. These patients have an abnormal peripheral T and B cell repertoire, but how this is affected by abnormalities in the composition of the pre-immune repertoire vs. antigen-mediated selection and homeostatic proliferation in the periphery is unknown.

Objectives: In order to investigate whether mouse models with hypomorphic mutations in the RAG1 CTD recapitulate the phenotype observed in patients with CID-G/AI, and to study how these mutations affect repertoire composition, cell selection and survival during T and B cell development, we generated three mouse models carrying homozygous RAG1 mutations (F971L, R972Q, and R972W), corresponding to human mutations (F974L, R975Q, R975W) previously reported in patients with late-onset combined immune deficiency with granuloma and/or autoimmunity (CID-G/AI).
Methods: Mice were generated using CRISPR/Cas9 mediated gene editing. T and B cell development, including apoptosis was studied by flow cytometry. Immunoglobulins in naïve mice, BAFF levels and specific antibody responses were measured by ELISA. Serum IgM autoantibodies were measured using a microarray (UTSW). Analysis of T cell receptor (Trb) repertoire in several sorted T cell populations and immunoglobulin heavy chain (Igh) repertoire in pre-B cells was performed by Adaptive Biotechnologies. In order to be able to detect both DJ and VDJ rearrangements, pro-B cells and spleen B cells were sequenced using high-throughput genome-wide translocation sequencing-adapted repertoire sequencing (HTGTS-Rep-seq). Analysis of Vk-Jk rearrangements in pre-B cells was performed by PCR amplification.
Results: Immunological characterization showed partial development of T and B lymphocytes, with persistence of naïve cells, preserved serum immunoglobulin, but impaired antibody responses and presence of autoantibodies, thereby recapitulating the phenotype seen in patients with CID-G/AI.
By using high throughput sequencing, we identified marked skewing of Igh V and Trb V gene usage in early progenitors, with a bias for productive rearrangements after selection occurred, and increased apoptosis of B cell progenitors. This suggested that more alleles remained in germline configuration. Moreover, in the rearranged Igh loci, the distal V gene segments were preferentially rearranged already at the earliest stages of B cell development, a finding that has not been previously reported. In addition, rearrangement at the Igh locus was impaired, and polyreactive IgM antibodies were detected.
Conclusions: In conclusion, this study demonstrates that hypomorphic Rag1 mutations reported in CID-G/AI cause abnormalities of the primary B and T cell repertoire. These changes may affect survival and selection of T and B cells, and thereby contribute to the immune dysregulation often seen in patients with CID-G/AI.
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**Submission ID#416066**

**Obstetric and Gynecological Outcomes in Patients with STAT3 Deficient Hyper IgE Syndrome**

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Introduction/Background: Autosomal dominant Hyper IgE syndrome (AD-HIES) is a primary immunodeficiency due to loss of function STAT3 mutations. Disease manifestations include recurrent skin and pulmonary infections, eczema, mucocutaneous candidiasis, as well as

various musculoskeletal and vascular abnormalities. Little is known of gynecologic-obstetric complications, but with improved therapies women are living longer making reproductive health more pertinent.
Objectives: To learn more about obstetric and gynecological health in women with STAT3 loss of function.
Methods: We prospectively interviewed and retrospectively reviewed medical records of adult women with AD-HIES evaluated at the NIH between 2000-2017.
Results: Of 61 patients aged 18-66 years (mean 35 years), 47 women were interviewed, and chart reviews were performed on 14. Age of menarche in our cohort was consistent with the national average (13.05 vs. 12.5 years). Five of 30 patients (16.7%) reported having worsening lung symptoms with menstruation, and 14 of 34 participants (41%) reported worsening eczema during menstruation. With regard to routine health maintenance, 21 of 30 women reported having regular cervical cytology testing; 7 (23%) reported an abnormal result. Of these 7, 5 had HPV that responded to treatment or was HPV only not requiring treatment; 2 had reactive changes due to yeast and 1 ASCUS that was subsequently normal. Mastitis and breast abscesses occurred in 14 women. Eleven women reported vulvar cysts/abscesses requiring drainage. Nine women had progestin-releasing IUDs placed, in some to suppress menses-associated vulvar eczema/abscess flares; no infectious complications were reported from progestin IUD use.
Over 30% of women chose not to conceive given underlying disease. Of 16 women with pregnancies, 15 women had 26 live births, 6 of 16 (38%) had miscarriages, and 3 of 16 (19%) experienced recurrent pregnancy loss. Three women whose pulmonary symptoms worsened during pregnancy were diagnosed with progression of parenchymal lung disease post-partum. One woman experienced worsening of skin manifestations. Other reported postpartum complications included one wound infection (after Caesarean) and one hemorrhage leading to hysterectomy.
Conclusions: As women with AD-HIES are living longer, significant infectious and disease-related exacerbations related to both menstruation and pregnancy were observed in this patient population. It is important to focus on maintenance of their gynecologic and obstetric health and carefully monitor for these morbidities. Finally, while these women may choose to attempt pregnancy, the risk of recurrent pregnancy loss and worsening disease warrants discussion.

**Submission ID#425074**

**RNA sequencing identifies Aichi virus 1 as the cause of chronic infection with lymphoproliferation in a patient with X-linked agammaglobulinemia**

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Introduction/Background: Deleterious mutations in the Bruton tyrosine kinase gene (BTK) cause X-linked agammaglobulinemia (XLA) through B cell maturation arrest.
Objectives: We here present an XLA patient with a complicated course in whom we detected Aichi virus 1 (AiV1).
Methods: Case report: The patient was diagnosed with XLA at age 3 years, based on agammaglobulinemia with absent B cells and a known pathogenic mutation in BTK (c82C>T, p.R28C). He had been suffering from recurrent respiratory and gastrointestinal infections since the age of 2 months. He was started on immunoglobulin (Ig) substitution, which resulted in complete control of infections with IgG trough levels at 8 g/L. However, at 6 years of age he developed unexplained fever, refractory temporal epilepsy, hepatitis, progressive nephromegaly with chronic renal failure, splenomegaly, episodic diarrhea and growth failure. Ultrasound identified multiple focal lesions in the liver, spleen and kidney. A liver biopsy showed severe chronic hepatitis with initial perisinusoidal fibrosis. Serial kidney biopsies showed variable oligoclonal cytotoxic T cell infiltrates, suggestive of chronic viral infection. Standard diagnostics failed to reveal a pathogen in blood or stool samples and on biopsies.
Results: We finally resorted to RNA sequencing on a kidney biopsy sample. This technique identified AiV1, a Kobuvirus of the family Picornaviridae, with a high read number. Subsequently PCR confirmed the presence of AiV1 in both liver and spleen. Results for cerebrospinal fluid, blood and feces are pending.
Conclusions: AiV1 is a picornavirus responsible for self-limiting gastroenteritis in humans. Confirmatory PCRs on blood, CSF and stool samples are ongoing. However, given the unequivocal result of the RNA sequencing and confirmatory PCRs in other affected organs, we believe that the complications in this XLA patient can be explained by AiV1 chronic infection. These findings confirm the potential of next generation sequencing techniques to identify infectious agents in patients with primary immunodeficiency.

