**RESEARCH**                                                                 **Open Access**

CrossMark

# Implementation of next generation sequencing into pediatric hematology-oncology practice: moving beyond actionable alterations

Jennifer A. Oberg[1†] , Julia L. Glade Bender[1,6†], Maria Luisa Sulis[1,6†], Danielle Pendrick[1], Anthony N. Sireci[2], Susan J. Hsiao[2], Andrew T. Turk[2], Filemon S. Dela Cruz[1,6,7], Hanina Hibshoosh[2,6], Helen Remotti[2], Rebecca J. Zylber[1], Jiuhong Pang[2], Daniel Diolaiti[1,7], Carrie Koval[3], Stuart J. Andrews[2], James H. Garvin[1,6], Darrell J. Yamashiro[1,2,6], Wendy K. Chung[1,4,6], Stephen G. Emerson[4,5,6], Peter L. Nagy[2,8], Mahesh M. Mansukhani[2,6] and Andrew L. Kung[1,6,7*]

## Abstract

**Background:** Molecular characterization has the potential to advance the management of pediatric cancer and high-risk hematologic disease. The clinical integration of genome sequencing into standard clinical practice has been limited and the potential utility of genome sequencing to identify clinically impactful information beyond targetable alterations has been underestimated.

**Methods:** The Precision in Pediatric Sequencing (PIPseq) Program at Columbia University Medical Center instituted prospective clinical next generation sequencing (NGS) for pediatric cancer and hematologic disorders at risk for treatment failure. We performed cancer whole exome sequencing (WES) of patient-matched tumor-normal samples and RNA sequencing (RNA-seq) of tumor to identify sequence variants, fusion transcripts, relative gene expression, and copy number variation (CNV). A directed cancer gene panel assay was used when sample adequacy was a concern. Constitutional WES of patients and parents was performed when a constitutionally encoded disease was suspected. Results were initially reviewed by a molecular pathologist and subsequently by a multi-disciplinary molecular tumor board. Clinical reports were issued to the ordering physician and posted to the patient's electronic medical record.

**Results:** NGS was performed on tumor and/or normal tissue from 101 high-risk pediatric patients. Potentially actionable alterations were identified in 38% of patients, of which only 16% subsequently received matched therapy. In an additional 38% of patients, the genomic data provided clinically relevant information of diagnostic, prognostic, or pharmacogenomic significance. RNA-seq was clinically impactful in 37/65 patients (57%) providing diagnostic and/or prognostic information for 17 patients (26%) and identified therapeutic targets in 15 patients (23%). Known or likely pathogenic germline alterations were discovered in 18/90 patients (20%) with 14% having germline alternations in cancer predisposition genes. American College of Medical Genetics (ACMG) secondary findings were identified in six patients.

(Continued on next page)

---

* Correspondence: kunga@mskcc.org
†Equal contributors
[1]Department of Pediatrics, Columbia University Medical Center, New York, NY 10032, USA
[6]Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY 10032, USA
Full list of author information is available at the end of the article

Oberg *et al. Genome Medicine* (2016) 8:133

Page 2 of 19

(Continued from previous page)

**Conclusions:** Our results demonstrate the feasibility of incorporating clinical NGS into pediatric hematology-oncology practice. Beyond the identification of actionable alterations, the ability to avoid ineffective/inappropriate therapies, make a definitive diagnosis, and identify pharmacogenomic modifiers is clinically impactful. Taking a more inclusive view of potential clinical utility, 66% of cases tested through our program had clinically impactful findings and samples interrogated with both WES and RNA-seq resulted in data that impacted clinical decisions in 75% of cases.

**Keywords:** Whole exome sequencing, RNA sequencing, Precision medicine, Pediatric oncology

## Background

The outcomes for children with cancer have steadily improved to the present time when more than 80% of all pediatric oncology patients are cured [1]. Nonetheless, cancer remains the leading cause of disease-related death in children. Moreover, this success has come at a price; two-thirds of all survivors have some long-term sequelae attributable to their treatment [2]. Together, the requirement to further improve existing outcomes and to decrease toxicity underscores the need for the current national initiative in precision medicine to include pediatric oncology patients.

Many of the advances in pediatric oncology have resulted from the implementation of risk-stratified treatment strategies that incorporate histological, anatomical, and molecular prognostic and predictive determinants into the choice of therapies for individual patients [3]. Changes in ploidy, chromosomal segmental changes, and specific gene alterations are routinely utilized to guide treatment intensity in pediatric oncology [4]. Therefore, the tenants of precision medicine are intrinsic to the practice of pediatric oncology.

Recent advances in massively parallel sequencing allow for more comprehensive approaches to determine the abnormalities contributing to tumorigenesis. Initial implementation of next generation sequencing (NGS) technologies focused on the identification of actionable alterations, with estimates in the range of 5% to nearly 100% depending on disease histology and evolving definitions of "actionable" [5–15]. The utility of these technologies, however, extends well beyond the identification of actionable alterations and determining the value of these technologies should be more inclusive and consider the broad clinical impact of testing.

