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Abstracts

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Poster Hämatologie

H01

F3AK treatment of aggressive lymphoma cells results in induction of apoptosis in vitro

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Background: Diffuse large B cell lymphoma (DLBCL) is the most common lymphoma entity in adults. The fact that the incidence of DLBCL is still increasing and that approximately one-third of patients relapses or does not attain remission, indicates the requirement to develop novel therapeutic strategies. The synthetically produced chalcone derivative F3AK showed growth inhibitory effects on various colon cancer cell lines. Hence, we aimed to determine the in vitro effect of F3AK known to possess anti-tumoral properties on aggressive lymphoma cells.

Methods: Therefore, we cultured seven different lymphoma cell lines (Karpas422 and SuDHL4 as GCB-DLBCL models, RI-1 and U2932 as ABC-DLBCL models, BL-2 and Raji as Burkitt lymphoma models, and Jurkat as model for T-NHLs) with F3AK followed by MTS assays to determine cell growth, cell cycle analysis and apoptosis assays (Annexin V-staining and PARP-cleavage).

Results: After 72 hours of cell exposure to F3AK in culture, concentration-dependent growth inhibition with IC50 values ranging 675nM – 2073 nM in all investigated cell lines was observed. Furthermore, culturing of cells with F3AK resulted in more than 50 % of lymphoma cells staining positive for Annexin V and exhibited a SubG1 one peak after 24 h in a concentration of 1000 nM and 500 nM for all investigated lymphoma cell lines.

Conclusions: These preclinical data indicate that F3AK possesses pro-apoptotic effects on aggressive lymphoma cells. Thus, this agent should be further investigated regarding potential as novel anti-lymphoma therapy.

H02

Nr4a1 possesses immune suppressive function in Myc-driven lymphomagenesis

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Background: In aggressive lymphomas, low expression of NR4A1 is associated with poor lymphoma-specific survival and its overexpression suppresses lymphoma cell growth in vivo indicating its tumor suppressive properties. The aim of this study was to comprehensively study the function of Nr4a1 loss in lymphomagenesis.

Methods: Therefore, we intercrossed the EμMyc lymphoma mouse model with the Nr4a1-/- mouse and monitored them until the onset of disease. Furthermore, we transplanted lymphoma cells of EμMyc Nr4a1-/- and EμMyc Nr4a1+/+ mice into immune-competent C57BL/6 mice and immune-deficient Fox Chase SCID beige mice. Finally, we performed co-culture cytotoxicity assays using OVA peptide-pulsed EμMyc Nr4a1+/+ and EμMyc Nr4a1-/- lymphoma cells and OVA targeting CD8+ T cells and measured T cell-mediated lymphoma cell lysis after 4 h, 8 h, 16 h und 24 h, respectively.

Results: We observed that loss of Nr4a1 leads to an accelerated lymphomagenesis in vivo, concomitant with increased expression of immune checkpoint components. Immunocompetent, but not immune-deficient mice, transplanted with Nr4a1-deficient lymphoma cells exhibited rapid lymphoma development, reduced survival, and upregulation of immune checkpoints. Interestingly, in our co-culture experiments using the OVA peptide-pulsed lymphoma cells and OVA targeting CD8+ T cells, we observed a massively diminished lymphoma cell lysis in the EμMyc Nr4a1-/- setting after 16 h and 24 h.

Conclusions: Our data suggest that Nr4a1 plays a critical role in regulating the licensing of immune evasion in aggressive lymphomas by regulating immune checkpoint expression. Thus, it might act as a potential target to restore anti-lymphoma immune responses.

H03

Diphenyleneiodonium possesses cell growth inhibitory effects on aggressive lymphoma cells

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Background: Diffuse large B-cell lymphoma (DLBCL) possess a pronounced genetic and clinical heterogeneity. Transcription profiling identified a DLBCL subgroup that exhibits increased expression of genes involved in mitochondrial oxidative phosphorylation (OXPHOS) and which is insensitive to B-cell receptor inhibition. Therefore, we aim to investigate whether OXPHOS inhibition could be therapeutically targeted.

Methods: We used seven different lymphoma cell lines (Karpas422 and SuDHL4 as GCB-DLBCL models, RI-1 and U2932 as ABC-DLBCL models, BL2 as Burkitt lymphoma model and Jurkat as model for T-cell non-Hodgkin's lymphoma). These cells were cultured with Diphenyleneiodonium (DPI) – an OXPHOS-inhibitor – followed by cell-growth assays as well as cell-cycle analysis and apoptosis assays.

Results: After 72 hours of DPI exposure, a concentration-dependent growth inhibition in all investigated cell lines was detected by MTS assay. Especially, DLBCL cell lines exhibited low IC50 values ranging from 12 nM to 40 nM, whereas IC50 values for BL2 (149 nM) and Jurkat (640 nM) cells were higher ($p = 0.071$). Furthermore, DPI treatment (250 nM, 50 nM and 10 nM) of SuDHL4 and BL2 cells induced a cell cycle arrest in the S phase in comparison to untreated cells. In Jurkat and U2932 cells, a change in cell cycle distribution was also observed but to a lower extend. Interestingly, in these four cell lines no apoptotic effects were detected.

Conclusions: These preclinical data indicate that DPI treatment causes cell growth inhibition, which is mediated by changes in cell cycle distribution. Thus, pharmacologic inhibition of OXPHOS might serve as a novel therapeutic approach for further DLBCL therapies.

H04

Brusatol inhibits cell growth of aggressive lymphoma cells in vitro by inducing apoptosis

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Background: Aggressive lymphomas represent the most common lymphoid malignancies in adults with an increasing incidence. Despite great advances in therapy, treatment fails in more than one-third of patients, indicating the need for novel therapeutic strategies. Thus, in this study, we investigated the potential of Brusatol to treat aggressive lymphomas.

Methods: The effect of Brusatol was studied in seven lymphoma cell lines (GCB- and NGCB-DLBCL, T-ALL and Burkitt lymphoma). Cells were treated with increasing Brusatol concentrations to determine IC50 values. After 24 hours Brusatol treatment (50 and 250 nM), apoptotic assays (Annexin V staining, Caspase-3 cleavage, PARP cleavage) and cell cycle analysis were performed. Furthermore, samples from untreated and Brusatol-treated cells were collected for Western blot analysis.

Results: In all seven cell lines, Brusatol inhibited cell growth in a concentration-dependent manner. GCB-DLBCL and Burkitt lymphoma cell lines exhibited a higher sensitivity to Brusatol in all apoptotic assays. Interestingly, Western blot analysis of Brusatol-sensitive cell lines showed decreased protein levels of BCL2, BCLXL, and MCL1. Furthermore, in the above-mentioned cell lines, a reduced p53 and MYC protein expression were detected after treatment. Notably, cell lines with higher MYC levels are more sensitive to Brusatol treatment.

Conclusions: Our data indicate that Brusatol is able to efficiently induce cell death in aggressive lymphoma cells by reducing the expression of pro-survival proteins. Interestingly, cells with MYC overexpression were especially sensitive to this compound. Together, our study suggests that Brusatol represents a promising agent to develop novel anti-lymphoma therapies.

H05

Distinct chemokine receptor expression profiles are associated with the clinicopathological features of follicular lymphoma

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Background: Follicular lymphoma (FL) is a heterogeneous disease. Recently, progression of disease within 24 months (POD24) was shown to associated poor prognosis. Currently, there exists no marker identifying FL with later POD24. Since chemokine receptors (CCR) play a key role in lymphomagenesis, we comprehensively studied the expression pattern of these receptors in FLs with and without POD24.

Methods: We performed an expression analysis of mRNA levels of 17 CCRs (CCR1-CCR10, CXCR1- CXCR5, XCR1, and CX3CR) in tissue biopsies of 279 FL patients with POD24 and without POD24 and correlated results with patients' outcome. Non-neoplastic tonsils were included as controls.

Results: In our expression analysis we observed that the CCR expression profiles of FL differed substantially from those of non-neoplastic tonsils, with a higher expression of CCR8 (70-fold, $p = 0.008$), and lower expression of CCR7 (2-fold, $p = 0.018$) in FL. By comparing the CCR expression profiles of FL with POD24 to FL without POD24, we found a lower expression of CCR5 (5.5-fold, $p = 0.019$), CXCR2 (5-fold, $p = 0.027$) and CXCR3 (563-fold, $p < 0.001$) in FLs with POD24. By comparing the CCR pattern to clinical data of FL patients, we observed that high expression of CCR3, CCR4, CCR7, and CCR10 was associated with worse lymphoma-specific survival.

Conclusions: Overall, our results suggest that a distinct chemokine receptor pattern might be associated with early progression of FL. Thus, several receptors might serve as a useful clinical prognostic marker for risk stratification and might be potential novel therapeutic targets in future lymphoma therapy.

H06

Distinct expression signatures of chemokine receptors in primary CNS lymphoma

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Background: Chemokine receptors (CRs) mediate the migration and activation of lymphocytes through binding of their ligands. Previous studies have shown that there are important contributions of CRs to the development, progression, and dissemination of lymphoid malignancies.

Aim: Due to the limited knowledge on the expression profile in primary central nervous system lymphoma (PCNS-L), we aimed to comprehensively study the expression patterns of CRs in primary central nervous system lymphoma.

Methods: For this purpose, expression analysis of 19 well-characterized CRs in biopsies of patients with PCNS-L ($n=28$), and cDNA isolated from tonsils of healthy donors ($n=5$) was performed by using semiquantitative real-time PCR (qPCR). Germinal centre B cells (GC-B, $n=5$) were included as non-neoplastic controls.

Results: The chemokine receptor expression profile of PCNS-L substantially differed from those of non-neoplastic GC-B, with a higher expression of CCR1 (42-fold, $p=0.01$), CCR4 (72-fold, $p=0.009$), CCR9 (8.9-fold, $p=0.054$) and CXCR6 (4.8-fold, $p=0.096$), lower expression of CCR6 (2.9-fold, $p=0.007$) and de novo expression of CCR5, CXCR2 and XCR1 ($p<0.05$) in PCNS-L, respectively. By comparing the CR pattern to clinical data of PCNS-L patients, we observed that high expression of CCR2 ($p=0.045$), CCR6 ($p=0.021$) and CXCR4 ($p=0.026$) were associated with worse lymphoma specific survival using univariate analysis.

Conclusions: Our data indicate that a distinct CR expression pattern is implicated in the development of PCNS-L and that several receptors might serve as a useful clinical prognostic marker and represent a potential novel therapeutic target for lymphoma therapy.

H07

Impact of distinct expression profiles of eukaryotic initiation factors on the pathogenesis and prognosis of primary central nervous system lymphomas

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Background: Primary central nervous system lymphoma (PCNSL) is a rare, but aggressive extranodal lymphoma type. The current standard treatment is high-dose methotrexate-based induction chemotherapy. However, relapses with poor prognosis are frequent within treated patients. Since our group clearly demonstrated that eukaryotic initiation factors (eIFs) were significantly associated with clinical course of aggressive lymphomas we aimed to comprehensively study these factors in PCNSL.

Methods: mRNA expression levels of 16 eIFs were analyzed by quantitative real-time PCR in tissue biopsies of 31 PCNSL patients. As controls, non-neoplastic germinal center B-cell (GC-B) specimens were included ($n=5$). We compared eIF expression in PCNSL to non-neoplastic controls and correlated expression levels with the patients' clinical course.

Results: Analysis of mRNA expression revealed a higher expression of EIF1A (5.5-fold; $p=0.027$), EIF2B3 (4 fold; $p=0.013$) and EIF3D (9.3 fold, $p=0.028$) and a lower expression of EIF2A (3.4 fold; $p<0.001$), EIF4BP1 (2 fold; $p=0.002$) and EIF4G3 (5.4 fold; $p=0.004$) in PCNSL compared to GC-B. Interestingly, by comparing the expression level of eIFS to clinical course, we observed that 7 out of 16 eIFs were associated with survival ($p<0.033$): high expression of EIF1, EIF2B4, EIF2B5, EIF2S1, EIF3L, EIF4A2, and EIF5 was associated with poor lymphoma-specific survival.

Conclusions: Our data indicate that eIFs may play an important role in pathogenesis of PCNSL. Thus, the expression pattern of specific eIF subunits might serve as a useful clinical prognostic marker for risk stratification. Additionally, it seems that certain eIFs might represent novel targets to treat PCNSL in future.

H08

Distinct chemokine receptor expression profiles in de novo DLBCL, transformed follicular lymphoma, Richter's transformed DLBCL, and germinal center B-cells

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Background: Chemokine receptors (CR) play a key function in the development of B-cell lymphoma. However, they have not been intensively studied in diffuse large B-cell lymphoma (DLBCL), transformed follicular lymphoma (tFL), and Richter syndrome (RS). Thus, we aim to comprehensively investigate CR expression profiles in these lymphoma entities.

Methods: We performed an expression analysis of 18 chemokine receptors on mRNA levels by using RQ-PCR, as well as of seven chemokine receptors by immunohistochemistry in the aggressive component of RS, de novo DLBCL, and tFL. Germinal center B-cells (GC-B) served as non-neoplastic controls.

Results: The chemokine receptors expression profiles of de novo DLBCL, tFLs and RS substantially differed from those of GC-B, with at least 5-fold higher expression of 14 out of 18 investigated CRs (CCR1-CCR9, CXCR1, CXCR2, CXCR6, CX3CR1, and XCR1) in the three lymphoma subtypes. Moreover, de novo DLBCL and tFL exhibited at least 22-fold higher expression of CCR5, CCR8, and CXCR6 compared to RS. No significant difference in CR expression profile between de novo DLBCL and tFL was detected. Furthermore, in de novo DLBCL and tFLs low expression of CCR8 and CXCR2 and high expression of CCR7 were associated with a poor cancer-specific survival.

Conclusions: Our data indicate that the chemokine receptor expression profile of RS differs substantially from those of de novo DLBCL, tFL and GC-B. Thus, these multiple deregulated CRs might serve as useful diagnostic and prognostic tools and might be considered as clinical markers of high value.

H09

Renal function in patients with BCR-ABL-negative myeloproliferative neoplasms

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Background: Chronic kidney disease (CKD) is a general risk factor for thrombosis and overall mortality. However, little is known about the incidence and impact of CKD in patients with BCR-ABL-negative myeloproliferative neoplasms (MPN) encompassing polycythemia vera (PV), essential thrombocytosis (ET) and primary myelofibrosis (PMF). The aim of this study was to characterize the renal function at diagnosis and during the disease course in a cohort of MPN patients.

Methods: In this retrospective study we determined overall survival (OS) in patients diagnosed with MPN at the Medical University of Graz between 1988 and 2019 and analyzed their renal parameters at time of diagnosis ($n=214$) as well as during their disease course up to ten years ($n=127$).