**Submission ID#421053**

**Characterization and successful treatment of a novel autosomal dominant immune dysregulatory syndrome caused by a JAK1 gain-of-function mutation.**

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Introduction/Background: Janus Kinase 1 (JAK1) plays an essential, non-redundant role in the JAK/STAT signaling cascade, a key pathway in the control of hematopoiesis and immune function. Significant progress has been made in elucidating the role of JAK1, but gaps in our knowledge still persist. To date, somatic gain-of-function mutations in JAK1 have been linked to T-cell acute lymphoblastic leukemia.
Objectives: To understand and treat human JAK1 gain-of-function mutations.
Methods: Research study protocols were approved by our institutional review board. Four members of the family (the affected children and their parents) were enrolled. Written informed consent for genetic testing and participation was provided by the parents for their children. Genetic, bioinformatic, biochemical and immunological investigations were performed.
Results: We describe the first known patients carrying a germ-line gain-of-function mutation in JAK1. The clinical phenotype includes severe atopic dermatitis, markedly elevated peripheral blood eosinophil counts with eosinophilic infiltration of the liver and gastrointestinal tract, hepatosplenomegaly, autoimmunity, and failure to thrive. Functional analysis established the gain of function phenotype caused by the muta-

tion and in vitro studies demonstrated that the enhanced signaling could be controlled by ruxolitinib, an approved JAK1/2 inhibitor. Informed by these experimental data, the patients were treated with ruxolitinib with remarkable improvement in a variety of clinical end-points, including hematological profiles and growth parameters.
Conclusions: This characterization of a human JAK1 gain-of-function mutation expands our current understanding of the role of JAK1 in eosinophil biology, hematopoiesis and immune function.

**Submission ID#428687**

**Chronic Granulomatous Disease, Ornithine Transcarbamylase Deficiency and X-inactivation**

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Introduction/Background: Chronic Granulomatous Disease (CGD) is a primary immune deficiency characterized by defects in the NADPH oxidase enzyme complex resulting in a susceptibility to a narrow spectrum of bacteria and fungi. Mutations in CYBB encoding gp91phox and located at Xp21.1 are associated with the most common form of CGD. Deletions and rearrangements in this region are associated with other genetic diseases such as McLeod Syndrome (XK), Retinitis pigmentosa (RPGR), Duchenes muscular dystrophy (DMD), Ornithine transcarbamylase deficiency (OTC), and X-linked mental retardation (TSPAN7). Patient phenotype depends on the extent and position of the deletion, creating a "contiguous X-chromosome gene deletion syndrome.
Objectives: We report a four-year-old female, who presented with symptoms of X-linked CGD and OTC deficiency with a large, contiguous multi-gene deletion on the X-chromosome.
Methods: We report a four-year-old female, who presented with symptoms of X-linked CGD and OTC deficiency with a large, contiguous multi-gene deletion on the X-chromosome.
The patient is the second born of a set of non-identical triplets from a clomiphene assisted pregnancy at 35 weeks gestation. (weight at birth: 5lbs 2oz). After a 7 day stay in the NICU, and CPAP for one day she was discharged home. By the second month, she began having problems gaining weight and had persistent vomiting. At 3 months, she was admitted with a pneumonia diagnosed as methicillin resistant Staphylococcus aureus by lung biopsy. During that hospitalization, the mother noted an enlarging lesion on the infants left hand which was biopsy proven Serratia marscecens osteomyelitis and was treated with intravenous Cefepime for 6 weeks. At this point a dihydrorhodamine assay (DHR) showed only 15.1% positive cells (NL 80-100%) and she was diagnosed as a carrier of X-linked CGD. Bactrim was initiated for antibacterial prophylaxis but it was discontinued due to recurrent diarrhea; she was unable to tolerate antifungal prophylaxis as well due to liver function abnormalities. She continued having frequent vomiting episodes, irritability, failure to thrive and development delay. Ulcerations in esophagus were confirmed by EGD and colonoscopy, probably related to persistent emesis. Diarrhea was unresolved. At 30 months of life she was admitted with acute encephalitis, a serum ammonia level of 218 and elevated urine orotic acid.
Results: Given the demonstrated carrier status for CYBB and apparent OTC deficiency, comparative genomic hybridization was performed, revealing a deletion from Xp21.1 to Xp11.4 This 3.9MB loss includes 18 genes that are known to cause disease. Since the diagnosis, the patient has been on reduced protein diet, resolving her diarrhea, she has subsequently grown and is on 5th percentile for weight and height. Her DHR is now 28% positive. At 3 years she was diagnosed with cone rod dystrophy. Currently she is on Bactrim for CGD prophylaxis and L-citrulline for the OTC deficiency. She continues to gain weight, and has shown great improvement in her development delay and no new CGD-related infections have recurred.
Conclusions: This case reminds us that X-linked carriers with large deletions may be symptomatic and genetic analysis to determine other affected genes can be important for medical management.

**Submission ID#420697**

**Clinical Characteristics and Genetic Profiles of 40 Patients with Wiskott-Aldrich Syndrome (2004-2016): a Single Center Experience in China**

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Introduction/Background: Wiskott-Aldrich syndrome (WAS) is a rare and severe X-linked disorder with variable clinical phenotypes correlating with the type of mutations in the WAS gene. The long-term prognosis of this syndrome is generally poor, with hematopoietic stem cell transplantation (HSCT) remaining the only curative choice. The syndrome is poorly characterized in China.
Objectives: We retrospectively reviewed patients with WAS referred to our hospital from 2004 to 2016, and summarize their clinical manifestations and genetic features.
Methods: Sixty-four children suspected to be WAS from 62 unrelated families were enrolled in this study. The clinical data of children were reviewed in the present study. Distribution of lymphocyte subsets from peripheral blood and WAS protein (WASP) expression in peripheral blood mononuclear cells was examined by ow cytometry (FCM). WASP mutations were identified by direct sequencing of PCR-amplified genomic DNA
Results: Among 725 patients with Primary Immunodeficiency Diseases (PID), 40 (5.52%) were finally diagnosed as WAS with gene identified. The mean time of diagnosis was 6.25 months (range, 0.4-9.63). The common onset clinical manifestation was diarrhea, and most patients had recurrent upper respiratory tract infection, otitis media, pneumonia, and skin abscess. One patient had Nephrotic syndrome and no patient with malignancy. All patients had classical WAS phenotype with WAS clinical scores 3-5. Total 36 mutations in WASP were identified, including 14 novel mutations. Six patients received HSCT, five survived, with one died because of GVHD. Compared with the other 24 patients without WASP mutations, WAS patients had lower numbers of CD4+ T cells and B cells, and higher EOS and IgE level. There was a negative association between the number of B cells and the WAS clinical scores.
Conclusions: In China, diagnosis of WAS has improved over the last decade, although a much higher number of cases had been expected. Establishing more diagnostic centers dedicated to the care of PID will facilitate early, correct diagnosis and better care of WAS in China.