In 2014, we implemented a clinical NGS platform for pediatric oncology patients. The Precision in Pediatric Sequencing (PIPseq) Program utilizes NGS of tumor and germline in a CLIA-certified (Clinical Laboratory Improvement Amendments of 1988) environment and includes interrogation of both DNA and RNA. We conducted a retrospective review of our first 101 consecutively sequenced patients utilizing the PIPseq pipeline and report here our experience with integrating clinical NGS into pediatric hematology-oncology practice and describe the broad clinical utility of genomically informed cancer medicine.

## Methods

### The PIPseq pipeline

To achieve more comprehensive genome-level analysis in our pediatric oncology patients, we utilized three CLIA-certified, CAP (College of American Pathologists), and New York State Department of Health approved assays. When possible, we utilized a cancer whole exome sequencing test (cWES) comprising WES of tumor and normal tissue (buccal swab or peripheral blood) and RNA sequencing (RNA-seq) of tumor tissue. This assay was optimized for fresh or frozen specimens. When sample adequacy was a concern, we also utilized a directed cancer gene panel assay which sequenced 467 cancer-associated genes and was optimized for use with formalin fixed paraffin embedded (FFPE) material (Columbia Comprehensive Cancer Panel, CCCP). If a constitutionally encoded disease was suspected (e.g. familial hemophagocytic lymphohistiocytosis), we performed constitutional WES from the patient and both parents (trio) when available.

Tissue for sequencing was obtained either from archived blocks (FFPE) or frozen tissue blocks from the Department of Pathology. DNA and RNA extraction and sequencing were performed in a CLIA-certified laboratory. Variant calls were independently made on tumor and germline material and somatic variants determined by subtraction. Copy number variation (CNV) was determined from the WES data, fusion transcripts were identified from the RNA-seq data, and relative gene expression was determined by comparison to a model built from 124 transcriptomes. A mix of tissues was used to generate the model including normal white blood cells, lung, liver, brain, glioma, and cell lines.

After initial review by a molecular pathologist, all results were reviewed in a multi-disciplinary molecular tumor board. Participants included representation by molecular pathology, pediatric oncology, cytogenetics, medical genetics, and cancer biology. For each patient, a report was issued containing variant calls, CNV, fusions, and overexpressed genes. Variants were assigned a tier based on disease-association and separately a tier based on level of evidence for clinical actionability (described below). Reports were delivered to ordering oncologists and posted to the electronic medical record (EMR) in

Oberg et al. Genome Medicine (2016) 8:133

Page 3 of 19

accordance with patient opt-in/opt-out preferences selected at the time of informed consent.

## Patients and informed consent for clinical sequencing

Between January 2014 and April 2016, NGS was performed on tumor and/or normal tissue from 101 high-risk patients by the Laboratory of Personalized Genomic Medicine at Columbia University Medical Center (CUMC). This represented approximately 32% of the total patients in our clinical practice. High-risk patients were defined as those having a prognosis of <50% overall survival at 5 years, outlier clinical phenotype, rare cancer without standard of care therapy, suspected cancer predisposition, or relapsed disease. A request for constitutional WES, cWES and RNA-seq, or targeted cancer panel testing was made at the discretion of the referring oncologist in consultation with the PIPseq team [16].

Participants signed consent for WES or cWES either as part of an Institutional Review Board (IRB)-approved protocol (IRB nos. AAAB7109, AAAJ5811) or they signed the clinical consent (http://pathology.columbia.edu/diagnostic/PGM/oncologytests.html). Written consent for clinical WES and cWES testing was obtained after the risks and benefits had been explained to the patient and/or caregiver, which include the potential disclosure of medically actionable secondary findings, defined as germline disease-causing mutations unrelated to the condition for which sequencing was being performed. Patients could opt in or opt out of the following: learning secondary findings and/or having these results appear in the EMR; having their samples and/or data stored for future research, both with or without identifiers; and future contact. Results not reported included carrier status, variants of uncertain significance (VOUS) in secondary findings except as related to cancer, and mutations related to adult-onset conditions for which the genetic link is either unclear or for which no known intervention is of proven benefit (e.g. Alzheimer's disease). IRB approval was obtained for this retrospective analysis of de-identified patient and clinical genomics data (IRB nos. AAAP1200 and AAAQ8170).

## Clinical sequencing

Testing required at least 200 ng of DNA for WES, at least 50 ng of DNA for targeted DNA sequencing, and at least 3000 ng of RNA for transcriptome analysis (Additional file 1: DNA and RNA extraction). The entire assay was a CLIA-certified assay. The laboratory-developed test used general purpose reagents and the Agilent WES ver.5 + UTR baits. Specifically, WES was performed using the Agilent SureSelectXT All Exon V5 + UTRs capture kit for library generation and sequenced on the HiSeq2500 using paired-end 125 cycle × 2 sequencing (two tumors, two normal and two transcriptomes, pooled together and run in two lanes). Targeted DNA sequencing was performed on a 5.59 Mb Custom Agilent SureSelectXT library, targeting 467 genes, and sequenced on a HiSeq2500 using paired-end 125 cycle × 2 sequencing (seven samples per lane). RNA was sequenced using the TruSeq Stranded Total RNA LT Sample Prep Kit with 125 cycles × 2 paired-end sequencing on the HiSeq2500.