Results: Renal insufficiency as defined by a CKD stage ≥3 was found in 21.9 % of MPN patients with the lowest incidence in ET patients (6.9 % vs. 27.9 % (PV) vs. 27.3 % (PMF)). Reduction of the estimated glomerular filtration rate during disease course did not differ between disease entities. CKD stage ≥3 at diagnosis resulted in reduced OS (65.3 vs 86.5 % and 45.6 vs. 74.6 % at 5 and 10 years, respectively, $p<0.001$). In multivariate analysis, lower OS was associated with PMF diagnosis, JAK2 V617F mutational status, male gender and higher age.

Conclusions: Significant CKD can be found in around one fifth of MPN patients and may contribute to an adverse disease outcome. However, prospective studies are needed to determine the exact role of CKD on the clinical course of MPN patients.

H10

Novel diagnostic markers and potential therapeutic targets in acute myeloid leukemia

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Background: Despite recent therapeutic progress, the prognosis of patients with acute myeloid leukemia (AML) is still dismal in many patients and novel treatment approaches are urgently needed. In this study we aimed to characterize novel surface markers for their diagnostic as well as therapeutic potential in AML.

Methods: Surface expression of recently described markers was determined in 104 diagnostic AML samples by flow cytometry and expression levels were correlated to distinct genetic and cytogenetic aberrations.

Results: Among nine tested markers, we found high surface expression of the G-protein coupled receptors CD97 and CD312, the complement receptor C3AR and the chemokine receptor CCR1 (CD191) in the majority of AML samples. While CD191 was expressed at levels comparable to normal hematopoietic stem and progenitor cells, the other three markers revealed significantly increased expression levels on AML blasts. Among AML subgroups, CD312 expression was higher in CD34-positive AML samples ($p=0.0325$). In contrast, C3AR and CD191 expression was significantly upregulated in CD34-negative AML samples ($p=0.0472$; $p=0.0118$). Concerning genetically defined subgroups we found increased expression of CD312 and C3AR in AML samples with mutated NPM1 ($p=0.0112$; $p=0.0212$), while CD97 was high in all subgroups. Finally, C3AR expression was also increased in samples with mutated FLT3 ($p=0.0455$).

Conclusions: We identified CD97, CD312 and C3AR as promising diagnostic markers as well as potential therapeutic targets in AML. While CD97 may represent a suitable marker in all AML patients, CD312 and C3AR may be most useful in distinct AML subgroups, such as NPM1-mutated patients.

H11

Mimicking leukemia loss-of-function mutations via CRISPR/Cas9 base editing

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Background: CRISPR/Cas9 is a genome-editing tool commonly used for gene knock-out (KO). Usually, error-prone non-homologous end joining (NHEJ) of Cas9-induced DNA double strand breaks (DDB) is employed for the formation of insertions and deletions (indels). Unfortunately, frequent DDBs lead to apoptosis of fragile primary cells, such as hematopoietic stem and progenitor cells. Base editing (BE), a new CRISPR-based application, allows for modification of single nucleotides without the need for a DDB. BE can be used to introduce in-frame STOP codons via C→T transitions, thereby leading to gene KO and mimicking of loss-of-function (LOF) mutations. Here, we employ BE to KO genes commonly affected by somatic LOF mutations in acute myeloid leukemia (AML) such as TP53, RUNX1, STAG2, SMC1A, ASXL1 and TET2.

Methods: We used plasmid- and mRNA-based BE to introduce C→T transitions for the formation of premature in-frame STOP codons in various leukemia cell lines. SgRNA were selected based on C→T conversion predictions using publicly available tools. BE was analyzed by Sanger sequencing and impact on protein expression was analyzed using Western Blot.

Results: We could show successful introduction of in-frame STOP codons in TP53, RUNX1, STAG2, SMC1A, ASXL1 and TET2. Furthermore, isogenic single cell-derived clones with hetero- and homozygous LOF could be generated.

Conclusions: We could show that CRISPR/Cas9-BE is a powerful tool to mimic LOF via the introduction of in-frame premature STOP codons. The established protocol in leukemia cell lines will serve as the basis for translation into primary hematopoietic cells.

H12

Die Lebensqualität erwachsener Personen nach einer allogenen hämatopoetischen Stammzelltransplantation – ein Rapid Review

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Grundlagen: Der größte Teil der Bemühungen der Gesundheits- und Krankenpflege konzentriert sich auf die Phase nach der allogenen HSCT. Um gesundheitsbezogenen Interventionen zu setzen, ist die Lebensqualität (LQ) von Patient*innen ein wichtiges Kriterium. Das Ziel war es festzustellen, welche Faktoren die LQ von erwachsenen Personen mit einer hämatologischen Erkrankung nach einer allogenen HSCT (poststationär) beeinflussen.

Methodik: Zur Beantwortung der Forschungsfrage wurde ein Rapid Review durchgeführt. Es wurde eine systematische Literaturrecherche in vier medizinischen Datenbanken sowie eine Handsuche in Google Scholar und Referenzlisten durchgeführt. Die Studien wurden nach Ein- und Ausschlusskriterien gewählt und kritisch bewertet. Die Ergebnisse wurden in Kategorien entsprechend den Lebensqualitätsdimensionen nach dem konzeptuellen Modell der gesundheitsbezogenen Lebensqualität von Ferrans et al. (2005) übergeordnet geclustert und narrativ beschrieben.

Ergebnisse: Aus insgesamt 1802 Studien konnten 24 Studien inkludiert werden. Charakteristika der Umgebung (u. a. Arbeitslosigkeit), Charakteristika des Individuums (u. a. niedriger Bildungsstand, höheres Alter, persönliches Erscheinungsbild, Geschlecht), Biologische Faktoren (u. a. akute/chronische Graft-versus-host-disease), Symptome (u. a. Fatigue, Wunden im Mund, Schlafstörungen, Schmerzen, Geschmacksveränderungen, Übelkeit, Erbrechen, Appetitverlust), niedriger funktionaler Status (u. a. reduzierte Muskelkraft, physische Schwäche), allgemeine niedrige Gesundheitswahrnehmung und allgemeine Lebensqualität (u. a. kurzer Zeitraum nach der allogenen HSCT, bis zu einem Jahr nach der allogenen HSCT) sind Faktoren, welche die LQ signifikant verringern.

Schlussfolgerungen: Eine allogenen HSCT ist mit Risiken verbunden, die sich auf die LQ auswirken können. Faktoren wie die Unfähigkeit soziale Rollen einzunehmen, Symptombelastungen sowie emotionale Belastungen wie posttraumatische Erlebnisse sollen bei Patient*innen nach einer allogenen HSCT mittels standardisierten Instrumenten erhoben und individuell durch qualifiziertes diplomiertes Pflegepersonen in Ambulanzen betrachtet werden.

H13

DNA methylation profiling reveal mechanisms of relapse specific gene expression in acute myeloid leukemia

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Although most acute myeloid leukemia (AML) patients respond to chemotherapy and achieve a complete remission, most patients will eventually relapse. To uncover mechanisms of therapy resistance in AML, we integrated matched genetic, epigenetic, and transcriptomic profiles of AML patients during the course of disease. Samples collected at initial diagnosis, complete remission, and relapse were analyzed by exome sequencing, transcriptome sequencing and 450K methylation profiling. Epigenetic regulators including DNMT3A or IDH1/2, TET1/2, and WT1 („ITW" genes) are commonly mutated in AML at diagnosis (DNMT3A: 54 %, ITW: 40 %). While mutations in these genes never get lost at relapse, they are frequently gained (DNMT3A: 54 %, ITW: 50 %). Mutations in DNMT3A were associated with global hypermethylation, whereas ITW mutations were associated with hypomethylation and shorter time to relapse, indicating a more aggressive disease. At relapse, the number of differentially methylated CpG sites was reduced profoundly, indicating a more specific epigenetic regulation. In contrast, the number of timepoint-specific differentially expressed genes was significantly increased at relapse ($n = 499$ vs. $n = 687$, P = 1.7E-06). At diagnosis, these genes are enriched for biological functions such as „cell division" and „cell cycle", while at relapse, functions such as „inflammatory response" and „regulation of response to stimulus" are enriched, indicating a regulatory shift from tumor development and survival at diagnosis towards a potential response to the tumor, chemotherapy and infectious complications at relapse. Taken together, our approach enables to track the evolution of AML at multiple molecular levels during the course of disease and to identify potential molecular mechanisms of relapse development.

H14

Autologous stem cell transplantation as rescue therapy for prolonged cytopenia after treatment with CD19-targeted chimeric antigen receptor modified T-cells: two case reports

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Background: CD19-targeted chimeric antigen receptor (CAR) modified T-cells is an established treatment in relapsed or refractory large B-cell lymphomas. Although prolonged cytopenias beyond day 30 after CAR T-cell infusion can be observed with most CAR T-cell products, the underlying mechanisms are still unclear. We present two patients, who received autologous blood stem cell infusions in prolonged cytopenias.

Case presentation: Patient A was diagnosed with nodal marginal zone B-cell lymphoma with transformation into diffuse large B-cell lymphoma (DLBCL). He received an autologous blood stem cell transplantation, but relapsed 106 days later. Patient B suffered from DLBCL. She relapsed early after receiving 6 cycles of R-COMP. Both patients received anti-CD19 CAR T-cell infusion (Yescarta®) after lympho-depleting chemotherapy with cyclophosphamide and fludarabine. However, both recipients experienced prolonged severe pancytopenia beyond day 30 including G-CSF refractory neutropenia. We administered transfusions of stored autologous blood stem cells, in patient A 48 days and in patient B 33 days after CAR T-cell infusion.

Results: Leukocyte engraftment (ANC > 500/µL) supported by G-CSF stimulation occurred in patient A 3 days, in patient B 11 days after stem cell support, platelet engraftment with platelets over 20.000/µL was observed in patient A 40 days and patient B 17 days after autologous stem cell rescue. No adverse events were recognized.

Conclusions: Autologous stem cell infusion seems to be a rescue therapy for prolonged cytopenia after CAR T-cell-therapy. With the reduction of duration of cytopenia, it could lower risk of infectious complication and reduce the duration of hospitalization for CAR T-cell recipients.

H15

Retrospective analysis of cases of indolent or advanced mastocytosis in Upper Styria 2004–2021

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Background: Mastocytosis is a rare disease caused by the accumulation of dysfunctional mast cells in the skin or internal organs. Criteria for diagnosis and different subforms are well established, a targeted therapy aimed ad KIT is available, but mastocytosis may be underdiagnosed, as it can occur secondary to another condition. The true incidence is not known so far. We reviewed 19 cases of patients admitted to our department in the period 2004–2021 to investigate the occurence and clinical presentation in our region.

Methods: Internal Database Seach (medical records, laboratory archive).

Results and Conclusions: We examined 19 cases of suspected mastocytosis in our outpatient clinic. 12 patients had advanced mastocytosis, 5 of them had systemic mastocytosis with associated hematological neoplasia (SM-AHM). 3 patients with suspected mastocytosis had only increased tryptase levels or mast cell counts but mastocytosis was not detected. 4 patients were diagnosed with indolent mastocytosis.

We found that patients with advanced mastocytosis were about 10 years older than patients with indolent mastocytosis (66 versus 56 years). Patients with advanced mastocytosis also had more comorbidities, they often had malabsorption, and

they had more anaphylactic reactions in their history (but cutaneous symptoms were less pronounced); they always met the histopathological criteria (Main Criteria) of pathologic spindle-shaped mast cells whereas tryptase levels were not always elevated and KIT-mutation D816V was present in less than 50 %. Systemic mastocytosis remains an interesting and intriguing disease and diagnosis requires a high index of suspicion.

H16

Identification of risk factors for failed humoral response to SARS-CoV-2 mRNA vaccination in allogeneic HSCT recipients

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Background: Both mRNA-based Covid-19 vaccines, BNT162b2 (BioNTech/Pfizer) and mRNA-1273 (Moderna), effectively decrease the risk for severe Covid-19 disease in the general population. However, vaccination success is less likely in immunocompromised individuals.

Methods: To assess the rate of humoral response to a full (two-fold) primary mRNA vaccination, defined by manufacturer cut-off >= 7.1 BAU/ml (SARS-CoV-2 IgG II Quant assay, Abbott), and identify clinical and laboratory parameters associated with failed humoral response in recipients of an allogeneic hematopoietic stem cell transplant (HSCT), 167 HSCT recipients were evaluated in this single-center retrospective study. Variables studied included time since HSCT to vaccination, time between vaccination to titer measurement, age of recipient and donor, ongoing anti-tumor therapy, individual immunosuppressives, type of GHVD-prophylaxis, sex, donor/recipient relationship, and vaccine type, and the immunologic parameters (assessed closely to vaccination), CD4, CD8, CD19, and NK blood-cell counts, immunoglobulin levels (IgA, IgG, and IgM), both by univariate and multivariate analyses.

Results: Thirty-seven HSCT recipients (22 %) developed no detectable antibody-response. Median time from HSCT to vaccination was 10.2 months (range, 2.5–88.9) in non-responders, while it was 35.3 months (3.0–215.1) in responders ($p<0.001$). By multivariable analysis, a higher CD19 (B cell) count was associated with humoral vaccination response adjusted odds ratio (aOR) 3.3 per 100 B-cells/mcl (CI 95 % [1.78–6.20], $p<0.001$). Concurrent immunosuppression with mycophenolate mofetil (MMF) plus/minus a calcineurin inhibitor decreased the probability of response (aOR 0.04, CI 95 % [0.01–0.24], $p<0.001$).

Conclusions: Our findings may contribute to a more profound understanding of risk factors for failure of mRNA-based SARS-CoV-2 vaccination in HSCT recipients.

H17

Single-cell RNA-sequencing reveals sub clonal architecture in peripheral T cell lymphoma

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Background: Peripheral T cell lymphomas (PTCL) are a heterogeneous group of rare lymphoid malignancies. Current anthracycline-based treatments achieve a complete remission of approximately 60 % of patients, but with a high rate of relapse or disease progression. With a 5-year OS of 32–45 %, there is an unmet need for deeper understanding of disease mechanisms and improved treatment.

Methods: Here, we performed single-cell RNA sequencing on patient samples of clinical lymph node biopsies from 12 different patients suffering from PTCL, T-follicular-helper type or not otherwise specified T-cell lymphoma. The single-cell transcriptomic data was further integrated with histopathology reports and clinical information. Tumor microenvironment cell types were identified by established marker genes and validated by flow cytometry and immunohistochemistry. Bioinformatic calculations for the detection of large copy number variations from RNA expression distinguished malignant from non-malignant T cells.