**Submission ID#426369**

**CMV, EBV and HHV-6 Outcomes in Hematopoietic Stem Cell Transplantation (HSCT) for Primary Immunodeficiency**

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Introduction/Background: Herpesviridae infection after HSCT for hematologic malignancies, specifically cytomegalovirus (CMV), Epstein-Barr virus (EBV) and human herpes virus-6 (HHV-6), have been associated with various outcomes including post transplant lymphoproliferative disorder (PTLD), graft-versus-host disease (GVHD) and mortality (1-3). Patients with primary immunodeficiency may have different incidence and outcomes with respect to Herpesviridae given their differences in age at transplant, conditioning choices and underlying disease susceptibility to this viral family.
Objectives: The objective of this study was to describe the incidence and outcomes of CMV, EBV and HHV-6 post HSCT for the primary immunodeficiency population.
Methods: A single center retrospective chart review of primary immunodeficiency registry patients (Research Ethics Board Protocol no. 1000005598) who received HSCT from January 2000 December 2016 was undertaken. Patients who received gene therapy were excluded. Antiviral prophylaxis was given according to institutional protocol. Demographic and clinical data were collated and analyzed with Microsoft Excel . The primary outcome was incidence and time to DNAemia for CMV, EBV and HHV-6.
Results: Sixty one patients who underwent HSCT for primary immunodeficiency from January 2000-December 2016 were reviewed. Diagnoses are noted in Table 1. The average age of transplant was 18.0 months (range 1.9-93.8). 51 transplant recipients received busulfan and cyclophosphamide conditioning (2 with anti-thymocyte globulin (ATG), 2 with alemtuzumab added), 3 transplants received busulfan, fludarabine and either ATG or alemtuzumab, and 1 received ATG alone. Six transplants were unconditioned. 70.5% (43/61) of patients developed GVHD requiring systemic immune suppression.
The overall incidence of CMV, EBV and HHV-6 post HSCT for primary immunodeficiency was 9.8% (6/61), 59.0% (36/61) and 24.6% (15/61) respectively. In those with severe or profound combined immune deficiency the incidence of CMV was 7.0% (3/43), EBV 53.5% (23/43), and HHV-6 18.6% (8/43). In the 6 patients with chronic granulomatous disease, CMV incidence was 17% (1/6), and EBV and HHV-6 were both 50% (3/6). In recipients with pre-transplant negative PCR for EBV, CMV and HHV-6 (R-), time to DNAemia post transplant is seen in Figure 1.
PTLD was seen in 2 patients with RAG1 and IL2R, both of whom were D+R- for EBV status with EBV DNAemia, and one of whom died attributed to PTLD. Two year mortality for all patients was 23% (14/61), with mortality 24.4% (11/45) for those with either CMV, EBV or HHV-6 DNAemia vs 18.8% (3/16) in those without (p=0.64). Disseminated CMV prior to transplant was attributed to cause of death for one case.
Conclusions: CMV incidence was rare, likely from screening for CMV negative HSCT donors. CMV, EBV and HHV-6 DNAemia was not associated with differences in mortality in this cohort. EBV incidence was common, and PTLD incidence was similar to previously published outcomes for HSCT for other disease (2). The advent of cytotoxic T cell therapy for EBV may help abrogate this risk.

| Immune Deficiency | Number of Patients |
|---|---|
| ADA | 7 |
| Artemis | 1 |
| CD3δ | 2 |
| CGD | 6 |
| CID* | 4 |
| Coronin 1A | 1 |
| DNA ligase IV | 1 |
| IL10Rα | 1 |
| IL2Rγ | 12 |
| IL7Rα | 2 |
| IPEX | 1 |
| Jak3 | 2 |
| Rag1 | 3 |
| RelB | 2 |
| RMRP | 3 |
| SCID* | 8 |
| STAT1 | 1 |
| ZAP70 | 4 |

**Table 1.** Primary immunodeficiency diagnoses of HSCT recipients from 2000-2016.*Underlying genetic etiology not yet found.

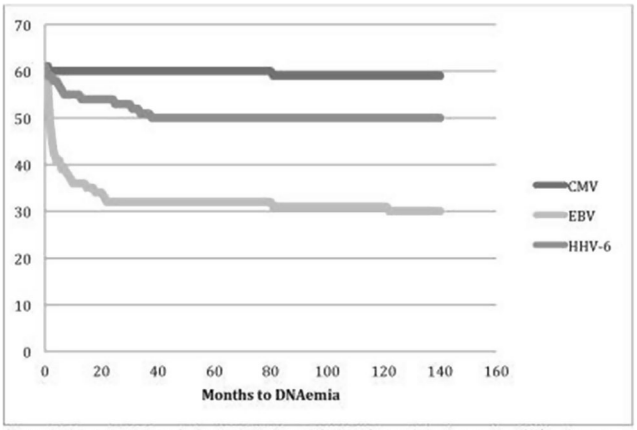


**Figure 1. Time to DNAemia for EBV, CMV and HHV-6 for recipient negative (R-) primary immune deficiency patients post HSCT.**

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**Submission ID#426912**

**Daratumumab controls life-threatening post-HSCT autoimmune haemolytic anaemia**

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Introduction/Background: New-onset AIHA occurs in 2-6% of pediatric patients post-HSCT. Incomplete immune recovery may predispose to immune dysregulation following HSCT including autoimmune cytopenias. Although prednisolone or other immunosuppressive drugs control most episodes, some patients respond incompletely to first or second line therapies including rituximab.
Objectives: We describe an innovative therapy for post-BMT AIHA refractory to proteasome inhibition. Three patients responded to anti-CD38 antibody (daratumumab) therapy after failing treatment with bortezomib.
Methods: We retrospectively evaluated data from three patients treated with Daratumumab for post-transplant AIHA. Patients 2 and 3 were treated according to the positive response reported for Patient 1.
Results: AIHA occurred between 4-9 months following HSCT. Daratumumab was curative in 2 patients, the third one had only transient response and relapsed 5 months after this treatment had been initiated. Following daratumumab patients no longer required any PRBC transfusions.
Conclusions: In potentially life-threatening AIHA in the context of HSCT daratumumab may be an effective rescue therapy in combination with rituximab.

**Submission ID#421664**

**Early post-natal thymus development is strictly dependent on the level of Foxn1 expression in TEC**

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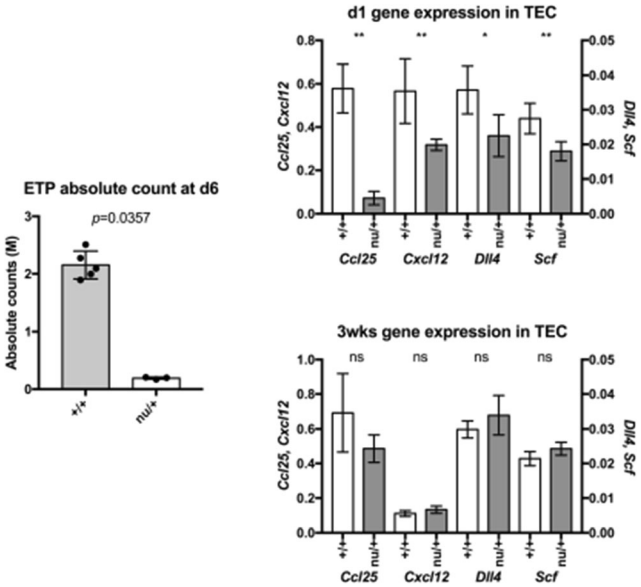
Introduction/Background: FOXN1 is the master regulator gene for the development and maturation of the thymic epithelial cells (TECs). By inducing the expression of chemokine receptors such as CCL25 and CXCL12, FOXN1 also allows the migration of early thymic progenitor (ETP) cells from the bone marrow. Lack of FOXN1 leads to the nude (nu)/severe combined immunodeficiency (SCID) phenotype in humans and mice. Recently, a number of newborns have been identified with low T cell receptor excision circles (TRECs) at birth, associated with heterozygous FOXN1 mutations. These infants present severe T cell lymphopenia early in life, but in most cases their immune system gradually normalizes. However, the role of FOXN1 haploinsufficiency in causing this phenotype is unclear.