## Sequencing analysis

DNA sequencing reads were de-multiplexed and converted to fastq files using CASAVA from Illumina. Mapping and variant calling of tumor and normal samples was performed using NextGene (v.2.3.4; Softgenetics, State College, PA, USA), which uses a modified Burrows-Wheeler transform (BWT) alignment method. Sequences were mapped to GRCh37 ("hg19"), retaining reads with a median quality score of 20 or higher, with no more than three ambiguous bases, a minimum number of 25 called bases per read, and trimming reads when three consecutive reads fell below a quality score of 16. Alignment and variant calling was performed using paired-end reads with a minimum of 10 reads, at least three variant reads, and a minimum variant allelic fraction of 10% for tumor and 5% for normal was required to call a variant. The variant calling module was set to "detect large indels." The variant calling algorithm showed a 99.6% agreement with single nucleotide polymorphisms on an oligonucleotide microarray and over 96% sensitivity in inter-laboratory comparison and a 96% detection rate for heterozygous variants in a 40/60% mixture of samples. For small indels, the lab detected 93% of all variants detected by another laboratory in inter-laboratory comparison, with the greatest disagreement in insertions greater than 10 bp.

Variants were subject to filtering. In normal DNA, variants were passed through a "reference range filter" of cancer predisposition genes, genes relevant to pharmacogenomics, and variants relevant to patient care; a "reportable range filter" which includes COSMIC (cosmic70 provided by Annovar) variants in the patient's mutation report file and variants in genes recommended by the American College of Medical Genetics (ACMG) for the reporting of secondary findings [17]; as well as a frequency filter, which includes variants whose minor allele frequency in the 1000 Genomes (phase 1, version 3, release date 23 November 2010) is less than 1%. Somatic mutations in the tumor were identified by subtracting all variants called in normal tissue (output at minor allelic fraction of ≥5%) from variants called in the tumor (output at a minor allelic fraction of ≥10%). The approach maximized the number of variants output to minimize the likelihood of filtering out actionable mutations prior to molecular tumor board discussion (Additional file 1: Supplementary Methods; Somatic Variant Calling Strategy).

Oberg *et al. Genome Medicine* (2016) 8:133

Page 4 of 19

Variants in the tumor were further characterized as homozygous, compound heterozygous, somatic, and "disruptive" (loss of function, namely, nonsense, frameshift, or splice site). Spreadsheets with the various categories were presented to molecular pathologists for review. Quality statistics for WES and cWES are presented in Additional file 2: Table S1. Targeted DNA sequencing was performed to an average 500X depth and analyzed as above. All DNA sequencing results were manually reviewed by molecular pathologists to prioritize variants for presentation at the multi-disciplinary tumor board and subsequent reporting of consensus variants. For mutation statistics, the list of "tumor-specific" variants obtained by comparison of vcfs was filtered for variants with at least 30X coverage in tumor and either a "quality score" ≥20 or a variant allelic fraction ≥25% in the tumor.

### Copy number variation

CNV was identified using EXCAVATOR (v.2.2; https://sourceforge.net/projects/excavatortool) software [18]. For samples with greater than 95% of targeted nucleotides present at least 10X in the reference normal and at least 90% covered 30X in the corresponding tumor sample, EXCAVATOR was run with parameters chosen for moderate sensitivity (assuming a tumor percentage of 0.8) and cutoff for loss set to a log2 ratio of –0.2. In addition, all high quality heterozygous variants with variant allelic fractions (VAFs) in the range of 45–55% and 90–100% in the normal sample were output. The allelic ratio at these genomic coordinates in the tumor was also output for viewing on the integrated genomic viewer to allow identification of copy number neutral loss of heterozygosity (LOH) and to support the CNVs identified by EXCAVATOR. The laboratory detected all chromosome arm changes seen on karyotyping, losses of 26 Mb and greater seen on array CGH, and reproducibly identified all CNVs that involved at least ten exons at 40% tumor fraction (Additional file 1: Supplementary Methods).