Results and Conclusions: Independent clustering of the transcriptional signature of over 52,000 cancer and microenvironment single-cells provided detailed knowledge of sub clonal architecture in PTCL and its dynamic tumor ecosystem. The Integration with publicly available scRNAseq data of reactive lymph nodes, discerened altered transcriptional signatures and distribution of certain nonmalignant T and B cell types, cycling T cells and distinct PTCL specific B cells. The transcriptional and chromosomal characterization on a single-cell level elucidated inter-patient heterogeneity and revealed transcriptional sub clones within patients. We identified transcriptional signatures of each sub clone and distinguished specific functional properties. These findings may lead to an explanation for residual sub clones responsible for early relapse in PTCL.

H18

Efficacy and safety of heterologous booster vaccination with Ad26.COV2.S after BNT162b2 mRNA Covid-19 vaccine in haemato-oncological patients with no antibody response

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Background: Patients with haemato-oncological malignancies are one of the high-risk groups for a severe course in case of Covid-19 infections. Furthermore, vaccination results in significantly lower response rates in haematological malignancies and lower antibody levels in patients with solid cancer. We investigated efficacy and safety of a heterologous booster vaccination with Ad26.COV2.S DNA vector vaccine in haemato-oncological patients without antibody response after double-dose BNT162b2 mRNA Covid-19 vaccine.

Methods: A total of 32 haemato-oncological non-responders to double-dose BNT162b2 received a heterologous booster vaccination with Ad26.COV2.S. Blood samples were assessed directly before the vaccination (T0) and 4 weeks after (T1). Safety assessment was performed using a standardised questionnaire.

Results: The overall response rate was 31 %, with a mean (SD) antibody titre of 693.79 (1096.99) BAU/ml. Patients with chronic lymphocytic leukaemia or lymphoma showed a significantly lower response rate (P = 0.048). Adverse events were reported in 29.6 % of patients, whereby 7.1 % were graded as severe, which includes grade III and IV events following CTCAE.

Conclusions: The heterologous booster vaccination with Ad26.COV2.S led to a serological response in 9 out of 29 patients without response after double-dose BNT162b2. Furthermore, the vaccination was safe in our cohort, leading to mainly mild local and systemic reactions. Overall, this vaccination regimen should be further evaluated to increase the response rate in the highly vulnerable population of haemato-oncological patients.

H19

Significance of renal comorbidity in patients with chronic myelomonocytic leukemia

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Background: Chronic myelomonocytic leukemia (CMML) is a rare hematopoietic malignancy which is mostly diagnosed in elderly patients who frequently have one or more comorbidities. The clinical significance of renal comorbidity in patients with CMML is poorly investigated.

Methods: Using data from the Austrian Biodatabase for CMML (ABCMML) we analyzed retrospectively the prevalence of renal comorbidity and potential correlations with clinical, phenotypic and genotypic features in patients with CMML.

Results: Data on renal comorbidity were available from 166 patient's charts. Increased creatinine values (>1.1 mg/dl) were found in 71 of 166 (43 %) patients. The median survival of patients with increased creatinine values was significantly shorter than in patients without impairment of renal function (20 vs. 51.6 months, $p<0.001$). Patients with increased creatinine values were older (median age 74 vs. 72 years, $p=0.005$), more often male (80 % vs. 63 %, $p=0.017$), had higher WBC counts (WBC ≥ 13 G/L, 61 % vs. 29 %, $p<0.001$), monocyte counts (AMC ≥ 10 G/L, 13 % vs. 2 %, $p=0.009$), LDH values (LDH ≥ 250 U/L, 67 % vs. 45 %, $p=0.016$) and had a more frequent occurrence of peripheral IMC (79 % vs. 52 %, $p=0.002$). There was a trend of a higher prevalence of CBL mutations (10 % vs. 6 %, $p=0.060$) and ASXL1 mutations (40 % vs. 24 %, $p=0.064$) in patients with renal impairment.

Conclusions: Our findings show a high prevalence of renal abnormalities in patients with CMML. Increased creatinine values were identified as a new prognostic marker. This finding may be important for the individualized management of this heterogenous group of patients.

H20

Cytokine levels impact on response to NETosis triggers

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Background: Recently, NETosis a neutrophil specific type of cell death which facilitates thrombogenesis has been implicated MPN. The pathogenesis of MPN and thrombosis are closely linked to inflammatory processes and their mediating cytokines. In this study we correlate cytokine profiles with NETosis rates induced in primary MPN patient derived neutrophils.

Methods: Neutrophils were isolated from blood samples of 37 MPN patients (PMF $n=10$; PV $n=12$; ET $n=15$). NETosis was triggered by PMA or Ionomycin and rates of released nucleosomes were determined by ELISA as surrogate marker of NETosis. Cytokines were quantified in serum samples obtained from the same patients by multiplex ELISA. Furthermore, primary neutrophils from 5 MPN patients were incubated with serum from 6 different MPN patients prior to NETosis induction. Donors of these serum samples had shown high ($n=3$) and low ($n=3$) rates NETosis rates in prior experiments. After pre-incubation NETosis was triggered and quantified by ELISA.

Results: Pre-incubation of donor neutrophils with different MPN serum samples resulted in altered NETosis rates compared to standard serum pre-incubation. For analysis of the observed cytokine profiles patients were classified as high or low responders to NETosis triggers or according to their mutational status, MPN-subtype and treatment, respectively. Significant differences were observed in IL-1b, IL-13, IL-17A, MIP-1a with regard to NETosis response.

Conclusions: Prior studies demonstrated cell-inherent differences between neutrophils derived from MPN-patients and healthy donors. Here we present evidence that the proinflammatory stimuli mediated by MPN serum further modulate NETosis response in MPN-neutrophils.

H21

AML and MDS patients with TP53 aberrations – a single cancer center experience

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Background: Mutations in TP53 gene are detected in 5–10 % cases of acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) with a higher representation in older patients and therapy related sub-types.

Methods: To compare the biological and clinical features of TP53 mutated AML and MDS patients, we have analysed the data from 84 TP53 mutated AML ($n=46$) and MDS ($n=38$) patients from our tertiary cancer center.

Results: A strong association with complex karyotype and therapy related subtype was observed in both AML and MDS patients. The immunophenotypic analysis of the blast cells from AML and MDS revealed no significant difference in expression of myeloid progenitor and aberrant markers. However, NGS analysis revealed a different co-mutational pattern. The median overall survival (OS) of the total cohort was 226 days (95 % confidence interval [CI], 131–300). MDS patients had a significantly better OS with a median of 345 days (95 % CI, 235–590) as compared to those with AML with a median of 91 days (95 % CI, 64–226). However, 60 % MDS patients transformed to AML, which substantially reduced their prognosis. Cox regression analysis revealed MDS, the type of treatment and the variant allele frequency (VAF) of TP53 mutations are the parameters significantly influencing OS.

Conclusions: In conclusion, data from our cohort confirm the adverse prognosis of TP53 mutated AML and MDS patients. However, they further indicate that TP53 mutated MDS patients have a significantly better OS than those with AML, which might be due to the differences in co-occurring mutations and/or the TP53 VAF.

Poster Onkologie

O01

Circulating tumor DNA correlates with tumor burden and predicts outcome in pancreatic cancer irrespective of tumor stage

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Background: Circulating tumor DNA (ctDNA) represents a promising tool for diagnosis, prognosis and treatment monitoring of several malignancies. Its association with tumor burden in pancreatic ductal cancer (PDAC), especially in localized disease, is not fully explored yet. We aimed to investigate the association of pretherapeutic ctDNA levels in localized and metastatic PDAC with tumor volume and clinical outcomes.

Methods: Liquid biopsy for ctDNA detection was prospectively obtained from patients with localized or disseminated PDAC prior to either resection or systemic treatment. Detection rates and levels of ctDNA (digital droplet PCR) were correlated to tumor volume, relapse rate and survival.

Results: 60 patients with localized and 47 patients with metastatic PDAC were included. ctDNA was detected in 10 % of localized and 57.4 % of metastasized PDAC samples. In localized disease, ctDNA detection significantly correlated with the numbers of involved locoregional lymph nodes ($p=0.030$). Primary tumor volume did not correlate with ctDNA levels in neither localized ($p=0.573$) nor metastasized disease ($p=0.878$). In disseminated disease, ctDNA levels correlated with total tumor volume ($p=0.026$) and especially with liver metastases volume ($p=0.004$), but not with other metastases. Detection of pretherapeutic ctDNA was associated with shorter DFS in localized PDAC (3.3 vs. 18.1 months, $p=0.000$), whereas ctDNA levels were associated with worse OS in metastatic PDAC (5.7 vs. 7.8 months, $p=0.036$).

Conclusions: ctDNA positivity indicates major nodal involvement or even presence of undetected distant metastases associated with early recurrence in localized PDAC. Moreover, it predicts worse clinical outcome in both localized and metastatic disease.

O02

Real-world study of cabozantinib in patients with advanced renal cell carcinoma (aRCC) after VEGF-targeted therapy (CASSIOPE): interim-data for patients who had also received prior nivolumab

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Background: Cabozantinib is a tyrosine kinase inhibitor approved in Europe as monotherapy for aRCC following prior VEGF-targeted therapy, or for treatment naïve patients with intermediate/poor risk. We report interim real-world data of cabozantinib in patients with aRCC who have received prior VEGF-targeted therapy and the checkpoint inhibitor nivolumab.

Methods: CASSIOPE (NCT03419572) is an ongoing, non-interventional study of cabozantinib in patients with aRCC who have received prior VEGF-targeted therapy; a pre-planned interim analysis was conducted when 50 % of patients had completed ≥3 months of follow-up. This post-hoc analysis assessed patient characteristics, best overall response (BOR; RECIST 1.1), dose modifications and tolerability at 3 months in patients who had also received prior nivolumab.

Results: Of 337 patients included in CASSIOPE, 154 (45.7 %) had received prior nivolumab (median age, 67.5yrs; 70.8 % male, 87.7 % clear-cell histology, 96.1 % metastatic disease; 80.8 % ECOG PS0–1). Of these patients, 58.4 % initiated cabozantinib at 60 mg/day; median daily dose during the study was 40 mg. Overall, 78.6 % of patients had dose modifications, 66.9 % due to adverse events (AEs) (reduction 46.8 % (any) and 43.5 % (due to AEs); interruption, 54.5 % and 46.8 %; discontinuation, 26.0 % and 14.3 %). AEs were reported in 94.8 % of patients, most commonly diarrhea (36.4 %) and PPE (25.3 %). During the first 3 months, 58 patients had an evaluable BOR: 39.7 % had partial response, 44.8 % stable and 12.1 % progressive disease (3.4 % not evaluable). There were 17 all-cause deaths (11.0 %).

Conclusions: This post-hoc-analysis suggests that cabozantinib is broadly tolerable and may offer tumor response in patients previously treated with VEGF-targeted therapy and nivolumab.

O03

Blood-based B-cell subtypes as predictors of treatment response during treatment with immune checkpoint inhibitors: a prospective longitudinal pan-cancer study

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Background: Immune checkpoint inhibitors (ICIs) have revolutionized systemic treatment of different cancer types. This study aimed to evaluate peripheral blood B-cell subtypes as potential predictors of ICI treatment response.

Methods: 39 cancer patients who received ICIs were included in this prospective single-center cohort study. Patients had a first blood-draw at the date before treatment-initiation and a second 8–12 weeks at the time of first response evaluation. B-cell subtypes were quantified and characterized by fluorescence-activated cell sorting (FACS). Disease control rate (DCR) and objective response rate (ORR) were considered co-primary endpoints. Parametric and non-parametric hypothesis tests were used as appropriate and uni- and multivariable logistic regression models were implemented. Due to the hypothesis-generating character of the study, multivariable analysis was only adjusted for tumor type.

Results: In the overall cohort DCR was 48.7 % and ORR was 25.6 %. At baseline, there was no significant association of any B-cell subtype, with neither DCR nor ORR. After 8–12 weeks of ICI-treatment an increase in the frequency-of-parent of CD21-B-cells was a significant negative predictor of response as indicated by DCR (OR = 0.05, 95 % CI 0.00-0.67, $p = 0.024$) and ORR (OR = 0.09, 95 % CI 0.01-0.96, $p = 0.046$). Likewise, an increase of the frequency-of-parent of switched memory B-cells was significantly associated with reduced odds for DCR (OR = 0.06, 95 % CI 0.01-0.70, $p = 0.025$) Patients with an increase in the frequency-of-parent of naïve B-cells were more likely to respond indicated by DCR (OR = 12.31, 95 % CI 1.13-134.22, $p = 0.039$).

Conclusions: B-cell subpopulations may be linked to ICI-treatment response and may represent a novel predictive biomarker.

O04

Distribution and prognostic relevance of the ABO blood group system in non-metastatic renal cell carcinoma patients upon fifteen years of median follow-up

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Background: The ABO blood group system has been previously discussed as a risk factor to develop and as a prognostic factor in non-metastatic renal cell carcinoma (RCC). Controversial findings have been reported in different populations of RCC patients with rather short follow-up periods. This study aimed to clarify the distribution and prognostic role of ABO blood groups upon 15 years of median follow-up in non-metastatic RCC patients.

Methods: We evaluated the distribution and prognostic significance of ABO blood group system in a screening cohort ($n=405$) and a validation cohort ($n=1473$) of non-metastatic RCC patients, who underwent curative nephrectomy between 1998 and 2012 at two tertiary academic centers. Cancer-specific- (CSS), metastasis-free- (MFS), as well as overall survival (OS) were assessed using the Kaplan-Meier method, univariable- and multivariable Cox regression models were applied, respectively.

Results: In the screening and validation cohort, blood groups were not associated with any clinical endpoints (for the validation cohort: CSS (HR = 1.233; 95 %CI 0.998-1.523, $p=0.052$), MFS (HR = 1.161; 95 %CI 0.952-1.416, $p=0.142$) and OS (HR = 1.037; 95 %CI 0.890-1.208, $p=0.641$), respectively). Compared to 250.298 healthy blood-donors of the Styrian state, the distribution of blood groups was (624 (42.4 %) versus 106.861 (42.7 %) in group A, 191 (13 %) versus 34.164 (13.7 %) in group B, 575 (39 %) versus 93.579 (37.4 %) in group O and 83 (5.6 %) versus 15.694 (6.3 %), $p=0.467$).

Conclusions: The ABO blood group system could not be validated as a prognostic factor in predicting important clinical endpoints in non-metastatic RCC patients.

O05

Effects of immune checkpoint-inhibitors on bone turnover in cancer patients

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Background: Immune checkpoint inhibitors (ICI) represent the new standard of cancer care. Despite the widespread use, their effect on bone turnover remains unknown. The aim of this study is to prospectively assess markers of bone remodeling during ICI treatment in patients with advanced cancer.