Objectives: To analyze T cell development and TEC phenotype in nu/+ mice of various age, in order to investigate whether Foxn1 haploinsufficiency in mice results in impaired thymic development early in life, followed by progressive normalization, thereby recapitulating the human phenotype.
Methods: We analyzed 3 groups of nu/+ and +/+ littermates, divided by age: 1 day, 4-7 days, 3 weeks. The number of ETP and the distribution of cortical and medullary TECs (cTECs, mTECs) were analyzed by flow cytometry. Maturation of mTECs was further assessed by staining for MHC-II and AIRE. Real-time PCR was used to analyze the expression of Ccl25, Cxcl12, Dll4, and Scf, four key Foxn1 target genes. The study was performed in accordance to NIAID animal protocol LCIM 6E.
Results: At 1 day and 4-7 days of life, nu/+ mice showed a dramatic reduction of ETPs, both in terms of frequency and absolute numbers, as compared to +/+ mice. However, by 3 weeks of age, the frequency and count of ETPs were comparable in nu/+ and +/+ mice. A slight but significant reduction in the frequency and absolute count of mTECs was observed in all three groups of nu/+ mice. Additionally, the ratio between mTEC expressing high levels of MHCII (mTEChi) and those expressing low levels of MHCII (mTEClo) was always higher in +/+ mice as compared to nu/+ mice. Moreover, mTEChi cells in nu/+ mice expressed lower levels of AIRE, the gene crucial for thymic negative selection of autoreactive T cells. Finally, as compared to wild-type littermates, nu/+ mice showed reduced thymic expression of Ccl25, Cxcl12, Dll4, and Scf at day 1. Reduced expression of Ccl25 persisted at day 5, but normalized at 3 weeks.
Conclusions: These data indicate that Foxn1 haploinsufficiency in mice leads to impaired thymic colonization by ETPs and abnormalities of TEC differentiation and maturation early in life, followed by progressive normalization, thereby recapitulating what observed in newborns with heterozygous FOXN1 mutations. These observations have important implications for the management of these infants,

who should be monitored closely without rushing to definitive treatment for SCID.

**Submission ID#420864**

**Foxn1 haploinsufficiency leads to defective thymic development early in life implications for interpretation of newborn screening for SCID**

Yasuhiro Yamazaki, MD PhD[1], Marita Bosticardo, PhD[2], Cristina Corsino[3], Luigi D. Notarangelo, MD[4]

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[4]Chief, Laboratory of Clinical Immunology and Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health

Introduction/Background: FOXN1 is the master regulator gene for the development and maturation of the thymic epithelial cells (TECs). By inducing the expression of chemokine receptors such as CCL25 and CXCL12, FOXN1 also allows the migration of early thymic progenitor (ETP) cells from the bone marrow. Lack of FOXN1 leads to the nude (nu)/severe combined immunodeficiency (SCID) phenotype in humans and mice. Recently, a number of newborns have been identified with low T cell receptor excision circles (TRECs) at birth, associated with heterozygous FOXN1 mutations. These infants present severe T cell lymphopenia early in life, but in most cases their immune system gradually normalizes. However, the role of FOXN1 haploinsufficiency in causing this phenotype is unclear.
Objectives: To analyze T cell development and TEC phenotype in nu/+ mice of various age, in order to investigate whether Foxn1 haploinsufficiency in mice results in impaired thymic development early in life, followed by progressive normalization, thereby recapitulating the human phenotype.
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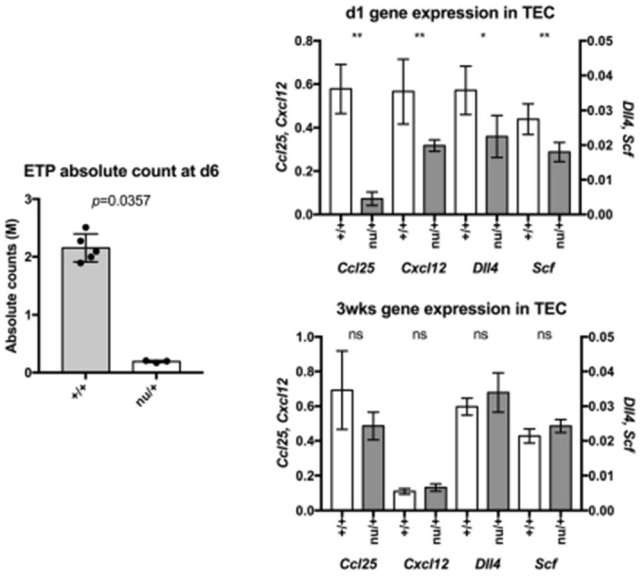


This study was supported by the Division of Intramural Research, NIAID, NIH.

**Submission ID#426679**

**IgG4 Related Disease (IgG4RD) in an Adolescent Male Misdiagnosed as AutoImmune Lymphoproliferative Syndrome**

Brianne Navetta-Modrov, M.D.[1], John Stone, MD, MPH[2], Vincent Bonagura, M.D.[3]
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Introduction/Background: Immunoglobulin G4RD is an immune-mediated disease most commonly seen in middle-aged and older men. Clinical features include autoimmune pancreatitis, salivary gland disease, orbital disease and retroperitoneal fibrosis. Pathologic features include a lymphoplasmacytic infiltrate enriched in IgG4-positive plasma cells and fibrosis in a storiform pattern. Laboratory evaluation usually reveals an elevated serum IgG4 concentration, and glucocorticoids are often used as treatment in early stages of disease. However, disease may recur off of steroids, and prolonged illness or steroid non-responsive disease is usually treated with Rituximab.
Objectives: The objective of this case presentation is to discuss a presentation of a IgG4RD is a young adult male.
Results: 16 year old male adopted from Thailand, initially presented to hospital with five days of intermittent abdominal pain, night sweats and worsening fatigue. CT Abdomen and Pelvis revealed bronchiectasis in lung bases, hepatic masses and soft tissue infiltration in the porta hepatis extending into the liver, pulmonary nodules (read as possible metastases), bilateral renal masses, retroperitoneal nodes and ileocolic intussusception secondary to possible lymphoma. One year prior to presentation he developed new onset bilateral cervical lymphadenopathy. Biopsy revealed a heterogenous lymphoid population and lymphoma was ruled out. He had a repeat lymph node biopsies during the admission, which was all negative for malignant cells, and was compatible with reactive lymphoid tissue with plasmacytosis. Immunophenotype showed 59% lymphocytes with normal CD4/CD8 ratio, and 8% CD4-,CD8-, TCR ++ T cells. Immunohistochemical stain of a lymph node fine needle biopsy showed