### Transcriptome analysis

For transcriptome analysis, fastq files from CASAVA were filtered for ribosomal RNA (rRNA) using Sort-MeRNA (v.1.7; http://bioinfo.lifl.fr/RNA/sortmerna/) and trimmed to remove poor-quality tails using Trim-Galore (v.0.2.7; http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) with settings to exclude reads of quality score <20 and read length <20. Remaining reads were mapped to GRCh37 (hg19) using Tuxedo Suite [19, 20] consisting of TopHat2 (v.2.0.8), BOW-TIE2 (v.2.1.0), and CUFFLINKS (v.2.1.1). Non-uniquely mapped reads were excluded before estimation of fragments per kilobase per million reads (FPKMs) by CUFFLINKS. Mutation calling was performed using NextGene software. At least 50 million uniquely

mapped reads were required with less than 5% DNA contamination. In addition, unmapped reads were analyzed using "FusionMap" (v.01/01/2015) to generate a list of fusions for review by molecular pathologists [21]. To identify alterations in gene expression, the median FPKMs of 8000 housekeeping genes were used as reference [22] and the relative expression of each gene was compared to 124 normal transcriptomes from various tissues (13 blood, 20 liver, 24 kidney, 17 lung, and 50 brain) (Additional file 1: Supplementary Methods).

### Data interpretation and reporting

Interpretation of clinical WES, RNA-seq, and CNV was conducted via a molecular tumor board with multi-disciplinary representation from pediatric oncology, pathology, cancer biology, molecular and clinical genetics, and bioinformatics. Following the tumor board, approximately 60 days after testing request, a tiered report was generated for clinical samples by pathology, sent to the referring physician and posted to the EMR according to the opt-in/opt-out selections of the patient consent. Only variants with good normal coverage (generally at least 30X) were detected on multiple independent fragments and were not excluded as likely benign were reported. For clinical testing, the report included variants that were justified by the literature as driver mutations (e.g. well characterized hot spot mutations); unambiguous loss of function mutations in tumor suppressor genes (i.e. nonsense or frame-shift mutations that resulted in loss of functional domains); mutations with published laboratory data documenting gain or loss of function in oncogenes and tumor suppressor genes, respectively; and previously reported fusions or fusions that were expected to have the same effect as previously reported fusions involving one of the partner genes. Certain exceptions for clinical testing were made. For example, if a variant was likely a strong driver (e.g. a known activating mutation of an oncogene) but had low coverage in the normal or appeared low-quality on review, the molecular pathologist still considered it but required independent confirmation by an orthogonal method prior to reporting.

The final clinical cWES report included: known tumor type-specific actionable somatic mutations (Tier 1); somatic mutations in targetable pathways, actionable somatic mutations in other tumor types, somatic mutations in well-established cancer genes (Tier 2); other somatic mutations in cancer genes (Tier 3); and somatic VOUS (Tier 4). Reporting of germline findings included: known pathogenic secondary ACMG variants [17]; secondary non-ACMG variants and selected VOUS in known cancer genes with commentary; and known variants that influence pharmacogenomics. Reports further included translocations, significantly overexpressed genes, and segmental CNV. A sample cWES report is presented in Additional file 3. Accession

Oberg *et al. Genome Medicine* (2016) 8:133

Page 5 of 19

number for all genes and fusions referenced in the paper are reported in Additional file 2: Table S2. Datasets are available through the cBioPortal for Cancer Genomics (http://cbioportal.org) [23, 24].

Clinical utility, defined as the ability of a molecular test result to provide information related to the care of the patient and his/her family members to diagnose, monitor, prognosticate or predict disease progression, and inform treatment [25], was used to evaluate the potential impact of findings from clinical sequencing. "Clinical impact" and "clinically impactful" are broad terms used throughout this paper to refer to any molecular test result that, when integrated with a patient's history, symptoms, and other clinical findings, informed the medical team's assessment or management of the patient. These clinically meaningful results were subcategorized into the following five categories to evaluate the potential clinical utility of tumor and germline alterations: (1) diagnostic; (2) prognostic; (3) identification of a therapeutic target; (4) other clinically impactful information, including pharmacogenomics or findings that resulted in a significant refinement of a therapeutic plan (e.g. choice of donor or withdrawal of recommendation for bone marrow transplantation); and (5) recommendations for health maintenance interventions or genetic counseling for the patient and other at-risk family members. Genetic alterations were considered targetable if: (1) an FDA approved drug or experimental drug was available that inhibited the target directly or inhibited its downstream signaling pathway; or (2) there was preclinical evidence to support efficient targeting of the aberrant function of the mutated gene and/or potential clinical benefit; and (3) there was some age-appropriate information on dosing. Targetable somatic mutations were further categorized using a five-tier system previously described by Wagle et al. [26] and Harris et al. [15]. This sub-tiering system uses the strength of preclinical and clinical data as evidence to support the potential clinical benefit of targeting an altered gene with a specific therapeutic agent.