Methods: Patients with inoperable cancer and no bone metastases (assessed per PET-CT or bone scan) receiving single-agent PD1 or PD-L1 inhibitor were enrolled. Levels of C-terminal telopeptide (CTX) and osteocalcin (OCN), markers of bone resorption and formation respectively, as well as calcium, vitamin D and parathormone were measured in serum samples collected before each ICI application for six months.

Results: Between 02/2020 and 02/2021 eight patients have been enrolled and seven included in the current analysis. Median age was 70 years (range 58–77), 85 % were male. Lung cancer was diagnosed in 42 % of the patients, 70 % received a PD1 inhibitor. With a median observation time of 15 weeks (range 6–15), CTX levels significantly decreased after the first ICI application (from 0.59 ± 0.49 to 0.46 ± 0.45; $p=0.018$) to return to baseline thereafter. In contrast, OCN progressively increased overtime (from 16.47 ± 5.72 to 22.38 ± 7.18, $p=$ n. s.). No significant changes in calcium, Vit. D and PTH levels were observed.

Conclusions: Despite the small number of patients, our pilot study shows a decrease of CTX/OCN ratio, thus suggesting for the first time a positive effect of ICI on bone turnover in cancer patients. Patients' enrollment is ongoing, correlative preclinical studies are planned.

O06

ALYREF regulates glucose metabolism and tumorigenesis in colorectal cancer

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Background: Cancer cells are particular vulnerable to energy restrictions due to their increased demand for glucose going along with their limited flexibility for modifying their means of ATP generation in response to changes of environmental conditions and energy source availability. Recent studies provide evidence that colorectal cancer also seems to be a

suitable target for energy restriction-based approaches. The nuclear export factor ALYREF has been implicated in human cancers, though the biological role and molecular mode of action in colorectal carcinogenesis have not been elucidated yet.

Methods and Results: High ALYREF expression was significantly associated with poor survival in colorectal cancer patients (HR = 2.71, (1.05-7.02), $p < 0.039$, $n = 126$). Gain and loss of function experiments, clearly demonstrated that ALYREF expression effects colorectal cancer cell growth, glycolytic activity and tumor growth in vitro and in vivo xenograft tumors. Whole transcriptome analysis proposed a molecular link between ALYREF and the metabolic gene insulin receptor substrate 1 (IRS1). Mechanistically, we could demonstrate that ALYREF is a transcriptional co-regulator of IRS1 and that increased ALYREF expression – which is found in colorectal cancer tissue – is augmenting IRS1 levels causing the observed anti-tumorigenic effects by reducing cellular metabolism – limiting the access of the vitally needed glycolytic pathway for CRC cells.

Conclusions: Overall, our study identified ALYREF as a novel driver of colorectal carcinogenesis by impacting cellular glucose metabolism and therefore, representing a potential therapeutic target for energy restriction-based treatment approaches.

O07

The RNA-binding protein ALYREF is promoting triple negative breast carcinogenesis through CPSF6 -mediated selective regulation of the short NEAT1 isoform

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Background: Triple negative breast cancer (TNBC) is a subtype with poor prognosis due to its underlying aggressive biology. A better understanding of the molecular basis of TNBC might help to develop new therapeutic strategies.

Methods and Results: In this study, we identified the RNA-binding protein ALYREF amplified in 5 % of breast carcinomas, significantly up-regulated in TNBC, and associated with poor prognosis in two large independent breast cancer cohorts. In vitro and in vivo characterization of ALYREF showed that its expression significantly influenced cellular growth, apoptosis and mitochondrial energy metabolism as well as breast tumorigenesis in orthotopic mouse models. Transcriptional profiling, phenocopy and rescue experiments identified ALYREF as a molecular driver of the short isoform of the lncRNA NEAT1. Mechanistically, we found that ALYREF binds to the NEAT1 promoter region to enhance the global NEAT1 transcription. ALYREF stabilizes CPSF6, a protein that selectively activates the post-transcriptional generation of the short, oncogenic isoform of NEAT1, as well as exclusively binds and stabilizes this short NEAT1 isoform.

Conclusions: Overall, our study identified ALYREF as a novel TNBC-driving factor and indicates the significance of ALYREF as a promising therapeutic target in TNBC.

O08

A novel long non-coding RNA hypoxia associated-lncRNA1 is involved in cellular growth, apoptosis and patients' prognosis in clear cell renal cell carcinoma

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Background: Clear cell renal cell carcinoma (ccRCC) is mainly driven by alterations in the Von-Hippel-Lindau-HIF2 signaling cascade, which constitutively mimics hypoxic conditions and lead to gene activation in angiogenesis, proliferation and tumor metabolism. The role of long non-coding RNAs in these processes is poorly understood.

Methods: Using a model system consisting of ccRCC cell lines and hypoxic conditions, we profiled the expression changes of the whole transcriptome, the ncRNome and antisense transcripts to identify novel molecular players in cancer cell hypoxia. Based on this screening step, we started to study the human relevance of novel not yet characterized transcripts. To study the cellular functions regulated by those novel transcripts, we performed loss-of-function experiments using siRNAs and GapmeRs directed against a novel lncRNA called hypoxia-associated lincRNA1 (ha-lincRNA1).

Results: ha-lincRNA1 was associated with poor overall survival in two independent ccRCC cohorts comprising more than 500 patients. Decreased expression of ha-lincRNA1 significantly reduced cellular growth in three independent ccRCC cell lines. This effect was principally achieved through the activation of apoptosis, which was confirmed by increased levels of annexin V, caspase 3/7, and cleaved PARP. Interestingly, despite the observed influence on apoptosis activation, reduced levels of this lncRNA led to a significantly increased cellular migration.

Conclusions: In conclusion, this is the first report suggesting the crucial role of ha-lncRNA1 in ccRCC.

O09

MicroRNA miR-4646 is a novel tumor suppressive factor in triple negative breast cancer

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Background: Triple negative breast cancer (TNBC) is a breast cancer subtype that stands out as particularly aggressive

and difficult to treat. As non-coding microRNAs (miRNAs) have been found to contribute to virtually all types of human cancer, the aim of this study was to identify a novel miRNA involved in the tumorigenesis of TNBC.

Methods and Results: Based on a previously published genome-wide expression study, we analyzed the prognostic value of the mammosphere-associated miRNA miR-4646 in the Cancer Genome Atlas (TCGA) dataset and found low expression of miR-4646 to be associated with poor survival in TNBC patients. In vitro growth assays showed that overexpression of miR-4646 resulted in a decrease of TNBC cell numbers whereas inhibition had the opposite effect. Moreover, increased caspase 3/7 activity and cleavage of PARP hinted at the induction of apoptosis. Cancer cell migration assays showed decreased/increased migration upon overexpression/inhibition, respectively. In addition, we found that miR-4646 impedes the in vitro tube formation ability of endothelial cells and reduces aldehyde dehydrogenase activity, a marker for cancer stemness, in TNBC cells. To unravel the molecular mechanisms underlying this tumor suppressive phenotype, we performed an RNAseq analysis, target predictions and luciferase-based interaction assays where we identified GRAMD1B, a protein involved in cholesterol homeostasis, as a direct target of miR-4646.

Conclusions: To conclude, the results of this study revealed miR-4646 as a novel tumor suppressive factor in TNBC.

O10

Impact of metastases location and number of affected location on survival in de novo metastatic pancreatic cancer

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Background: Despite metastatic pancreatic adenocarcinoma (PC) is a devastating disease, there seem to be significant differences in individual disease courses and clinical outcome among a subgroup of patients. Especially, the location of metastases (lung only versus liver versus peritoneum) or the number of affected organs might reflect differences of underlying biology of PC.

Methods: Data from 431 consecutive patients with de novo diagnosed metastatic adenocarcinoma of the pancreas, treated between 2004 and 2020 at a single academic center, were evaluated. The primary endpoint was overall survival, which was analyzed by Kaplan-Meier curve analysis, and both univariate and multivariate Cox proportional models.

Results: Overall, the median survival was 5 months (range 0–89 months) in our cohort, and at the landmark of 2-years 50 patients (11.6 %) were still alive. We observed in 19 (4.4 %) patients lung metastases only, and in 342 (79 %) patients one organ region affected by metastases. Lung metastases only (median survival 13 months (95 % CI 8.7–17.2 months)) compared to other metastases location (median survival 5 months (95 % CI 4.1–5.8 months, $p = 0.031$, log-rank test)) as well as only one affected anatomical region (compared to two or more) were associated with a favorable prognosis in those patients.

Conclusions: Based on the aggressiveness of metastatic spread, there seem to be differences in patient outcome and probably the underlying biology in PC patients.

O11

Impact of major changes in the treatment landscape of metastatic renal cell carcinoma on overall survival: a real-world comparison of three historical cohorts

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Background: The treatment landscape of metastatic renal cell carcinoma (RCC) has dramatically evolved over the past decades. Whether real-life cohorts reflect the significant differences in overall survival (OS) found in controlled clinical trials is undefined yet. The aim of this study was to compare patient outcomes of three historical treatment cohorts.

Methods: 914 patients with metastatic RCC diagnosed and treated at the Medical University of Graz between July 1985, and September 2020 were retrospectively enrolled and assigned to three treatment-eras („interferon“, „TKI“, „immunotherapy“) based on the EMA-approval of sunitinib (July 27th, 2006) and nivolumab (June 26th, 2015) in metastatic RCC treatment. Patients who lived or developed metastases after the respective date were assigned to the respective treatment-era. OS was considered the primary endpoint. Kaplan-Meier-analysis, log-rank tests and uni- and multivariable Cox-models were implemented.

Results: Median OS was 2.6 years in the overall study population and was 2.4 and 1.7 years in the interferon- and TKI-era. OS was significantly higher in patients of the immunotherapy-era as compared to the interferon- and TKI-era (all $p < 0.001$). Patients treated in the immunotherapy-era had a significantly better prognosis (HR = 0.46, 95 %CI 0.35-0.60, $p < 0.001$). There was no difference in the TKI-era cohort (HR = 1.16, 95 %CI 0.93-1.46, $p = 0.191$) compared to the time span of pure interferon treatment. Subgroup analysis stratified by IMDC prognostic groups showed significantly longer OS in the immunotherapy-era as compared to TKI and interferon.

Conclusions: Advances in metastatic RCC treatment during the recent decades had a major impact on patient outcomes in a real-life population in Austria.

O12

MicroRNA-22-3p represents a potential novel therapeutic approach for treating colorectal cancer metastases

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Background: Metastatic colorectal cancer (CRC) is still an incurable disease in many patients. Molecular networks between non-coding RNAs may facilitate the discovery of novel drug vulnerabilities in this disease. In this study, we identified miR-22-3p as a direct interaction partner of the apoptosis-regulating long non-coding RNA FLANC, and thus further characterized and evaluated the therapeutic value of miR-22-3p in CRC metastases.

Methods: In silico and in vitro analyses revealed miR-22-3p as a potential interaction partner of FLANC. In a series of in vitro experiments and molecular analyses, we characterized the fundamental biological role of miR-22-3p in CRC. We further explored the therapeutic potential of miR-22-3p in a mouse model of colorectal cancer liver metastases.

Results: miR-22-3p expression was downregulated in CRC tumor tissue as compared to healthy colon mucosa. In four different CRC cell lines, miR-22-3p reduced cellular growth through induction of apoptosis. Whole proteome and gene set enrichment identified PAK1 and apoptosis-related pathways to be influenced by miR-22-3p. In vivo pharmacological targeting of CRC metastases by administration of nano-liposomal encapsulated miR-22-3p mimics significantly reduced the number of metastases, corroborated by reduced proliferation and induction of apoptosis. Importantly, these effects were observed without evident tissue toxicity or pro-inflammatory effects in the mouse model.

Conclusions: Our study identified miR-22-3p as a part of apoptosis-regulating non-coding RNA network and potential therapeutic approach in treating metastatic CRC.

O13

Double-stranded RNA molecules trigger cancer cells' antiviral response and lead to the upregulation of immune checkpoint molecules

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Background: The efficacy of immunotherapy is improved in combination with chemotherapy or radiotherapy in certain types of cancer. Although this synergistic effect is commonly explained by an immunostimulatory effect of respective measures, the underlying molecular mechanisms are still poorly characterized. Here, we studied the impact of double stranded RNA (dsRNA) species on the expression of immune checkpoint (IC) molecules in biliary tract cancer (BTC) cells.

Methods and Results: BTC cell lines, which were exposed to miR-200c-3p mimic (23-mer dsRNA), displayed upregulated expression levels of immunosuppressive IC molecules IDO1, PD-L1, and LGALS9. This could not be replicated using a shorter miR-141-3p mimic (22-mer dsRNA). Subjecting cells to Poly (I:C) – a dsRNA analog with sizes ranging from 1.5 kb to 8 kb – resulted in similar effects as with the miR-200c-3p mimic. Both, exposure to miR-200c-3p mimic and Poly (I:C) lead to the activation of dsRNA sensing machinery, the upregulation of interferons, and the induction of antiviral response genes in BTC cell lines – effects that could be reversed by simultaneously inhibiting key players of the respective pathways.

Conclusions: Considering the above-mentioned results together with supporting literature, we suggest a model in which an active immune response against cancer cells leads to cell lysis and resulting accumulation of dsRNA species in the tumor microenvironment. Sensing of dsRNA species with certain lengths by yet unharmed cancer cells leads to the upregulation of interferons, which signal to surrounding tumor cells, culminating in the upregulation of IC molecules to suppress the ongoing immune cell attack.

O14

The long non-coding RNA NORAD and its relevance on PARP inhibitor sensitivity in triple negative breast cancer

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Background and Methods: Triple negative breast cancer (TNBC) stands for the most aggressive breast cancer subtype and is frequently associated with mutations in BRCA1/2 genes. Patients with mutated BRCA1/2 genes can be treated with approved PARP (Poly (ADP-ribose) polymerase) inhibitors. PARP is involved in base excision repair and its inhibi-

tion in homologous recombination-deficient cells leads to an increased cell death. The long non-coding RNA Activated by DNA Damage (NORAD) has been implicated in molecular mechanisms associated to carcinogenesis. As NORAD function is maintaining genomic stability, we examined if combining NORAD knock-down and PARP inhibitor treatment lead to an increased cell death in TNBC cells.

Results: The lncRNA NORAD is significantly up-regulated in cancerous tissue of TNBC patients compared to healthy tissue and associated with poor progression-free survival (HR = 1.76, (1.3 – 2.39), *p*-value: 0.00024; *n* = 417). To identify the role of NORAD in breast carcinogenesis, we used a gapmer-mediated silencing in three independent TNBC cell lines to find a reduced cellular growth in cells with decreased NORAD levels. A combination of NORAD knock-down and the PARP inhibitor olaparib influenced the expression pattern of several DNA repair proteins. To analyze the consequences of this combination in wild type and in BRCA1/2 KO cell lines we generated BRCA1/2 mutated cell lines by CRIPSR/Cas9 based approach, and results will be presented.