a mixture of CD20+ B and CD3+ T cells, abundant CD138+, IgG+ plasma cells(PCs) , with some IgG+ PCs(CT of the neck and chest was completed for possible staging and revealed cervical lymphadenopathy, nasal polyposis, a soft tissue mass vs. enlarged lateral rectus muscle (left orbit), and mediastinal lymphadenopathy. A diagnosis of Autoimmune Lymphoproliferative Syndrome (ALPS) was presumed by the hematologic/oncologic team given his elevated IgG (6,682 mg/dL) and elevated vitamin B12 (>2000) with multiorgan involvement. ALPS panel revealed a mutation in FASLG (Allele 1, c.-2C>T) which is a variant of uncertain clinical significance. ALPS Criteria/Scorewas + for 1/4 criteria (CD3+CD25+/HLA DR Ratio < 1.0). He had been referred to the NIH for further management of ALPS before initial presentation to our office.
Immunologic work up revealed immunoglobulins of IgG: 6,600 mg/dL, IgA: 83 mg/dL, IgM: 71 mg/dL; 13/23 protective streptococcal titers (> 1.3 mcg/mL); HIB: 0.46 mg/L; negative Quantiferon gold, and HIV-serology. At that point IgG4RD vs. Castleman disease was suspected instead of ALPS. Further work up showed a normal IL-6 level, and Human Herpes Virus 6 was also negative. IgG subsets showed an elevated IgG1 (2670 mg/dL), IgG2 (1450 mg/dL), IgG3 (355 mg/dL) and normal IgG4 (20.9 mg/dL). Excisional lymph node biopsy was recommended to rule out Castleman syndrome or IgG4RD. A left salivary gland was removed and showed mainly IgG4 positive PCs with no multicentric lymphocytic infiltrates. Dilution of the patients serum to determine if there was a prozone affect that minimized the IgG4 level showed an elevated IgG4 level of 2700.9 mg/dL. This is in the 1st percentile of all cases of IgG4RD in terms of serum IgG4 concentration. He was diagnosed with IgG4RD, a form previously called Mikulicz disease, which is comprised of lacrimal and parotid gland enlargement. Rituximab was given initially because of the severity of his disease. After two cycles he showed decreased lymphadenopathy, notable weight gain and marked decrease in fatigue.
Conclusions: IgG4RD is a rare and complex immunologically based disease process rarely seen in children or adolescents. Patients with IgG4RD often undiagnosed at initial evaluation. Normal serum IgG4 levels are seen in 40% of patients with IgG4RD and thus a normal IgG4 level should not be used as a biomarker to make the diagnosis of IgG4RD or in treating this disease. Furthermore, the possibility of having a prozone affect in measuring IgG4 needs to be considered and evaluated. Meticulous correlation of clinical, pathologic, and imaging findings is required to make the diagnosis.

**Submission ID#422323**

**Modelling human immune deficiency from novel missense mutations with orthologous heterozygous mutations engineered in mice by CRISPR/Cas9**

Bahar Miraghazadeh, PhD[1], Chelisa Cardinez[2], Elizabeth da Silva, MD[3], Rochna Chand[4], Ainsley Davies[5], Gaetan Burgio, MD, PhD[6], Carola Vinuesa, MD, PhD[7], Matthew Cook, ACT[8]

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[2]Honors, PhD candidate student, John Curtin School Medical Research
[3]FRACP FRCPA, The Canberra Hospital
[4]Honors, Research assistant, John Curtin School Medical Research
[5]Honors, PhD student, John Curtin School Medical Research
[6]Head of transgenesis Unit, John Curtin School Medical Research
[7]Head of department of immunology and infectiuos diseases, John Curtin School Medical Research
[8]Head of translational research and the center for personalized immunology, John Curtin School Medical Research

Introduction/Background: Next generation sequencing has resulted in substantial progress in identification of Mendelian immune deficiency syndromes. In some cases, however, putative causal mutations occur in single kindreds, or even individual patients. Under these circumstances, functional analysis of patient derived cells combined with in vitro analysis of genetically manipulated cell lines can provide additional evidence in support of genetic causation, but this might not be conclusive.
Objectives: Understanding how genetic defects result in complex syndromes of immune deficiency and immune dysregulation can be impossible to achieve in vitro. One method for overcoming these obstacles is to generate accurate mouse models of human immune deficiency
Methods: Mouse models of human immune deficiency are a valuable tool in which the murine genome is engineered to introduce a mutation orthologous to that discovered in the patient. We have applied this strategy to elucidate causation and mechanism of immunological defect in several mutations affecting the NF-kB pathway.
Results: So far, defects in both canonical and non-canonical pathways of NF-kB activation have been shown to cause immune deficiency, often associated with immune dysregulation. We describe a known defects and novel putative defect identified in the canonical NF-kB pathway
Conclusions: CRISPR-cas9 mouse models can be used to elucidate mechanism of disease and provide compelling evidence that mutations are causative.

**Submission ID#427095**

**Novel Epigenetic Immune Cell Quantification Suitable For Primary Immune Deficiencies And Immundysregulatory Disorders**

Janika Schulze, MSc[1], Udo Baron, PhD[2], Jeannette Werner, PhD[3], Konstantin Schildknecht, Dipl.-Math.[4], Andreas Grützkau, PhD[5], Nicholas Harre, PhD[6], Stephan Borte, MD, PhD[7], Sven Olek, PhD[8], Rosa Bacchetta, MD[9]

[1]PhD student, Ivana Türbachova Laboratory for Epigenetics, Epiontis GmbH, Berlin, Germany
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[6]LRSP, Division of Stem Cell Transplantation and Regenerative Medicine, Department of Pediatrics, Stanford University Medical School, CA, USA
[7]Clinical Research Coordinator, ImmunoDeficiencyCenter Leipzig (IDCL) at Municipal Hospital St. Georg Leipzig, Leipzig, Germany
[8]CEO, Ivana Türbachova Laboratory for Epigenetics, Epiontis GmbH, Berlin, Germany
[9]Associate Professor, Division of Stem Cell Transplantation and Regenerative Medicine, Dept Pediatrics, Stanford University School of Medicine

Introduction/Background: Primary immune deficiencies (PID) with or without immundysregulation are rare diseases resulting from monogenetic aberrations leading to either infections or autoimmune manifestations or both. Early diagnosis and treatment are crucial for reducing morbidity and mortality. Beside genetic diagnosis not commonly yet performed, current standard methods for early diagnosis are dependent on either fresh samples or limited to certain cell types. To overcome those limitations, especially in newborn screening, a novel technology of methylation-based qPCR can be applied. Among the known epigenetic modifications, DNA demethylation is the most stable and genomic loci with highly cell type specific demethylation sites can be identified. Differential methylation can be measured from blood samples of limited availability, or with suboptimal storage. We previously showed that thymic-derived T

regulatory cells (Tregs) are precisely quantified by measuring demethylation of the Treg-specific-region of FOXP3. More recently, we have identified highly cell type-specific DNA regions of demethylation for further cell populations.
Objectives: This novel technology allows the implementation of differential immune phenotyping into the newborn screening procedure. Here, we aimed at epigenetically quantify multiple immune cell types in different biological samples including dried blood spots and samples from patients with PID with immundysregulation, where currently no approach is available.
Methods: Using cell type-specific demethylation sites, we developed epigenetic qPCRs for quantification of T-, Treg, B-, NK-, monocyte and granulocyte cell population. Epigenetic qPCR is applicable for relative and absolute quantification in whole blood and dried blood spots using isolated, bisulfite converted DNA.
Results: We demonstrated >95% concordance with flow cytometric analyses of the same fresh blood samples from healthy subjects. We have validated the method in 30 children with symptoms of immundysregulation resulting from monogenic defects, including for example FOXP3, CD25, STAT1, CTLA4, and leading to Treg/Teffector cell imbalance where Treg deficiency has been difficult to assess by flow cytometry. Furthermore, we tested 250 dried blood spot (Guthrie card) samples from healthy newborns and 30 patients with diverse PIDs and correctly identified 29/30 PID patients, indicating that this method holds promise for newborn screening.
Conclusions: The method we established for immune cell quantification based on epigenetic cell type-specific markers, is feasible and reliable in biological samples either fresh, frozen or archived, including dried blood spot. The analysis of further immune cell types introduces an innovative opportunity to diagnose a variety of PIDs and immunodysregulatory disorders as early as newborn screening.