## Results
### Patients
Demographic and clinical characteristics are presented in Table 1 and Fig. 1. Molecular characterization was performed on 120 samples (85, primary disease; 35, relapse/refractory disease) from 101 consecutive cases (mean age, 9.3 years; median age, 8.0 years; range, 2 weeks – 26 years). Patients aged over 18 years in this cohort were initially diagnosed with a pediatric disease under the age of 18 years. Testing included: full cWES (tumor, germline, and transcriptome; $n = 63$); cWES without transcriptome ($n = 19$); transcriptome only ($n = 3$); targeted tumor panel sequencing ($n = 13$); and

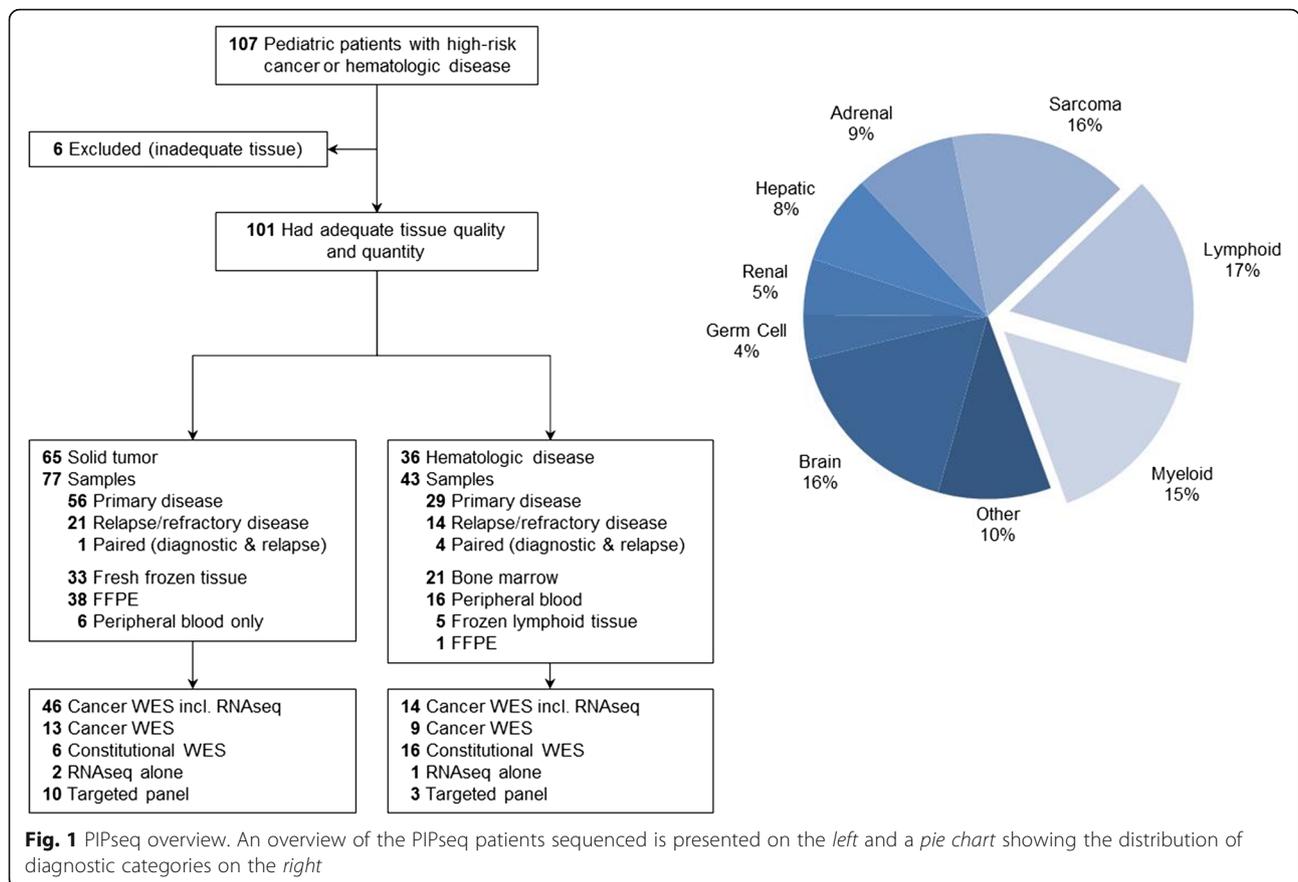**Table 1** Patient and sample characteristics (n = 101)

| | n (%) |
|---|---|
| Diagnostic category | |
| Solid tumors | 65 (64) |
| Hematologic conditions | 36 (36) |
| Gender | |
| Male | 60 (59) |
| Female | 41 (41) |
| Age (years) | |
| 0–5 | 35 (34) |
| 6–12 | 30 (30) |
| 13–17 | 20 (20) |
| ≥18 | 16 (16) |
| Samples tested (n = 120) | |
| Primary disease | 85 (71) |
| Relapse/refractory | 35 (29) |
| Platform (n = 120) | |
| Cancer WES with transcriptome | 63 (53) |
| Cancer WES | 19 (16) |
| Constitutional WES | 22 (18) |
| Transcriptome only | 3 (2) |
| Targeted panel (467 genes) | 13 (11) |
| Normal tissue source (n = 104) | |
| Blood | 78 (74) |
| Buccal swab | 23 (23) |
| Unaffected tissue | 3 (3) |

*WES* whole-exome sequencing

constitutional WES (proband and parental blood) ($n = 22$). For constitutional WES, trios (proband and both parents) were performed in 18/22 cases, 3/22 cases only had one parent available for testing, and in one case only the proband was tested post-mortem. Eighty-four patients underwent single platform testing, while multiple sequencing platforms were used for 17 cases (36 samples). Cases were predominantly pediatric patients with solid tumors (64%) (Fig. 1; Additional file 2: Table S3). Sarcoma ($n = 17$) was the most common diagnostic sub-category followed by brain tumors ($n = 16$). Patients with lymphoid disease ($n = 17$) comprised the majority of hematological conditions (Fig. 1; Additional file 2: Table S3).