Conclusions: First data suggest a role of NORAD in TNBC and combining a targeting approach of NORAD and PARP inhibitors might significantly impact DNA repair mechanisms.

O15

Trends of public interest in palliative care, euthanasia, and advanced health care directives in Austria, Germany, and Switzerland using big data from Google

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Background: Austria and Germany decided to liberalize their laws restricting assisted suicide, thus reigniting the debate about the implementation of its practice and how it should be embedded. This study examines whether the public awareness concerning end-of-life decisions changed with the implementation of palliative care services and new governmental regulations.

Methods: We searched for policies, laws, and regulations promulgated or amended in Austria, Germany, and Switzerland between 2004 and 2020 and extracted data on the search volume from Google Trends for palliative care, euthanasia, and advanced health care directives as a surrogate of public awareness and interest in end-of-life decisions.

Results: The enactment of laws regulating advance health care directives coincided with a significant drop in the volume of searches for the topic of euthanasia in all 3 countries (Austria: –24.48 %, $P = 0.02$; Germany: –14.95 %, $P < 0.001$; Switzerland: –11.75 %, $P = 0.049$). Interest in palliative care increased with the availability of care services and the implementation of laws and policies to promote palliative care (Austria: 22.69 %, $P = 0.01$; Germany: 14.39, $P < 0.001$; Switzerland: 17.59 %, $P < 0.001$). The search trends for advance health care directives showed mixed results.

Conclusions: Our results demonstrate that legal measures securing the autonomy of patients at the end of life may lower the search activities for topics related to euthanasia and assisted suicide. Hence, palliative care may be a meaningful way to raise awareness of the different options for end-of-life care and to guide patients in their decision-making process regarding the same.

O16

The BCL-2 family member BOK promotes KRAS-driven lung tumorigenesis in a p53-dependent manner

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Background: A variety of cancer entities are driven by KRAS mutations, which remain difficult to target clinically. Survival pathways, such as resistance to cell death, may represent a promising treatment approach in KRAS-mutated cancers. Based on the frequently observed genomic deletions of BCL-2-related ovarian killer (BOK) in cancer patients, we explored the function of BOK in a mutant KrasG12D-driven murine model of lung cancer.

Methods and Results: Using KrasG12D/+ Bok-/- mice, we observed an overall tumor-promoting function of BOK in vivo. Specifically, during tumor development, loss of BOK resulted in a lower tumor burden, with fewer, smaller, and less advanced tumors. Furthermore, we found that loss of BOK reduced prolif-

eration both in KrasG12D-driven lung cancer cell lines in vitro as well as in KrasG12D-driven tumor lesions in vivo. Cell cycle analysis revealed that Bok-deficient cells show longer G2 duration, indicative of persistent DNA damage. Indeed, we detected higher levels of phosphorylated histone γ-H2AX, an indicator of DNA double-strand breaks, in Bok-deficient lesions. By generating an independent p53-deleted second mouse model (KrasG12D/+ Tp53Δ/Δ Bok-/-), we identified that this phenotype was entirely dependent on the presence of functional p53. This was also confirmed in cell lines in vitro.

Conclusions: Taken together, our data points towards a role of the BCL-2 family member BOK in promoting the development of lung adenocarcinomas, as Bok-deficient mice have lower tumor burden. We found that this effect was mediated by defects in proliferation, and was entirely dependent on functional p53.

O17

Frequency and distribution of PIK3CA and ESR1 mutations in endocrine sensitive and endocrine resistant primary breast carcinomas and corresponding metastasis

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Background: The PIK3CA gene encodes the α-catalytic subunit of PI3K. Mutated PIK3CA is a target for therapy with alpelisib and fulvestrant in HR+/HER2- breast cancer (BC). The registrational trial of alpelisib included patients with HR+/HER2- BC harboring PIK3CA mutations in exon 8, 10 and 21.

Methods: We investigated frequency and distribution of PIK3CA and ESR1 mutations in endocrine sensitive and resistant primary BCs and corresponding distant metastases to derive insights into the mutation spectra and elucidate differences in the mutation frequencies between endocrine sensitive and resistant tumors and primaries versus metastases. Primaries and metastases of 44 women with HR+HER2- disease were analyzed by NGS. The carcinomas were endocrine-sensitive ($n=11$), primary endocrine-resistant ($n=13$) or secondary endocrine-resistant ($n=20$).

Results: In the endocrine-sensitive cohort 54.4 % (12/22) of the samples harbored PIK3CA mutations (PIK3CAmut) and 4.5 % (1/22) ESR1 mutations (ESR1mut). In the primary endocrine-resistant group 40.7 % (11/27) were PIK3CAmut and 23.1 % (6/22) ESR1mut. The secondary endocrine-resistant tumors exhibited in 40,0 % (16/40) a PIK3CAmut and in 7.5 % (3/40) ESR1mut phenotype. In 66,8 % of tumors the PIK3CAmut were located in exons 8, 10 and 21. In the other samples the PIK3CAmut were distributed amongst exons 2 to 19. The discrepancy of PIK3CAmut and ESR1mut between primary versus metastasis was 29.2 % (26/89) and 17.8 % (16/89).

Conclusions: PIK3CA mutations occur frequently outside of exon 8, 10 and 21. Their biological significance and utility as a therapeutic target are not yet elucidated. The mutation status of PIK3CA and ESR1 may differ between primary carcinomas and their metastasis.

NGS analysis was supported by Novartis.

O18

Cannabinoids reduce melanoma cell viability and do not interfere with commonly used targeted therapy in metastatic melanoma

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Background: Cannabinoids are mainly used for recreational purpose. However, due to its appetizing effects they can also be used for oncological patients with tumor cachexia. Additionally, there are some hints in the literature that cannabinoids might have some anti-cancerous effects. For this reason, cannabinoids are also taken by patients with metastatic disease under conventional oncological therapies. The aim of the study was to study the underlying mechanism how cannabinoids mediate their pro-apoptotic effects in metastatic melanoma in-vivo and in-vitro. Additionally, we aimed to study cannabinoids' value beside conventional targeted therapy in-vivo.

Methods: Several melanoma cell lines were treated with different concentrations of cannabinoids and anti-cancerous efficacy was assessed by proliferation and apoptosis assays. Subsequent pathway analysis was performed using flow cytometry, proliferation, apoptosis, and confocal microscopy data. Efficacy of cannabinoids in combination with the MEK inhibitor trametinib was studied in NSG mice in vivo.

Results: Cannabinoids reduced cell viability in multiple melanoma cell lines in a dose-dependent way. The effect was mediated by CB1, TRPV1 and PPARα receptors whereby pharmacological blockade of all three receptors protected from cannabinoid-induced apoptosis. Cannabinoids initiated apoptosis by mitochondrial cytochrome c release with consecutive activation of different caspases. Essentially, cannabinoids significantly decreased tumor growth in vivo and were not inferior to the clinically used MEK inhibitor trametinib.

Conclusions: Cannabinoids could reduce cell viability in several melanoma cell lines, initiate apoptosis via the intrinsic apoptotic pathway by cytochrome c release and caspase activation and do not interfere with commonly used targeted therapy.

O19

Curtailing metabolic flexibility by fasting to overcome sorafenib resistance in hepatocellular carcinoma

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Background: Rewired metabolism in cancer can reveal therapeutically exploitable vulnerabilities. Sorafenib, the widely used drug for advanced hepatocellular carcinoma (HCC) provides limited extension in median overall survival due to primary or acquired resistance. We explored whether nutrient deprivation can be used as adjuvant therapy to sorafenib in the treatment of resistant HCC.

Methods: We used in vitro and in vivo HCC models to investigate synergistic interaction of nutrient restriction and sorafenib. We probed cell viability and death as well as several growth pathways. Cellular metabolism was interrogated through oxidative phosphorylation, glycolysis, and glucose uptake assays. CRISPR/Cas9-mediated p53 knock-out and proteomics analyses were used to reveal p53-dependent mechanisms. Finally, we established an HCC mouse model with conditional, liver-specific p53 knock-out to investigate fasting/sorafenib synergism in non-resistant, orthotopic HCC.

Results: We show in HCC cells, xenografts, and in patient-derived HCC organoids that fasting can sensitise resistant HCC to sorafenib. Sorafenib acts non-canonically as an inhibitor of mitochondrial respiration, causing resistant cells to depend on glycolysis for survival. Fasting, through glucose reduction and impeded AKT/mTOR-signalling, prevents this Warburg shift. Affecting glucose transporter and pro-apoptotic protein expression, p53 is necessary and sufficient for the sorafenib-sensitising effect of fasting. Moreover, in an orthotopic sorafenib-sensitive HCC mouse model, p53 is also crucial for improvement of sorafenib efficacy by fasting.

Conclusions: Our data suggest fasting and sorafenib as rational combination therapy for HCC with intact p53 signalling.

O20

Histopathological features and prognosis of early lobular breast cancer

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Background: Invasive lobular breast cancer (ILC) is the second most common histological subtype in breast cancer. The aim of this study was to analyze clinico-pathological features and their impact on overall survival (OS) and disease-free survival (DFS) of early ILC patients treated at our institution.

Methods: We collected clinico-pathological features of 344 early ILC patients diagnosed and treated between 2006 and 2021. We stratified the tumors according to hormone receptor (HR) and HER2 status. As DFS we defined the time between diagnosis and occurrence of metastasis, loco-regional disease, second breast cancer, or death from any cause.

Results: The mean age of patients was 63.2 (25th percentile: 52; 75th percentile: 74). 334 patients (97,1 %) were hormone receptor positive (HR+), 26 (7,6 %) were HER2 positive (HER2+) and 6 (1,7 %) were triple negative. In HER2+ disease, 24 patients (92,3 %) were HR+. There was no significant difference in OS and DFS between HR+ and HER2+ disease or between the ER+/PR+ and the ER+/PR- group. OS and DFS were significantly reduced in triple negative ILC ($p < 0.0001$, respectively). Ki-67 ≥ 15 % and larger tumor size were significantly associated with shorter DFS and OS.

Conclusions: In our analysis 97,1 % were HR+. Larger tumor size and Ki-67 ≥ 15 % as well as triple negative subtype of ILC had a significantly adverse impact on DFS and OS. In the HER2+ subgroup, there was no difference in OS and DFS, either due to targeted treatment effect or the lack of dependence of ILC on HER2 signaling, which remains to be studied.

O21

BRCA1/2 Keimbahnmutationen beim Mammakarzinom – eine retrospektive monozentrische Studie

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Grundlagen: Für ca. 5–7 % aller Brustkrebserkrankungen sind Mutationen im BRCA1 oder BRCA2 Gen verantwortlich. Wenn nachgewiesen, bedeuten diese Mutationen Risiko für weitere Malignome, sind aber auch prädiktiv für den Einsatz von zielgerichteten Therapien wie der PARP Inhibitoren. Ziel dieser Arbeit ist es, die Häufigkeit der nachgewiesenen BRCA1/BRCA2 Keimbahnmutationen an der Klinischen Abteilung für Onkologie in Graz systemisch zu erfassen.

Methodik: In dieser retrospektiven, monozentrischen Studie wurden Brustkrebs-PatientInnen berücksichtigt, die

an unserer Abteilung im Zeitraum von 2015–2020 behandelt und wegen gestellter Indikation genetisch getestet wurden. Klinisch-pathologische und genetische Daten wurden in einer RedCap Datenbank erfasst.

Ergebnisse: Von 1360 PatientInnen wurden 203 (15 %) genetisch beraten und 189 (14 %) getestet. Die Anzahl der jährlich durchgeführten Testungen ist von 17/Jahr (2015) auf 45/Jahr (2020) gestiegen. Bei 40 PatientInnen (21 %) wurde eine BRCA1/2 Mutation nachgewiesen, davon waren 28 (70 %) BRCA1 und 12 (30 %) BRCA2 positiv. Durchschnittsalter der BRCA1-PatientInnen bei der Erstdiagnose betrug 44 Jahre und der BRCA2-PatientInnen 50 Jahre. Von BRCA1-PatientInnen waren 22 (79 %) triple-negativ und 5 (18 %) Hormonrezeptor-positiv. Von BRCA2-PatientInnen waren 5 (42 %) triple-negativ und 7 (58 %) Hormonrezeptor-positiv.

Schlussfolgerungen: Die Anzahl der genetischen Testungen und damit entdeckten Mutationen ist in den letzten Jahren kontinuierlich gestiegen. Aufgrund der Zulassung der PARP Inhibitoren in der metastasierten und demnächst in der frühen Erkrankung wird es notwendig werden, den Anteil der zu testenden PatientInnen weiter zu erhöhen, um möglichst vielen PatientInnen diese Therapieoption ermöglichen zu können.

O22

Treatment associated changes in the inflammatory microenvironment composition of brain metastases

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Background: Radio- and immunotherapy were postulated to have synergistic efficacy in brain metastasis (BM) treatment due to the immune modulating properties of radiation.

Methods: BM samples from treatment naïve patients (group 1) and from patients treated with whole brain radiotherapy (WBRT) (group 2), stereotactic radiosurgery (SRS) (group 3), combined WBRT and SRS (group 4), or prophylactic cranial irradiation (group 5) before BM resection were analyzed according T cell subsets.

Results: Specimens from 81 patients with BM from different solid tumors were available for analysis. Group 1 presented with statistically significantly higher CD3+ (median: 492.6 cells/mm^2), CD8+ (median: 116.3 cells/mm^2) and LAG3+ (median: 17.6 cells/mm^2) TIL densities than group 2 (CD3+ median: 55.5 cells/mm^2; LAG3+ median: 4.6 cells/mm^2), group 3 (CD3+ median: 67.7 cells/mm^2; CD8+ median: 40.6 cells/mm^2) and group 4 (CD3+ median: 38.28 cells/mm^2; p-value < 0.05; Kruskal Wallis test). No significant changes of the inflammatory microenvironment in group 5 compared to the other groups, and in PD-L1 expressions between the groups were observed (p-value > 0.05; Kruskal Wallis test). Of 24/81 (29.6 %) patients matched samples of initial resected BM and recurrent BM were available. All investigated T cell subsets were numerically lower in recurrent BM from patients treated with radiation therapy between resections than in recurrent BM from patients without radiation treatment between BM resections (p > 0.05; Mann-Whitney U-test).

Conclusions: Our data indicate an immunosuppressive effect of radiotherapy on BM, as evidenced by decreased T cell infiltration in radiated versus non-radiated BM specimens. Future clinical studies should focus on the optimal timely sequencing of immune modulating therapies and radiotherapy.