**Submission ID#419105**

**Pancytopenia and immunodeficiency with MDS in an infant due to SAMD9L mutation**

Blachy J. Davila Saldana, MD[1], Erin DeVitt, MSN, CPNP-AC/PC[2], Sameeya Ahmed-Winston, CPNP, CPHON, BMTCN[2]

[1]Assistant Professor, Children's National Medical Center
[2]Nurse Practitioner, Children's National Medical Center

Introduction/Background: Pediatric Myelodysplastic Syndromes (MDS) are rare in children and typically have a poor prognosis. The Sterile Alpha Motif Domain-containing 9 (SAMD9) gene is located in chromosome 7q21.2 of the human genome. Gain-of-function mutations cause a newly described (Tesi et al, Blood. 2017.) syndrome of pancytopenia, immunodeficiency and MDS. The physiological functions of both SAMD9 and SAMD9L currently remain poorly understood. SAMD9 and SAMD9L are both classified as myeloid tumor suppressors, as they are localized within a microdeletion cluster associated with myeloid disorders, such as juvenile myelomonocytic leukemia (JMML), acute myeloid leukemia (AML), and myelodysplastic syndrome (MDS). (Schwartz et al, Nature, 2017).
Objectives: We present a 17 month old pancytopenic male who was diagnosed with Myelodysplastic Syndrome (MDS) with Monosomy 7 due to SAMD9L mutation.
Methods: Whole exome sequencing was performed on a male, who presented at 8 months of age with findings concerning for a bone marrow failure (BMF) syndrome despite a normal BMF genetic panel.
Results: The patient presented at 8 months of age, with severe pancytopenia, fevers, E. Coli bacteremia, pancolitis, and echtyma gangrenosum. Bone marrow showed severe aplasia with occasional macrophages.He was treated with antibiotics, as well as steroids, etoposide and cyclosporine for presumed hemophagocytic lymphohistiocytosis (HLH). A bone marrow failure and HLH genetic panels were normal. Repeat bone marrow biopsy after count recovery post treatment showed normal cellularity, dysplastic features in megakaryocytes and dyserythropoesis, and monosomy 7 in 16/20 metaphases. Whole exome sequencing revealed an SAMD9L mutation (V1551L).
Conclusions: This is one of the first reported cases of SAMD9L mutations causing MDS since its initial discovery earlier this year. SAMD9L mutation should be considered in patients who present with pancytopenia and monosomy 7 MDS. As new defects continue to be identified, further evaluation outside of typical BMF panels may be relevant.

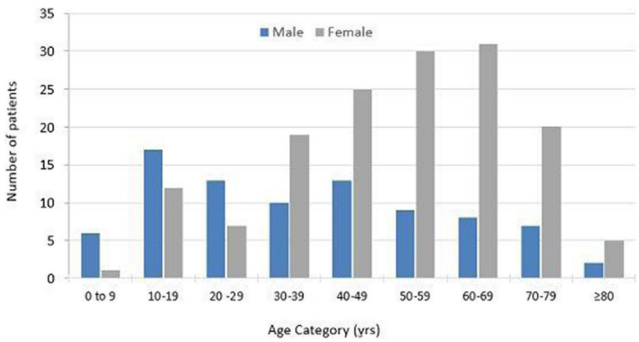
**Submission ID#428205**

**Primary Immune Deficiency Disease in Patients Over Age 60: An Analysis From a Proprietary Immunology Patient Registry**

Roger Kobayashi, MD[1], Ralph Shapiro, MD[2],Mark Stein, MD[3], Donald L. McNeil, MD[4]

[1]Clinical Professor, UCLA School of Medicine
[2]Director, Midwest Immunology Clinic
[3]Medical Director, Allergy Associates of the Palm Beaches
[4]President, Founder, and Principal Investigator, Optimed Research

Introduction/Background: Primary immune deficiency disease (PIDD) is typically considered a pediatric illness, although advances in treatment and diagnosis are changing this paradigm. Currently, data on PIDD in older patients are very limited.
Objectives: To characterize the prevalence of PIDD among older individuals using a patient database maintained by the Consortium of Independent Immunology Clinics (CIIC), comprised of 17 specialty immunology outpatient practices in the US.
Methods: Patients with PIDD were identified in the CIIC database using ICD-10 codes D80, D.80.3, D80.4, D80.5, D80.6, D81.1, D81.2, D82.0, D82.3, and D83.0. A total of 235 records from 11 geographically-diverse clinics were identified and characterized by age, gender, and PIDD diagnosis.




Results: Of the 235 PIDD patients in the CIIC registry, 73 (31%) were between 60-87 years of age (see Figure). Within this age group, most patients were female (n=56, 77%). The most common diagnoses among patients >60 years of age included Common Variable Immunodeficiency with Predominant Abnormalities of B-Cell Numbers and Function (D83.0; n=41, 56%) and Antibody Deficiency with Near Normal Immunoglobulins (D80.6; n=14, 19%). In comparison, the registry included 36 (15%) patients aged 0-19 years; this age group was predominantly male (n=23; 64%). The most common ICD-10 codes within the younger cohort were relatively evenly distributed between Hereditary Hypogammaglobulinemia (D80.0), Antibody Deficiency with Near Normal Immunoglobulins (D80.6), and Common Variable Immunodeficiency with Predominant Abnormalities of B-Cell Numbers and Function (D83.0).
Conclusions: Our data suggest that PIDD in patients over age 60 may be more prevalent than previously reported. Additional research is needed to corroborate these findings, further characterize the nature of PIDD in this

population, and determine whether there are unique diagnostic and treatment considerations within this demographic.