### Informed consent, cost, and reimbursement
All patients were consented to genomic analysis either through a research consent or clinical WES consent. Of the 101 cases, 67 were consented using the clinical cWES consent. Only four (6%) opted out of learning secondary findings and 21 (31%) opted out of having

Oberg *et al. Genome Medicine* (2016) 8:133

Page 6 of 19



**Fig. 1** PIPseq overview. An overview of the PIPseq patients sequenced is presented on the *left* and a *pie chart* showing the distribution of diagnostic categories on the *right*
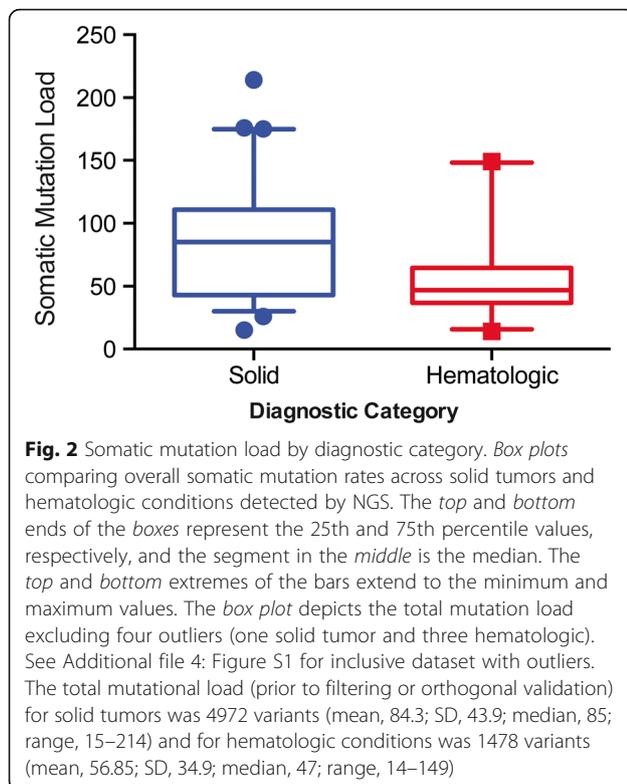
secondary findings in their medical record. All patients consented to have leftover samples stored. Only a single patient (2%) opted out of future contact (Additional file 2: Table S4).

As part of clinical implementation, we assessed the cost of cWES and RNA-seq and the reimbursement landscape. The total cost per case was calculated by summing the total variable cost (reagent cost, pathologist time) with the fixed cost per case (annual machine cost, annual maintenance, tech labor cost, informatics cost, space for NGS hardware, server time, NGS analysis lease, and data storage). The estimated cost of WES (tumor/normal) was $4459 and the cost of RNA-seq was $1764. These estimates do not include administrative overhead and billing for services.

The time to receiving final reimbursement decisions from third-party payers ranged between 6 months to 1 year. To date, we have received a decision for 56 patients with 45/56 (80%) receiving partial reimbursement. The average reimbursement by carrier type was as follows: commercial, $2747 (range, $770–6917); managed government plans, $2918 (range, $750–4555); and $0 from government plans. Patients and their families were not charged for sequencing or analysis.

## Genomic alterations in pediatric solid tumors and hematologic disorders

Over 150-fold and 500-fold average coverage was achieved by WES and targeted capture sequencing, respectively with >98% of the coding sequences having at least tenfold coverage. The mean mutational load across patients was 216.9 variants (SD = 829.3, median = 69), with a higher mean mutational load in solid tumors compared to hematologic malignancies (Fig. 2; Additional file 4: Figure S1). Genomic aberrations were reported in 92/101 patients (91%). After filtering, a total of 180 mutations (Additional file 2: Table S5) and 20 fusions were reported, 110 (including 10 fusions) from solid tumor samples (mean number of aberrations per sample, 2.91; median, 2.00; range, 1–6) and 90 (including 10 fusions) from hematologic samples (mean number of aberrations per sample, 5.2; median, 4.0; range, 1–12). The most commonly mutated gene was *TP53* (*n* = 9, 9%) in solid tumor samples and RAS pathway constituents (*NRAS: n* = 5, 5%; *KRAS: n* = 3, 3%) in hematologic samples (Fig. 3). In addition, significant changes in the pattern of genetic alterations were noted on serial sequencing of samples from individual patients at different time points during their therapy, reflecting clonal evolution. Awareness of these changes is important