O23

Existential distress and cancer progression. Protective role of the vagus nerve

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Background: Cancer patients face difficulties in many areas of life, which results in existential distress. Mostly, they experience loss of purpose and demoralization during their existential crisis. Cancer progression involves three important mechanisms: oxidative stress, inflammation and excessive sympathetic activity. Recent research suggests that the autonomic nervous system, and particularly the vagus nerve (indexed by heart rate variability, HRV), may affect tumorigenesis by modulating these mechanisms and thus slow tumor progression. The research aims at finding if the baseline level of HRV can predict the levels of immune biomarkers and cancer markers over 6 months and if this interaction could be moderated by the level of existential distress.

Methods: Metastatic Colorectal Cancer Patients at the Department of Clinical Oncology Graz will be evaluated with standardized psychological questionnaires including existential distress. The Heart Rate Variability will be measured via ECG, during practicing a sitting and standing breathing paradigm.

Results: There are currently no results as the recruitment process is only just at the beginning. Previous studies showed that lower HRV has been found in cancer patients compared with healthy population. (De Couck & Gidron, 2013, Kim et al., 2010). Moreover, the patients with advanced stage of cancer showed decreased HRV compared with patients in the early stages (De Couck & Gidron, 2013).

Conclusions: HRV could be used as a prognostic factor for cancer progression and manipulated to improve clinical outcome.

O24

EVI1 promotes proliferation and invasive properties of head and neck squamous cell carcinoma cells

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Head and neck squamous cell carcinoma (HNSCC) is a frequent cancer with a poor prognosis. So far, the EGFR inhibitor Cetuximab as the only approved targeted drug. A deeper understanding of the molecular and genetic basis of HNSCC is expected to identify additional targets for rationally designed therapeutics. The transcription factor EVI1, the major product of the MECOM locus, is an oncoprotein with roles in both hematopoietic and solid tumors. In HNSCC, high EVI1 expression was associated with an increased propensity to form lymph node metastases, but its effects in this tumor entity have not yet been determined experimentally. We therefore overexpressed or knocked down EVI1 in several HNSCC cell lines, and determined the impact of these manipulations on parameters relevant to tumor growth and invasiveness, and on gene expression patterns. Our results revealed that EVI1 promoted proliferation and migration of HNSCC cells. Furthermore, it augmented tumor spheroid formation, and the ability of tumor spheroids to displace an endothelial cell layer. Finally, EVI1 altered the expression of numerous genes in HNSCC cells, and these genes were enriched for gene ontology terms related to its cellular functions. In summary, EVI1 represents a novel oncogene in HNSCC that contributes to cellular proliferation and invasiveness.

O25

Predictors of Covid19 risk perception in a sample of Austrian cancer Patients: a cross sectional study

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Background: Beginning 2019 in China, the coronavirus spread rapidly worldwide and was declared a pandemic by the World Health Organisation. The rapid spread caused fear and anxiety. Especially in cancer patients, the prevalence of anxiety and other psychological disorders increased during the pandemic. In this study, we aimed to evaluate the factors associated with COVID-19 perceived threat in cancer patients.

Methods: This research is a part of international cross-sectional study examining risk perception, treatment adherence, personality during COVID-19 pandemic in cancer. An online survey ($n=1281$) was conducted worldwide in seven countries including Austria ($n=216$). In the current research, we investigated the factors potentially associated with cancer patients' perceived COVID-19 threat. Data was collected during COVID-19 outbreak, from June until November 2020. Multiple linear regression was used to examine predictors of COVID-19 threat perception in cancer patients.

Results: COVID-19 threat perception was positively associated with anxiety. However, it was negatively associated with confidence in safeguards, patients' physical state, and fighting spirit. Multiple regression analysis indicated that confidence in safeguards ($\beta=-0.219$, $P<0.001$), anxiety ($\beta=0.322$, $P<0.001$), patients' physical state ($\beta=-0.106$, $P=0.006$), and fighting spirit ($\beta=-0.383$, $P<0.016$), significantly predicted COVID-19 threat perception and explained 26.3 % of the total variance in COVID-19 threat perception.

Conclusions: Austrian cancer patients' COVID-19 risk perception was predicted by anxiety, confidence in safeguards, patients' physical state and fighting spirit. The study contributes to our understanding of factors important for screening and care of cancer patients during COVID-19 pandemic.

O26

Neoadjuvant and adjuvant treatment patterns and clinical outcomes of invasive lobular breast cancer

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Background: Invasive lobular carcinoma (ILC) is the second most common subtype of breast cancer, accounting for up to 15 % of the cases. The aim of this study is to descriptively analyze neoadjuvant and adjuvant treatment strategies as well as the outcome of patients with ILC.

Methods: Baseline and outcome data of patients with non-metastatic (stage I-III) ILC who were treated between 2006 and 2021 at a single academic institution were systematically collected. The primary outcome, disease-free survival (DFS), was defined as the time between diagnosis and local recurrence, second breast cancer, occurrence of metastasis or death. Secondary outcome was overall survival (OS).

Results: We analyzed 344 patients (median age: 64 years [25th–75th percentile: 52–74], median Ki-67: 10 % [7–20]). Few patients were treated in the neoadjuvant setting. $N=47$ (14 %) patients received neoadjuvant chemotherapy, $n=26$ (8 %) received neoadjuvant endocrine therapy. Fifty-nine patients (17 %) were treated with adjuvant chemotherapy, and $n=310$ (90 %) patients with adjuvant endocrine therapy. After a median follow-up of 6.9 years, we observed 104 DFS events and 77 OS events. This corresponded to a median DFS of 10.5 years, and median OS was not reached. Five-year DFS and OS estimates were 74 % (95 %CI: 68–79) and 85 % (95 %CI: 80–88), respectively.

Conclusions: In summary, we analyzed a large single-center cohort of patients diagnosed with early stage ILC treated at our department. The prevalences of neoadjuvant and adjuvant systemic therapies and the overall oncologic outcomes were consistent with previously published cohorts of ILC patients.

O27

Improvement in colorectal cancer outcomes over time is limited to patients with left-sided disease

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Background: Incidence and mortality of colorectal cancer (CRC) declined over the last decades. However, survival depends on the primary tumor location. It is unknown if all progress in outcomes vary depending on left-sided (LCRC) versus right sided (RCC) colorectal cancer. We compare incidence and mortality rates over time according to the primary tumor location.

Methods: Data from the Austrian National Cancer Registry spanning from 1983 to 2018 were used to calculate annual incidence and mortality rates and survival stratified by primary tumor localization and stage. Joinpoint regression with linear regression models were used on different subgroups to identify significant changes of incidence- and mortality slopes.

Results: A total of 168,260 (incidence-data set) and 87,355 cases (mortality data-set) were identified. Survival of disseminated RCC was worse compared to LCRC (HR 1.14; CI 1.106 – 1.169). Total and LCRC incidence- and mortality-rates declined steadily over time, whereas the rates of RCC did not. Incidence of disseminated RCC declined significantly less (slope -0.07; CI -0.086; -0.055) than in LCRC (slope -0.159; CI -0.183; -0.136); mortality rate of RCC was unchanged over time. Incidence and mortality of localized RCC remained unchanged over time, whereas both rates declined independently of stage in LCRC.

Conclusions: Biological differences between LCRC and RCC directly translate to the population level, which has not been evident for mortality, yet. Further, colorectal cancer outcomes during the last 35 years have preferentially improved in LCRC but not in RCC, indicating that the oncological progress made is limited to LCRC. It is necessary to define RCC as a distinct form of CRC and to focus on specific strategies for its early detection and treatment.

Klinische Studien

S01

AGMT_AIHA_Reg: PATIENT REGISTRY Autoimmune Hemolytic Anemia (AIHA) with corresponding Biobank

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This registry is a prospective and retrospective, multicentre collection of data on patients with AIHA in Austria. All disease characteristics, medical histories and also treatment sequences are documented in anonymised form. Additionally patients will be asked to complete the FACIT-Fatigue questionnaire. For documentation in the registry no further diagnostic or therapeutic measures are required than those already necessary in general. Participation in the project must not interfere with treatment routines. Data will be collected from all sites in Austria willing to participate. An estimated 100 patients are expected to be included; this number may be revised over time as interest and demand dictates. Within this project biomaterial of patients with AIHA in Austria will be collected in the AGMT biobank. This collection of biomaterial is optional.

Primary objective: The aim of the AIHA registry is to collect data regarding the following objectives of disease for all Austrian AIHA patients older than 18 years.

- Epidemiological evaluations
- Assessment of AIHA subtypes in Austria
- Assessment of specific characteristics and frequency of AIHA
- Patient care and treatment in Austria
- Treatments used, sequence of treatments
- Efficacy and toxicity
- Establishment of a central biobank to provide a basis for future AIHA related research (optional)

S02

AGMT_aMYELOIDr: AUSTRIAN MYELOID REGISTRY

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The Austrian Myeloid Registry (AMR) is a non-interventional study. It collects data from patients with the myeloid diseases like myelodysplastic syndromes (MDS), chronic myelomonocytic leukemia (CMML), acute myeloid leukemia (AML), primary myelofibrosis (PMF), chronic myeloid leukemia (CML), and other rarer disease subtypes. The AMR is multi-center database and collects data at various sites in Austria and potentially also at other centers in other countries in future. The registry has an electronic case report form (eCRF), where all data is entered by clinical trial personnel and/or physicians. The registry also consists of patients previously documented in the Austrian Registry of Hypomethylating Agents. The registry is intended as a long-term project. The initial medium-term goal regarding patient numbers will be 3,000 (incl. patients of HMA Registry) documented patients. The goal of the Austrian Myeloid Registry is to build a disease-specific registry aimed at assessing the therapeutic landscape of patients with myeloid diseases. Our intention is to advance our knowledge on the natural course of these diseases in untreated or best supportive care (BSC) treated patients, as well as the efficacy and toxicity and sequence of use of various treatments in a routine clinical setting.

Primary objective: To assess the treatment patterns (therapeutic landscape) of patients with myeloid diseases.

S03

AGMT_DISCOVER: Multicenter-randomized, double-blind-placebo-controlled, phase-III-clinical-trial to investigate efficacy + safety of Dronabinol in the Improvement of ChemOthErapy-induced and tumor-Related-symptoms in patients with locally-advanced or metastatic pancreatic-cancer during first-line-chemotherapy

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This is a multicenter, randomized, double-blind, placebo-controlled clinical trial to investigate the efficacy and safety of dronabinol in the improvement of chemotherapy-induced and tumor-related symptoms in patients with advanced pancreatic cancer during first-line chemotherapy.

Population: Adult patients (≥ 18 years) with diagnosis of locally advanced or metastatic pancreatic cancer, assigned to first-line chemotherapy with FOLFIRINOX or gemcitabine + Abraxane®.

Primary endpoint: The primary endpoint variable is the standardized area under the curve of the EORTC QLQ-C30 symptom summary score over the on-treatment period (scores at visits 1–9).

S04

PATIENT REGISTRY AGMT_LungCA_Reg: Lung Cancer Registry

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This registry is designed as ulticentre observational cohort of patients with lung cancer. It will be set up to collect real-world experience in the management of patients with this disease. This registry will collect data at various sites in Austria. The aim is to gain valuable insights on both efficacy and toxicity, as well as the sequence of use of various treatments in a routine clinical setting.

Indication: The registry will be made available for all disciplines and physicians caring for cancer patients and will include patients ≥ 18 years with locally advanced or metastatic lung cancer (advanced or metastatic stage patients in Austria (Stage III A-C and IV A-B NSCLC, limited disease (LD) and extensive disease (ED) SCLC)).

Primary objective:

- To describe the general characteristics of advanced or metastatic stage patients in Austria and molecular testing in patients with advanced or metastatic lung cancer
- To describe and characterize subgroups
- To describe treatment and outcome of treatment
- To describe patient outcome by means of overall survival and progression free survival
- To describe toxicity with a focus on immune related adverse events

Recruitment: 500 patients (this number may be revised over time as interest and demand dictates).

S05

PATIENT REGISTRY AGMT_MBC_Reg: Metastatic breast cancer in Austria

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This registry is a prospective and retrospective, multicenter collection of data on patients with metastatic breast cancer in Austria. All tumor characteristics, medical histories and also treatment sequences are documented in anonymized form. For documentation in the registry, no further diagnostic or therapeutic measures are required than those already necessary in general. Participation in the registry must not interfere with treatment routines. A written consent must be obtained prior to the input of data. No informed consent is required from deceased patients.

Indication:

- Histological evidence of breast cancer
- Histological and/or radiological evidence of metastases
- Metastasis within 10 years of registry initiation

Primary objective: Epidemiological evaluations (general characteristics of metastatic stage patients in Austria, assessment of metastatic stage breast cancer subtypes in Austria, assessment of the specific characteristics and frequency of metastatic breast cancer, data on survival of female patients with metastatic breast cancer in Austria) and therapy-specific evaluations

Recruitment: 2000–3000 patients

S06

AGMT_MM-2: Randomized Phase-II, 2-armed-study in transplant ineligible patients with newly-diagnosed-multiple-myeloma(NDMM) comparing Carfilzomib + Thalidomide + dexamethasone(KTd) versus Carfilzomib + Lenalidomide + dexamethasone(KRd) induction therapy with respect to response-rates and investigating a Carfilzomib(K) monotherapy-maintenance-strategy

Heinz Ludwig

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Design: This is a randomized, 2-arm phase-II, multi-center-study to evaluate the overall response rates in newly diagnosed, transplant ineligible patients receiving 9 cycles induction therapy with either KTd or KRd followed by randomization to either Carfilzomib maintenance treatment for 12 months or to obser-

vation only. Maintenance is given for 12 cycles or until progression of disease or intolerance, whatever occurs first.

Therapy regime: *Arm A)* KTd: K: 56 mg/m² weekly = day 1, 8, 15 of each cycle; (Note: C1D1 + 2 start with 20 mg/m² K, D 8 + 9 & 15 + 16 of C1: 27 mg/m²; C2 D 1, 2, 8, 9, 15 + 16: 27 mg/m²); Thalidomide 100 mg/day, day 1–28; dexamethasone: 40 mg/week, day 1, 8, 15, 22 or *Arm B)* KRd: K: 56 mg/m² weekly = day 1, 8, 15 of each cycle (Note: C1 and C2 see ArmA); Lenalidomide: 25 mg/day, day 1–21; Dexamethasone: 40 mg/week-day 1, 8, 15, 22 for a maximum of 9 cycles as induction therapy. Each cycle has 28 days. Primary endpoint is to show non-inferiority with respect to response rates between KTd and KRd.

Patients: A total of 146 adult patients (≥ 18 years) with newly diagnosed symptomatic MM will be enrolled in this study. Excluded are patients who are planned for an autologous-stem-cell-transplantation following induction, who are intolerable to IMiDs or Carfilzomib, are NYHA-class >II, present with PS ≥ 2, CrCl ≤ 30 ml/min, and/or neuropathy grade ≥ 2.