**Submission ID#427558**

**Survey of Canadian Patients with Primary Immunodeficiency**

Whitney Goulstone[1], Elizabeth Tough[2]

[1]Executive Director, Canadian Immunodeficiencies Patient Organization
[2]LPN, Alberta Health Services

Introduction/Background: Primary immunodeficiency diseases (PIDs) represent a significant collection of immune system disorders that increase susceptibility to infection, which in some cases are serious or life-threatening. Patients with PID often require immunoglobulin G (IgG, commonly referred to as Ig) replacement therapy to prevent infections and associated comorbidities. PID treatment, in addition to symptoms and associated social and emotional impacts, has a significant impact on patients quality of life (QoL). Information available on the real-world diagnosis, management, and outcomes of the Canadian PID patient population is limited.
Objectives: To better understand diagnosis and treatment of Canadian patients with PID, we surveyed Canadian patients with PID associated with the Canadian Immunodeficiencies Patient Organization (CIPO) The primary goal of the survey was to gain insight into the Canadian PID patient population with regards to demographics, diagnosis, treatment, including regimes, QoL, and communication and support .
Methods: The authors conducted a cross-sectional survey to measure health-related QoL in a cohort of patients with PID. Eligible participants were identified through the Canadian Immunodeficiencies Patient Organization (CIPO). The questionnaire consisted of 61 questions that covered patient-reported outcomes including diagnosis, QoL, treatment regimes, and communication.
Results: Surveys were returned by 149 patients with PID. Participants conveyed significant impact on QoL including personal, occupational, financial, emotional, and social impacts as a result of PID symptoms, risks, and treatment logistics, limitations, and side effects. The most common diagnoses were related to B-Lymphocyte disorders (60.4%). Common treatments include intravenous immunoglobulin (IVIg; in hospital) and subcutaneous immunoglobulin (SCIg; at home), in addition to antibiotics and antifungals as required. Respondents reported feeling average before treatment on a scale of 0-10 (mean 5.46 ± 2.23) with increased health after treatment (mean 6.66 ± 2.32). Respondents felt current treatment was convenient (mean 8.22 ± 1.94) and were comfortable with self-infusions (mean 6.96 ± 3.46).
Conclusions: Patients with PID are not uncommon in the Canadian community, and in these patients PID is associated with a significant impairment in QoL. Experiences range with regards to a particular treatments advantages and disadvantages, cost, travel, and convenience. Respondents hope to achieve improved QoL through the following solutions: better treatment, improved infusions, gene modification, more research and clinical trials, a cure, and education and outreach. Improved financial, medical, and social supports were also requested.

**Submission ID#424698**

**Two Years of success the Israeli Newborn Screening program for Severe combined immunodeficiency (SCID)**

Raz Somech[1], Shlomo Almashanu[2]

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Introduction/Background: Severe combined immunodeficiency (SCID), the most severe form of T cell immunodeficiency, is detectable through quantification of T cell receptor excision circles (TRECs) in dried blood spots (DBS) obtained at birth. For many professional, humanitarian and financial reasons, newborn screening (NBS) for SCID is warranted. Implementation of this screening test is highly important where high frequency of consanguinity is known to exist.
Objectives: Since October 2015, Israel has conducted national SCID NBS. This important, life-saving screening test is available at no cost for every newborn in Israel.
Methods: Herein, we describe two years results of the Israeli SCID newborn screening (NBS) program. Validation includes CBC, lymphocyte subsets, TREC (in a different method), T cell receptor (TCR) repertoire and response to mitogenic stimulation. Whole exome sequence (WES) for genetic detection, and next generation sequencing (NGS) to demonstrate TCR clonality are used as well, in some unsolved cases.
Results: Of 396,159 births screened, 719 (0.18%) had abnormal TREC in their first screen (448 terms, 271 pre-terms), 69 (0.016%) had a repeated abnormal TREC also in their second screen and were referred for a validation process. Fourteen SCID patients were diagnosed, so far, through the NBS program in its first years, revealing an incidence of 1:28,000 births in the Israeli population. Consanguine marriages and Muslim ethnic origin were found to be a risk factor in affected newborns, and a founder effect was detected for both IL7R and DCLRE1C deficiency SCID. Other diagnoses were made as follows: 8 cases were found to have T cell lymphopenia; 16 cases were diagnosed with syndromes; 4 cases were found to have secondary T cell lymphopenia; 10 cases were pre-term infants and 18 cases were considered as false positive with normal evaluation. Lymphocyte subset analysis and TREC quantification in the peripheral blood appear to be sufficient for confirmation of typical and leaky SCID and ruling out false positive results. Detection of secondary targets (infants with non-SCID lymphopenia) did not significantly affect the management or outcomes of these infants in our cohort. We could also report several perspectives regarding T cell development in non immunodeficient newborns that emerged from the accumulated data.
Conclusions: Already in a short term, TREC NBS in Israel has achieved early diagnosis of SCID and other conditions with T-cell lymphopenia, facilitating management and optimizing outcomes. This program has also enabled gaining insights on T cell development in health babies.

**Submission ID#454131**

**Diagnosis and Pre-Transplant Management of SCID Patients in the Era of Newborn Screening: A Survey of Practices in the Primary Immune Deficiency Treatment Consortium**

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[11]Allergy & Immunology Specialist, Division of Allergy and Immunology, Duke University Medical Center
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The Primary Immunodeficiency Treatment Consortium (PIDTC) recently reported that 42% of patients diagnosed with SCID via newborn screening (NBS) developed infection before hematopoietic stem cell transplant (HCT). Surprisingly, 76% of these infections were acquired during the interval between confirmation of the SCID diagnosis and HCT (Heimall, Blood 2017). To investigate further, in late 2017 we surveyed pre-HCT management practices for SCID patients at PIDTC centers. 51 physicians representing 43 North American centers responded, including 18 immunologists, 25 transplant specialists and 7 who identified as both. 33% of centers lacked a standard procedure for the management of infants with a positive NBS result. To confirm the SCID diagnosis, basic T, B and NK cell flow cytometry was performed by most. Testing for naïve T cells was generally included, but testing of lymphocyte mitogen proliferation was inconsistent. Specialists were notified of a patients positive NBS test at median age 7.5 days (2-30 days), and management of patients as SCID was started at median age 9 days (0-90 days). When unconditioned HCT was anticipated, 72% of respondents began planning as soon as possible after diagnosis, while 20% awaited genetic testing results before HCT.

Respondants consistently implemented pre-HCT prophylaxis with trimethoprim/sulfamethoxazole (84%), fluconazole (80%) and immunoglobulin infusions (96%), although timing of initiation varied. Palivizumab was used by 26%. There was little consensus regarding viral monitoring. Although most physicians screened for CMV by blood PCR (85%), only about half routinely screened for EBV or adenovirus. While 44% of physicians started prophylaxis against double-stranded DNA viruses in all patients, 46% did so only in selected situations, such as active genital HSV at the time of delivery, or when the mother was CMV-seropositive. Although all centers used only acyclovir as antiviral prophylaxis, doses and timing varied widely: from 25-90 mg/kg/day, divided into 2 or 3 doses, continued in most centers until immune reconstitution. Finally, 84% of physicians recommended that CMVseropositive mothers stop breast-feeding.

There was no consensus on where patients should reside prior to HCT. Hospital or home were favored equally, although need for a reliable family was indicated by 88% as a criterion for home-based management. For hospitalized patients, 62% of centers required patients to be in a reverse-isolation/positive-pressure room and over half required staff to wear gown, gloves, and mask. With regard to visitors, 75% required parents/relatives to perform hand hygiene. Approximately 75% did not require a gown, gloves, or mask for visitors. Finally, there was no consensus on allowing siblings or friends to visit, but the majority permitted grandparents or other adult relatives.