Oberg *et al. Genome Medicine* (2016) 8:133

Page 7 of 19



**Fig. 2** Somatic mutation load by diagnostic category. *Box plots* comparing overall somatic mutation rates across solid tumors and hematologic conditions detected by NGS. The *top* and *bottom* ends of the *boxes* represent the 25th and 75th percentile values, respectively, and the segment in the *middle* is the median. The *top* and *bottom* extremes of the bars extend to the minimum and maximum values. The *box plot* depicts the total mutation load excluding four outliers (one solid tumor and three hematologic). See Additional file 4: Figure S1 for inclusive dataset with outliers. The total mutational load (prior to filtering or orthogonal validation) for solid tumors was 4972 variants (mean, 84.3; SD, 43.9; median, 85; range, 15–214) and for hematologic conditions was 1478 variants (mean, 56.85; SD, 34.9; median, 47; range, 14–149)

for selecting an appropriate targeted therapy and assessing response to therapy.

### Evaluating potential clinical utility and targetable alterations

A genetic variant was considered targetable if: (1) an FDA-approved drug or experimental drug was available that inhibited the target directly or inhibited its downstream signaling pathway; or 2) there was preclinical evidence to support efficient targeting of the aberrant function of the mutated gene and/or potential clinical benefit; and 3) there was some age-appropriate information on dosing. Consistent with the published recommendations from the Association for Molecular Pathology [25], we evaluated clinical utility based on "the ability of a test result to provide information to the patient, physician, and payer related to the care of the patient and his/her family members to diagnose, monitor, prognosticate, or predict disease progression, and to inform treatment and reproductive decisions."

### Targetable somatic genomic alterations

Overall, 38/101 patients (38%) had at least one potentially targetable genomic alteration (Table 2). Specifically, 21/65 patients (32%) with solid tumors and 17/36 (47%) patients with hematologic conditions carried targetable alterations. Matched therapy based on genomic findings was received in 6/38 patients (16%).

Examples of targetable alterations include the identification of a *cKIT* (p.Asn655Lys) [27] mutation in a 7-year-old boy with acute myeloid leukemia (AML), who was subsequently treated with palliative imatinib and achieved a near-complete clearing of peripheral blood leukemic blasts with a sustained response for 9 months. The RNA expression data also led us to identify a *BCR-ABL1*-like [28] expression pattern in a 9-year-old girl with relapsed, refractory B-cell acute lymphoblastic leukemia (ALL). Subsequent analysis identified a *NUP214-ABL1* [29] fusion by real-time polymerase chain reaction (RT-PCR) and the addition of dasatinib to the third-line induction regimen resulted in a deep remission allowing for a curative bone marrow transplant. These results demonstrate the utility of comprehensive genomic characterization to identify clinically actionable alterations in pediatric oncology patients.
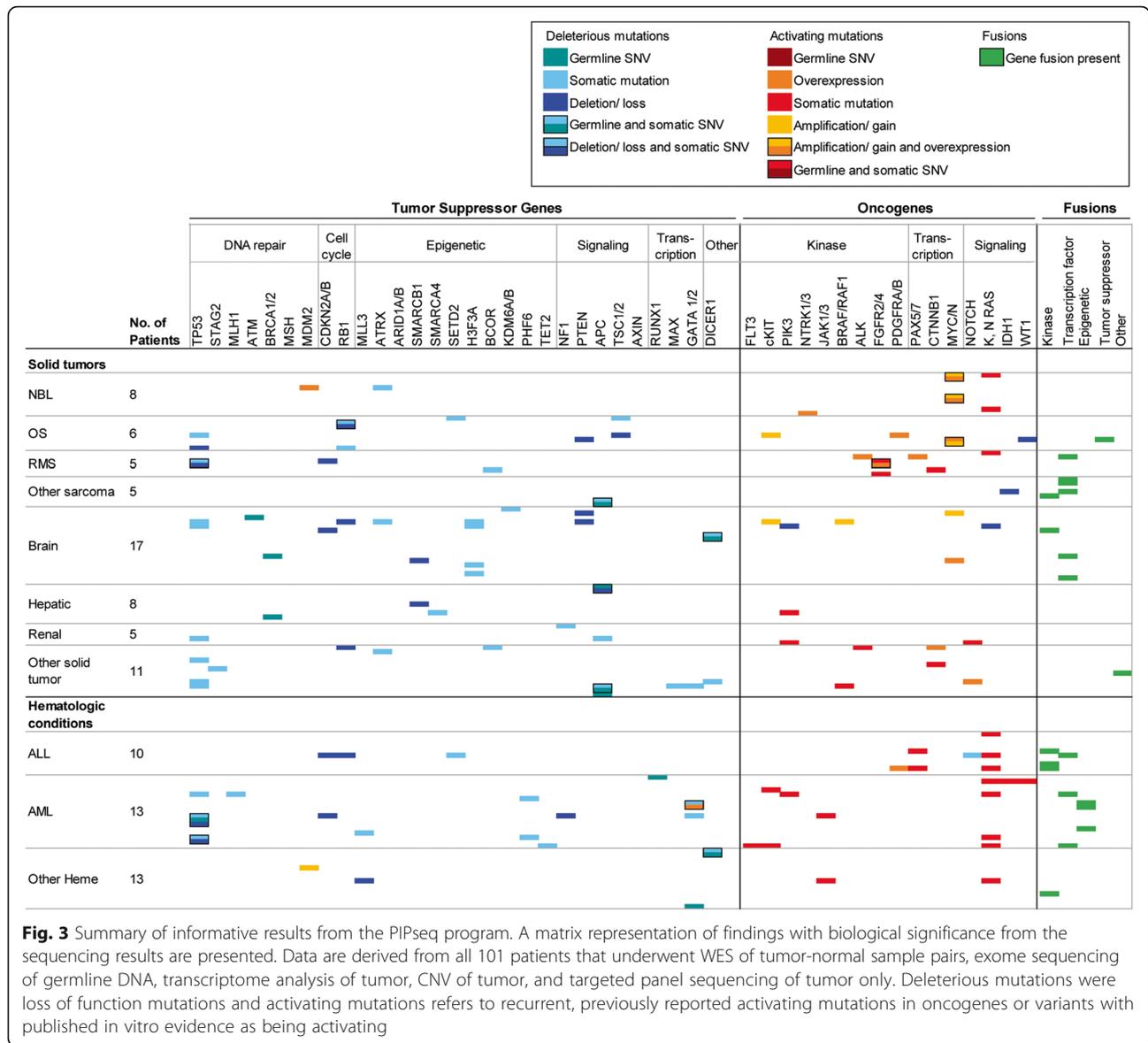
### Clinical impact of non-targetable somatic mutations