S07

AGMT_MM-4: Isatuximab in combination with Lenalidomide-Dexamethasone compared to Lenalidomide-Dexamethasone in elderly patients (aged ≥ 70 years) with newly diagnosed myeloma: a randomized phase II study (SGZ-2019-12650)

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Design: This is a prospective, multicenter, multinational, randomized, open-label, parallel group, 2-arm study evaluating the clinical benefit of isatuximab in combination with lenalidomide and low-dose dexamethasone followed by isatuximab and lenalidomide maintenance therapy as compared to lenalidomide and low-dose dexamethasone followed by lenalidomide maintenance therapy for the treatment of patients with newly diagnosed multiple myeloma 70 years of age or older.

Patient population: A total of 198 patients with newly diagnosed multiple myeloma aged ≥ 70 years meeting the criteria for inclusion as outlined below will be included.

Primary Objective: To demonstrate the benefit of isatuximab in combination with lenalidomide and low-dose dexamethasone followed by isatuximab and lenalidomide maintenance therapy in increasing the proportion of patients with MRD negativity as compared to lenalidomide and low-dose dexamethasone followed by lenalidomide maintenance treatment in patients with newly diagnosed multiple myeloma (NDMM).

S08

PATIENT REGISTRY AGMT_NGS_Reg: The use of genomic testing and the resulting medical decisions according to target identification

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This registry is designed as multicenter non-interventional (observational) cohort of oncology patients who received or plan to receive comprehensive genomic testing anytime on or after January 1, 2016. Patient medical, testing and treatment information will be obtained through extraction of data from existing patient medical charts. Longitudinal follow-up data, including survival and tumor progression, will also be extracted from patient medical charts. This patient follow-up data will be obtained until patient death or loss to follow-up.

For documentation in the registry, no further diagnostic or therapeutic measures are required than those already necessary in general. Participation in the registry must not interfere with treatment routines. Only routine data, which has already been recorded in the patient's medical chart, is transferred to the electronic Case Report Forms. To maintain patient confidentiality, each patient will be assigned a unique patient identifying number upon enrolment; this number will accompany the patient's medical and other registry information throughout the lifetime of the registry.

The goal of this registry is to landscape the clinical practice of molecular profiling in Austrian cancer patients with focus on identification of methods used, evaluation when the tests are performed in the course of the disease, and definition of the impact of the test result on the subsequent treatment decision.

S09

GELTAMO18-HL: BRESELIBET – BREntuximab Vedotin in SEcond LIne Therapy BEfore Transplant

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A randomized phase IIb study, evaluating efficacy of salvage therapy with Brentuximab Vedotin-ESHAP vs ESHAP in patients with relapsed/refractory classical Hodgkin's lymphoma, followed by Brentuximab Vedotin consolidation (instead of autologous hematopoietic stem cell transplantation) in those who attained a metabolic complete remission after salvage therapy.

Design: A phase IIb open label multi-center trial in patients with refractory/relapsed cHL.

Patients: The first part of the study randomizes 3 cycles of ESHAP as a standard of care therapy for those patients with primary refractory cHL and those patients relapsing after first-line therapy versus 3 cycles ESHAP-BV with BV at a dose of 1.8 mg/kg IV. The rationale behind this first part is the lack of prospec-

tive randomized comparisons between conventional salvage chemotherapy protocols and its BV-containing counterpart in terms of rate of metabolic CRs and ORR. In the second part of the trial, those patients that achieve a mCR will be consolidated with either 13 or 16 cycles of BV (depending on the prior treatment yes/no with BV together with the salvage second-line strategy) at the usual doses and time intervals (1.8 mg/kg iv in 30 min every 21 days). The objective of this second part is to try to avoid auto-HCT as well as early, mid and long-term toxicities associated to the procedure in a population of patients that have a better prognosis and substitute it by an anti-CD30 monoclonal-antibody drug conjugate that has demonstrated a beneficial effect as a consolidation strategy in the AETHERA Trial.

S10

GHSG_AERN: Abscopal effect of radiotherapy and nivolumab in relapsed Hodgkin lymphoma after anti-PD1 therapy

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The trial is a prospective, international, non-randomized, multicenter phase II investigator-sponsored trial for patients with relapsed or refractory cHL progressing while on treatment with an anti-PD1 antibody. A Simon's optimal two-stage design has been chosen with 9 patients to be evaluated for the primary endpoint in stage 1. If there are 1 or more stage-1-patients with an abscopal response to localized RT and 6 applications of nivolumab (ARR-6), 20 additional patients will be recruited into the second stage of the trial for a total of 29 patients to be evaluated for ARR-6.

Primary endpoint: Abscopal response rate (ARR-6) with abscopal response centrally confirmed as restaging result after RT to a single lesion and at least four but not more than six nivolumab infusions (RE-6 result). The primary objective of the trial is to show efficacy of the experimental treatment strategy. Secondary objectives are to further evaluate efficacy, show safety and feasibility and perform correlative studies.

Treatment: Nivolumab 240 mg i.v. at 2-weekly intervals combined with 20 Gy radiotherapy (RT) to a preferably progressive and not pre-irradiated single lesion. Nivolumab will be continued for a maximum of 18 months or until disease progression or unacceptable toxicity.

S11

GHSG_HD21: Treatment optimization trial in the first-line treatment of advanced stage Hodgkin lymphoma; comparison of 4–6 cycles of escalated BEACOPP with 4–6 cycles of BrECADD

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In the prospective, multicenter, randomized and open-label main trial, patients in the standard group are treated with either 4 or 6 cycles of escalated BEACOPP according to the results of the interim staging (PET-2 negative patients receive 4 cycles of escBEACOPP, PET-2 positive patients receive 6 cycles of escBEACOPP). Patients in the experimental group receive either 4 or 6 cycles of the BrECADD chemotherapy regimen, again according to the results of the interim staging (PET-2 negative patients receive 4 cycles of BrECADD, PET-2 positive patients receive 6 cycles of BrECADD).

In both groups, patients with PET positive residual tumor masses at the end of chemotherapy according to PET-4 or PET-6 are subjected to local irradiation with 30 Gy.

1500 patients up to 60 years of age were already randomized in the main study. In parallel, it is planned to recruit 85 older patients with advanced-stage classical HL. Primary objective of the main trial is to demonstrate reduced toxicity of the BrECADD treatment compared to the escalated BEACOPP treatment measured by treatment-related morbidity (TRMB objective). If reduced toxicity can be shown, the co-primary objective is to further demonstrate non-inferior efficacy of six cycles of BrECADD compared to six cycles of escalated BEACOPP, each followed by radiotherapy to PET-positive residual lesions, in terms of progression -free survival (efficacy objective).

The primary aim of the elderly cohort analysis is to generate a treatment protocol which is effective in advanced-stage classical HL and feasible in older patients eligible to receive polychemotherapy.

S12

ImbruVeRCHOP: Ibrutinib (Imbruvica®), Bortezomib (Velcade®) s.c., Rituximab, CHOP for the treatment of elderly patients (age 61–80 years) with CD20+ diffuse large B-cell lymphoma, IPI ≥2

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This trial is designated as a single arm multi-center prospective open phase I/II trial with a safety run-in phase, i.e. the phase I part of the trial, which is followed by the phase II part of the trial to evaluate the efficacy of Ibrutinib and Bortezomib s.c. in the treatment of higher-risk elderly DLBCL patients of different molecular subtypes and to correlate outcome with clinical, molecular and imaging-guided response parameters.

Population: Target group of the study are patients with untreated CD20-positive DLBCL-like aggressive Non-Hodgkin's lymphoma, 61–80 years of age with unfavorable risk profile (IPI ≥ 2). The number of patients planned to be included is 34.

Primary objective: The main objective of this clinical trial is to assess the efficacy of the treatment determined as the 2-year PFS for patients with DLBCL.

Primary endpoint: Primary endpoint is the 2-year progression-free survival (PFS) for all patients.

S13

DSHNHL_NIVEAU: Improvement of outcome in elderly-patients or patients not eligible for high-dose-chemotherapy with aggressive-Non-Hodgkin-Lymphoma in first-relapse or progression by adding nivolumab to gemcitabine, oxaliplatin + rituximab by CD20+-disease

Ulrich Jäger

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This trial is designed as an international, multicentre, randomised, open-label, treatment optimisation study, preceded by safety run-in phases conducted for B-cell and T-cell lymphoma separately. Aim of the phase-III trial is to test whether prognosis of patients with relapsed or refractory aggressive Non-Hodgkin Lymphoma not eligible for neither autologous nor allogeneic stem cell transplantation can be improved by combining nivolumab with (R)-GemOx.

Population: All patients with first relapse or progression of an aggressive Non-Hodgkin's lymphoma aged older than 65 years or older than 18 years with HCT-CI score > 2 are eligible for this study irrespective of their gender or stage of disease. There is no upper limit of age. Also patients not eligible for neither autologous nor allogeneic stem cell transplantation are eligible for this study.

Primary objective: Improvement of 1-yr PFS by nivolumab plus (R)-GemOx followed by nivolumab consolidation instead of (R)-GemOx alone. Primary endpoint is 1-yrs progression-free survival.

S14

Pola-R-ICE: Open-label, prospective phase III clinical study to compare polatuzumab vedotin + rituximab, ifosfamide, carboplatin + etoposide(Pola-R-ICE) with rituximab, ifosfamide, carboplatin + etoposide(R-ICE) alone as salvage-therapy in patients with primary refractory or relapsed diffuse large B-cell-lymphoma (DLBCL)

Richard Greil

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International, multicenter, open-label, two-arm, randomized, prospective, phase III study with Polatuzumab vedotin plus rituximab, ifosfamide, carboplatin and etoposide (Pola-R-ICE) versus R-ICE alone in second line treatment of diffuse large B-cell lymphoma (DLBCL).

Population: Male and female subjects 18 years or older suffering from first relapse or primary refractory disease of DLBCL.

Primary objective: The primary objective of this study is to investigate the following question in patients with relapsed or primary refractory DLBCL: Does salvage therapy with Pola-R-ICE improve event-free survival (EFS) compared to R-ICE alone?

Primary endpoint: The primary endpoint is EFS of patients with DLBCL at first progression or relapse. EFS is defined as the time between the day of randomization and the occurrence of any of the following events:

- Failure to achieve sufficient response in PET-CT (Deauville score 3 or less) at end of study treatment (metabolic CR)
- Disease progression (PD)
- Start of additional unplanned anti-tumor treatment (radiation therapy allowed)
- Relapse after achieving CR
- Death due to any cause

Patients who have not experienced any of these events by the time of analysis will be censored at the most recent date of disease assessment.

S15

ABCSG 45: Disruption of the C/EBP-PU.1 axis perturbs monocyte subset homeostasis and creates an MDS-promoting niche

Marija Balic, Christian Singer, Josef Thaler, Martin Schindl

Austrian Breast and Colorectal Cancer Study Group (abcsg), Vienna, Austria

A prospective, open, randomized, phase II study of carboplatin/olaparib in the pre-operative treatment of patients with triple-negative primary breast cancer which exhibit the features of positive homologous recombination deficiency (HRD) status.

Hypothese: geringere Residual Cancer Burden (RCB) unter Zugabe von Olaparib im Vergleich zu konventioneller Taxan/Anthrazyklin-Chemotherapie

Studienpopulation: 90 PatientInnen mit frühem TNBC und positivem HRD-Status

Einbringung: voraussichtlich bis Q3 2022

S16

ABCSG 55N/AMBHER: Description of patients with HER2 positive breast cancer undergoing neoadjuvant treatment and development of a dynamic composite risk score to predict the risk of distant recurrence

Marija Balic, Christian Singer, Josef Thaler, Martin Schindl

Austrian Breast and Colorectal Cancer Study Group (abcsg), Vienna, Austria

Hypothese: Entwicklung eines Modells zur Bewertung des Fernrezidiv-Risikos (Distant Recurrence)

Studienpopulation: ca. 500 PatientenInnen mit HER2+ frühem Brustkrebs (nach min. einer Dosis neoadjuvanter dualer HER2-Blockade), retrospektive PatientInnen können max. 5 Jahre nach Beginn der neoadjuvanten Therapie eingeschlossen werden.

Einbringung: voraussichtlich Q1 2022–Q2 2025

S17

ABCSG C08/Exercise II: Randomized trial of endurance exercise following adjuvant chemotherapy for colorectal cancer

Marija Balic, Christian Singer, Josef Thaler, Martin Schindl

Austrian Breast and Colorectal Cancer Study Group (abcsg), Vienna, Austria

Hypothese: bessere Wirksamkeit von Ausdauertraining im Vergleich zu üblicher körperlicher Aktivität, bezogen auf erkrankungsfreies Überleben

Studienpopulation: 100 PatientInnen mit lokal fortgeschrittenem Kolorektal-Karzinom nach adjuvanter Chemotherapie

Einbringung: voraussichtlich bis Ende Q4 2022

S18

ABCSG P02: A prospective randomized phase II trial of FOLFIRINOX alone versus FOLFIRINOX followed by radiochemotherapy in patients with locally advanced, primarily inoperable pancreatic cancer

Marija Balic, Christian Singer, Josef Thaler, Martin Schindl

Austrian Breast and Colorectal Cancer Study Group (abcsg), Vienna, Austria

Hypothese: Überlegenheit neoadjuvanter Chemotherapie gefolgt von neoadjuvanter Radiochemotherapie gegenüber alleiniger neoadjuvanter Chemotherapie in Bezug auf R0-Resektabilität

Studienpopulation: PatientInnen mit lokal fortgeschrittenem, primär inoperablem Pankreaskarzinom

Einbringung: voraussichtlich bis Ende Q1 2023

Weitere Informationen finden Sie unter https://www.abcsg.org/abcsg-studien/.