This survey revealed wide variability in the diagnostic pathway, viral surveillance, and isolation practices for SCID patients, although pre-transplant prophylaxis with immunoglobulin, fluconazole, and trimethoprim/sulfamethoxazole were utilized consistently. We conclude there is considerable opportunity to develop diagnostic and pre-HCT management pathways for SCID. Prospective tracking of management practices could reveal which are important for avoiding pre-HCT infections. Evidence-based practice guidance is needed to maximize the potential to bring each SCID patient identified via NBS to HCT infection-free.

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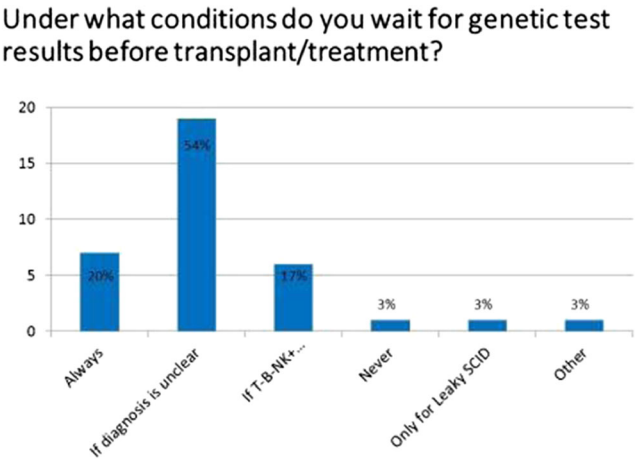
Graphic/Table
Center Reports on Waiting for Genetic Testing Prior to HCT




**Submission ID#451426**

**Dominant Negative IKZF1 Mutations Cause a Novel Combined Immunodeficiency**

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Ikaros/IKZF1 is an essential transcription factor expressed throughout hematopoiesis. In humans, somatic mutations in IKZF1 are linked to B-cell acute lymphoblastic leukemia (ALL), and germline heterozygous haploinsufficient mutations cause common variable immunodeficiency-like disorder with incomplete penetrance. Herein, we report seven unrelated patients with an earlyonset novel combined immunodeficiency associated with de-novo, fully-penetrant, germline heterozygous dominant negative mutations affecting amino acid N159 in IKZF1 DNA binding domain. Patients presented with different infections, but Pneumocystis jirovecii pneumonia was common to all. One patient developed a T-cell ALL. Additional findings included decreased B-cells, neutrophils, eosinophils and myeloid dendritic cells as well as T-cell and monocyte dysfunction. T-cells exhibited a profound naïve/recent thymic emigrant/ T-helper 0 phenotype and were unable to evolve into effector memory cells; monocytes failed to respond to different stimuli or facilitate T-cell activation. This new defect expands the spectrum of human IKZF1-associated immunodeficiency diseases from haploinsufficient to dominant negative.

**Table 1. Clinical features of patients with dominant negative IKZF1 mutations**

| | Mutation | Age at first symptoms | Infections | | | | Other manifestations | Outcome (age in years) |
|---|---|---|---|---|---|---|---|---|
| | | | Pneumocystis pneumonia (age) | Viral | Bacterial | Others | | |
| A1 | p.N159S | 2 m.o | yes (17 m.o) | Severe SRV bronchiolitis | *Klebsiella sp.* lung infection | Cryptosporidial cholangitis | No | HSCT Died (2) |
| B1 | p.N159S | 2 m.o | yes (2, 3 and 17 y.o) | No | Lung abscess, recurrent sinopulmonary infections, bronchiectasis Pulmonary *Mycobacterium avium complex* infection | Pulmonary aspergillosis | No | Alive (17) |
| C1 | p.N159S | 10 m.o | yes (10 m.o) | Influenza type A | Recurrent otitis media and pneumonia | No | No | Alive (4y) |
| D1 | p.N159S | 1.5y.o | yes (2 y.o) | Severe SRV bronchiolitis Adenoviral illness requiring cidofovir | Recurrent otitis media and pneumonia | No | Recurrent mouth ulcers | HSCT, alive and well |
| E1 | p.N159S | 1 y.o | yes (1y.o) | Recurrent HSV (oral, genital), uneventful chickenpox | *Streptococcus pneumoniae* meningitis; *Pseudomonas sp.* pneumonia; skin abscesses | Recurrent thrush; *Candida parapsilosis* fungemia | DVT/PE; pancreatitis | Alive (28) |
| F1 | p.N159S | 9 m.o | yes (20 m.o) | No | Severe pneumonia, otitis media, chronic sinusitis | Recurrent oral candidiasis | T-ALL | HSCT Alive and well (19) |
| G1 | p.N159T | 6 m.o | yes (6 m.o, 5 y.o) | Uneventful chickenpox, molluscum contagiosum, warts | No | Localized onychomyosis | Recurrent mouth ulcers | Alive and well (12) |

y.o, year old. m.o, month old, SRV syncitial respiratory virus, HSCT, hematopoietic stem cell transplantation, T-ALL T-cell acute lymphoblastic leukemia

**Submission ID#451334**

## IL2RB Deficiency Results in Early-onset Lymphoproliferation, Multisystem Autoimmunity, and Pervasive CMV Infection

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The beta subunit of the IL-2 receptor (IL2RB; CD122) is essential for IL-2 and IL-15 mediated signal transduction in a variety of hematopoietic cell types including T and NK cells. Here we report the clinical and immunologic phenotypes of two siblings born to consanguineous parents harboring a variant in IL2RB, resulting in autoimmunity, with lymphoproliferation of CD8+ T and CD56hi NK cells, and decreased regulatory T cell frequency. The proband presented with inflammatory enteropathy, failure to thrive, and disseminated CMV infection at 2 months of age, and later developed atopy, lymphocytic interstitial pneumonitis, and red blood cell autoantibodies. His younger sister presented with severe autoimmune hemolytic anemia and CMV viremia at 2 months of age, and later developed lymphocytic interstitial pneumonitis. Whole exome sequencing and chromosomal microarray studies revealed a homozygous deletion within the highly conserved WSXWS motif of the extracellular domain of IL2RB (c.665_673delCCTGGAGCC, p.Pro222_Ser224del). The deletion results in reduced IL2RB expression (cell surface and intracellular) in T and NK cells. The functional consequences of the defect include complete impairment of STAT5 phosphorylation through the IL-2 receptor but only partial impairment through the IL-15 receptor, as well as a compensatory increase in serum IL-2 and IL-15 levels (>100 pg/mL). CD8+ T cell proliferation responses to in vitro T cell receptor (TCR) stimulation were reduced compared to an age-matched, healthy control, but partially rescued by supraphysiologic levels of IL-2 and IL-15. Despite reduced CD8+ T cell proliferation responses, the proband displayed a T cell population skewed toward CD8+ T cells, with an oligoclonal expansion of effector memory cells. Arguing against effects of pervasive CMV infection alone, the sister displayed similar phenotypic and functional abnormalities at birth, prior to her CMV infection. Our data suggest that the identified hypomorphic mutation in IL2RB results in a survival advantage for those CD8+ T and CD56hi NK cells most sensitive to the exuberant serum IL-2 and IL-15 levels produced in response to the defect. These surviving CD8+ T and CD56hi NK cells have great lymphoproliferative potential, leading to multisystem autoimmunity and inflammatory complications. Therefore, we describe IL2RB deficiency as a novel primary immunodeficiency disease with prominent immune dysregulation and selective CD8+ T and CD56hi NK cell lymphoproliferation.