S19

ABCSG 49/POLAR: A phase III open-label, multicenter, randomized trial of adjuvant palbociclib in combination with endocrine therapy versus endocrine therapy alone for patients with hormone receptor positive/HER2-negative resected isolated locoregional recurrence of breast cancer

Marija Balic, Christian Singer

Austrian Breast and Colorectal Cancer Study Group (abcsg), Vienna, Austria

Hypothese: besseres invasiv-krankheitsfreies Überleben (iDFS) unter Zugabe von Palbociclib

Studienpopulation: 400 PatientInnen mit HR+, HER2-, isoliertem lokoregionären Brustkrebs-Rezidiv

Einbringung: voraussichtlich bis Q1 2023 (6 Länder)

S20

ABCSG 50/BRCA-P: A randomized, double-blind, placebo-controlled, multi-center, international phase 3 study to determine the preventive effect of denosumab on breast cancer in women carrying a BRCA1 germline mutation

Marija Balic, Christian Singer

Austrian Breast and Colorectal Cancer Study Group (abcsg), Vienna, Austria

Hypothese: Verringerung des Brustkrebs-Risikos durch präventives Denosumab

Studienpopulation: 2.918 BRCA1-Keimbahnmutationsträgerinnen

Einbringung: voraussichtlich bis Q4 2023 (7 Länder)

S21

ABCSG 56/SASCIA: Phase III postneoadjuvant study evaluating Sacituzumab Govitecan, an antibody drug conjugate in primary HER2-negative breast cancer patients with high relapse risk after standard neoadjuvant treatment

Marija Balic, Christian Singer

Austrian Breast and Colorectal Cancer Study Group (abcsg), Vienna, Austria

Hypothese: besseres invasiv-krankheitsfreies Überleben (iDFS) bei Behandlung mit Sacituzumab Govitecan

Studienpopulation: 1.200 PatientInnen mit HER2- Brustkrebs und Resttumor nach NACT

Einbringung: voraussichtlich bis Q4 2023 (8 Länder)

S22

ABCSG 57/ALPHABET: A randomized phase III trial of trastuzumab + ALpelisib +/– fulvestrant versus trastuzumab + chemotherapy in patients with PIK3CA mutated previously treated HER2+ Advanced BrEasT cancer

Marija Balic, Christian Singer

Austrian Breast and Colorectal Cancer Study Group (abcsg), Vienna, Austria

Hypothese: besseres progressions-freies Überleben (PFS) unter Zugabe von Alpelisib

Studienpopulation: 300 PatientInnen mit PIK3CA-mutiertem HER2+ Brustkrebs im fortgeschrittenen/rezidivierten Stadium (mit Trastuzumab und T-DM1 vorbehandelt)

Einbringung: voraussichtlich bis Q1 2025 (6 Länder)

Weitere Informationen finden Sie unter https://www.abcsg.org/abcsg-studien/.

Young Investigators-Meeting

Y01

Identification of lung cancer-specific tumor-associated neutrophils by single-cell RNA sequencing

Lena Horvath[1], Stefan Salcher[1], Gregor Sturm[2], Gerold Untergasser[1], Gabriele Gamerith[1], Marcus Kalb[1], Martina Sykora[1], Florian Augustin[3], Katja Schmitz[4], Agnieszka Martowicz[5], Sieghart Sopper[1], Zlatko Trajanoski[2], Dominik Wolf[1], Andreas Pircher[1]

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Background: Single cell RNA sequencing (scRNA-seq) unraveled the complexity of the tumor microenvironment (TME) and the importance of minor cell populations in non-small cell lung cancer (NSCLC). It identified a previously underestimated TME heterogeneity inter-individually, however data interpretation is impeded by small patient cohorts and missing power for rare cell populations. To overcome this limitation, we created a comprehensive transcriptome atlas of a large NSCLC cohort by merging publicly-available and own datasets.

Methods: Transcriptome data of 18 published plus our own datasets were selected, covering 182 NSCLC patients and 158 non-tumor controls. Data were obtained as raw counts, processed using Scanpy and integrated using the SCANVI algorithm. Cell-types were annotated based on unsupervised clustering and marker genes. Orthogonal validation of selected genes by flow cytometry and multiplex-immunohistochemistry was performed.

Results: Our atlas comprises the so-far largest scRNA-seq consortium covering 1.124.947 single cells and 31 cell types. It catalogues underestimated cell populations and displays neutrophils, known as largest myeloid population in NSCLC, to be underrepresented in most scRNA-seq studies due to technical reasons. This highlights that different sequencing platforms influence representation of cellular TME components. We define a unique tumor-associated neutrophil (TAN) transcriptomic signature, characterized by a CXCR4high, ORL1high, CXCR2low expression profile and depict TAN as the major VEGFA source in the TME.

Conclusions: We present the largest public-available NSCLC scRNA-seq atlas and focus on the first-time transcriptomic characterization of TAN. TAN show a distinct activated, pro-angiogenic signature and are missed by most standard scRNA-seq platforms.

Y02

BRAFV600E mutation in human hematopoietic stem and progenitor cells promotes monocyte/macrophage commitment and histiocytic features

Tommaso Sconocchia[1], Johannes Foßelteder[1], Angelika Schlacher[1], Lisa Auinger[1], Erdem Özkaya[1], Christine Beham-Schmid[2], Peter Schlenke[3], Heinz Sill[1], Armin Zebisch[1,4], Herbert Strobl[5], Andreas Reinisch[1,3]

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[5]Otto Loewi Research Center, Division of Immunology and Pathophysiology, Medical University of Graz, Graz, Austria

Background: MAPK pathway mutations are often found in rare histiocytic disorders such as Langerhans cell histiocytosis (LCH) and Erdheim-Chester disease (ECD). BRAFV600E is the most common mutation and can be detected in committed monocytes/macrophages, dendritic cells, and hematopoietic stem and progenitor cells (HSPCs). Although the cell of origin that gives rise to these disorders is currently unknown, it was recently suggested that LCH and ECD arise from BRAFV600E-mutant HSPCs. However, how BRAFV600E transforms HSPCs and causes histiocytic disorders is not fully understood. Here we aim to model BRAFV600E mutations in human HSPCs to gain further insight in the underlying pathomechanistic processes.

Methods: HSPCs were engineered by using a CRISPR-Cas9-based knock-in strategy. HSPCs were cultured either in semi-solid or liquid cultures and analyzed by microscopy and flow cytometry.

Results: We could successfully engineer HSPCs to express the heterozygous BRAFV600E mutation. Correct knock-in at the endogenous BRAF locus was confirmed by Sanger sequencing and by mutation-specific immunohistochemistry. Differentiation assays revealed that BRAFV600E HSPCs preferentially differentiate towards the monocytic lineage at the expense of the erythroid and granulocytic lineages. Interestingly, BRAFV600E

HSPCs cultured under stem cell-maintaining conditions sharply lose CD34 expression, gain the monocytic markers CD14 and CD11c, and form foamy macrophages.

Conclusions: Altogether, these data provide convincing evidence that the BRAFV600E mutation reprograms HSPCs to differentiate into abnormal mononuclear phagocytes. By recapitulating cellular phenotypes typically seen in Langerhans and non-Langerhans histiocytic disorders, our model further supports the recently proposed concept of an HSPC-origin in BRAFV600E-mutant histiocytic disorders.

Y03

Targeted introduction and correction of CALR mutations in human HSPCs sheds light on MPN pathogenesis

Johannes Foßelteder[1], Angelika Schlacher[1], Gabriel Pabst[1], Lisa Auinger[1], Christine Beham-Schmid[2], Karl Kashofer[2], Slave Trajanoski[3], Peter Schlenke[4], Heinz Sill[1], Albert Wölfler[1], Armin Zebisch[1,5], Andreas Reinisch[1,4]

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Background: Recurrent mutations in calreticulin (CALR) are common driver events in myeloproliferative neoplasms (MPNs) such as essential thrombocythemia (ET) and primary myelofibrosis (PMF). Mutant CALR causes constitutive thrombopoietin receptor (TPOR) signaling, leading to increased megakaryocyte formation in the bone marrow (BM). In addition, TPOR-independent mechanisms have been implicated in the pathogenesis of CALR mutant MPNs. However, these novel mechanistic insights have exclusively been generated in transgenic mouse models and cancer cell lines, limiting their clinical translation. We aimed to model human MPN pathogenesis using CRISPR-engineered human hematopoietic stem and progenitor cells (HSPCs) to circumvent these limitations.

Methods: We introduced and corrected CALR mutations in human HSPCs using a targeted CRISPR/Cas9-based knock-in strategy and analyzed their implications on HSPC function in vitro and in vivo.

Results: Introduction of CALR mutations caused TPO-independent growth and increased CD41+ megakaryocyte formation in vitro. Upon transplantation into immunodeficient NSG mice, CALR mutant HSPCs showed robust BM engraftment with enhanced CD41+ megakaryocyte formation in vivo. In addition, RNA-sequencing of CALR mutant HSPCs revealed upregulation of chaperones to increase ER stress resistance. Finally, ex vivo CRISPR-based genetic correction of CALR mutations in MPN patient-derived HSPCs abolished expression of mutant CALR protein and reduced pathologic megakaryocyte formation.

Conclusions: Our system faithfully recapitulates MPN phenotypes and allows for prospective investigation of human MPN pathogenesis. Furthermore, RNA-seq data sheds light on novel transforming mechanisms of mutant CALR in primary HSPCs. Finally, successful ex vivo gene correction of MPN-patient-derived HSPCs provides a novel, potentially curative, treatment approach.

Y04

Co-occurrence of mutations modifying RAS and EZH2 inactivation in chronic myelomonocytic leukemia causes amplification of RAS-AKT signaling and increases the sensitivity to AKT inhibitors

Franciska Telebar[1], Johannes Lorenz Berg[1,2], Bianca Perfler[1], Bernadette Bramreiter[3], Veronica Caraffini[1], Andreas Prokesch[4,5,6], Klaus Geissler[7,8], Thomas Penz[9], Michael Schuster[9], Andreas Reinisch[1,10], Albert Wölfler[1], Heinz Sill[1], Armin Zebisch[1,3]

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Background: Chronic myelomonocytic leukemia (CMML) is driven by mutations modifying the RAS oncogenes (RASmut). We have previously shown that RASmut in CMML frequently co-occur with inactivation of the histone protein modifier EZH2 (EZH2inact) and that this co-existence is associated with a dismal prognosis. Mechanistically, we demonstrated that RASmut/EZH2inact hyperactivates RAS-MAPK/ERK signaling (Berg JL, Leukemia 2021). Here, we aimed to analyze the effects of RASmut/EZH2inact co-occurrence on other RAS-downstream signaling cascades.

Methods: EZH2inact was established in the RASmut myeloid cell lines THP1, HL60, and U937 by lentiviral transduction of shRNAs or CRISPR/Cas9 gene editing. For mechanistic insights, RNA-sequencing and ChIP-sequencing data from RASmut/EZH2inact cells were analyzed. Activation of signaling pathways was assessed by (phospho-)immunoblots. Sensitivity to RAS-MAPK signaling inhibitors was tested by Annexin-V/7-AAD assays.

Results: Gene set enrichment analysis of RNA-sequencing performed in RASmut/EZH2inact cells revealed upregulation of genes activating Pi3K/AKT signaling. ChIP-sequencing data confirmed the EZH2-mediated changes in trimethylated histone H3 (H3K27me3) marks in the promoter region of AKT1/2. Immunoblots revealed that EZH2inact in RASmut cells indeed hyperactivated AKT signaling. Most importantly, this rendered

cells hypersensitive to pharmacological AKT inhibition, as shown by increased apoptosis induction after incubation with two different AKT inhibitors (GDC-0068; MK-2206).

Conclusions: Co-occurrence of RASmut and EZH2inact in CMML amplifies RAS-signaling via the AKT pathway. This hyperactivation causes increased dependence on this signaling cascade and renders leukemic cells more sensitive to pharmacological AKT inhibition. Therefore, these data might pave the way to a new therapeutic strategy in RASmut/EZH2inact CMML patients.

Y05

Functional cooperation of CEBPA and TET2 mutations in Acute Myeloid Leukemia

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Background: Mutations in the transcription factor CEBPA occur in 9–15 % of Acute Myeloid Leukemia (AML) patients and are frequently found together with loss-of-function mutations in the methylcytosine dioxygenase TET2 (34.8 %–44.4 % of CEBPA-mutated AML), resulting in adverse overall survival in affected patients. Thus, we hypothesized that combinatorial effects of both mutations specifically rewire cellular circuitries, influencing disease outcome. We aimed to elucidate the underlying molecular mechanisms through transcriptomic and epigenomic analyses.

Methods: We introduced Tet2-mutations into murine Cebpa-mutated cells using the CRISPR-Cas9 technology to generate a Cebpa-Tet2-mutated mouse model, which we characterized through RNA-, C/EBPα-ChIP-, ATAC- and Bisulfite-seq. This comprehensive dataset was used for in-depth comparative analysis and correlation with patient-data from the beatAML-collection.

Results: Cebpa-Tet2-co-mutated cell-lines had a strong proliferative advantage and Tet2 loss in the Cebpa-mutated AML mouse model shortened leukemia latency. Integration of transcriptomic and epigenomic data from all models with gene expression analyses in CEBPA-TET2-co-mutated AML patients identified the transcription factor GATA2 as a conserved target of the CEBPA-TET2 axis. While the Gata2 locus was bound by C/EBPα in multiple regions, its accessibility was reduced and its DNA methylation was increased upon Tet2 loss. RNAi-mediated silencing revealed a dose-dependent effect of Gata2-expression on leukemia cell fitness in vivo. Treatment with the demethylating agent 5-azacytidine restored Gata2-expression and caused a significant survival benefit in a Cebpa-Tet2 co-mutated AML model.

Conclusions: Our results show that mutational disruption of both CEBPA and TET2 in AML leads to deregulated GATA2-expression. Thus, restoration of GATA2 expression can be beneficial for AML patients with CEBPA- and TET2-mutations.

Y06

Disruption of the C/EBP-PU.1 axis perturbs monocyte subset homeostasis and creates an MDS-promoting niche

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Background: Hematopoiesis is a complex process that requires support from the bone marrow (BM) microenvironment as well as closely regulated cell-intrinsic fate decision mechanisms. One crucial player in both physiological and malignant hematopoiesis is the master regulator PU.1. Alterations in PU.1 expression have been shown to lead to the development of acute myeloid leukemia (AML), but little is known about its involvement in promoting preleukemic conditions such as myelodysplastic syndromes (MDS) and its interaction with the microenvironment.

Methods: We used knock-in mice with deficient C/EBP-driven PU.1 expression (subsequently called PU.1Ki/Ki) to specifically model monocyte-induced changes of the BM microenvironment.

Results: PU.1Ki/Ki mice display BM monocytosis and a shift towards the Ly6C+ inflammatory monocyte subset. Older mice (~ 1 year) develop an MDS-phenotype that is characterized by BM hypercellularity, leukopenia, thrombopenia and myeloid dysplasia. In addition, PU.1Ki/Ki mice display alterations of the BM microenvironment that manifest as osteoporosis and precede MDS onset. These niche changes propagate MDS development in wildtype (WT) BM in a series of transplant experiments. Conversely, disturbed monocyte subset homeostasis and niche changes are transplantable from the PU.1Ki/Ki stem cell level.

Conclusions: This study demonstrates that hematopoietic stem cells can regulate the composition of their own niche. The data suggest a new mechanistic concept in which MDS onset is promoted by a perturbed BM microenvironment secondary to dysregulated monopoiesis. Thus, targeting the microenvironment may be a putative therapeutic opportunity in MDS.

▸ For latest news from international oncology congresses see: http://www.springermedizin.at/memo-inoncology