While many studies have focused on actionable alterations, the potential clinical impact of non-targetable alterations was also evaluated. Genomic alterations identified by sequencing helped to confer a molecular diagnosis in 23 patients and identified prognostic, pharmacogenomic, and other significant health maintenance recommendations in 32 patients (Table 3). Although these findings do not meet the definition of "actionability," the clinical impact of such findings can be quite profound. For example, the identification of a *STAT5B* mutation [30] in a 5-year-old girl erroneously diagnosed with T-cell ALL helped to establish a diagnosis of gamma-delta T-cell lymphoma. Also, identification of a *PTPN11* mutation in a 4-year-old boy contributed to a change in his diagnosis from de novo AML to juvenile myelomonocytic leukemia (JMML) which evolved into AML [31].

The identification of resistance alleles likewise is not considered actionable, but may carry significant clinical implications. For example, in the 9-year-old girl with the relapsed *NUP214-ABL1* B-ALL, the finding of a *NT5C2* mutation associated with resistance to nucleoside analog therapies [32, 33] had clear implications for her salvage therapy. In aggregate, sequencing results were clinically informative for diagnostic, prognostic, or pharmacogenomic purposes in 38 patients (38%).

### Clinical impact of transcriptome and CNV analysis beyond target identification

Clinical impact by RNA-seq and CNV analysis was demonstrated in 23/33 patients (70%) (Table 3). Gene fusions confirming diagnosis were found in five patients: *BCR-ABL1* (chronic myeloid leukemia), *ASPSCR1-TFE3* (alveolar soft part sarcoma), *EWSR1-FLI1* in two patients

Oberg *et al. Genome Medicine* (2016) 8:133

Page 8 of 19



**Fig. 3** Summary of informative results from the PIPseq program. A matrix representation of findings with biological significance from the sequencing results are presented. Data are derived from all 101 patients that underwent WES of tumor-normal sample pairs, exome sequencing of germline DNA, transcriptome analysis of tumor, CNV of tumor, and targeted panel sequencing of tumor only. Deleterious mutations were loss of function mutations and activating mutations refers to recurrent, previously reported activating mutations in oncogenes or variants with published in vitro evidence as being activating

(Ewing sarcoma), and *EWSR1-WT1* (desmoplastic small round cell tumor). A novel *EML4-NTRK3* fusion found in a 2-year-old boy supported a change in diagnosis from undifferentiated sarcoma to infantile fibrosarcoma [15, 34]. In one patient, a *CBFA2T3-GLIS2* [35] fusion confirmed the diagnosis of acute megakaryoblastic leukemia (AMKL), was associated with poor prognosis, and supported the recommendation for a bone marrow transplant. A *PAX7-FOXO1* fusion was diagnostic and prognostic in a toddler with histologically defined solid alveolar rhabdomyosarcoma, but in whom FISH analysis using the FOXO1A (FKHR; 13q14.1) break-apart probe was repeatedly negative.

CNV was inferred from the WES data and relative gene expression was determined by reference to an averaged gene expression model. Segmental and gene

expression changes having prognostic implications were identified in 11 patients with a variety of diagnoses. Four patients diagnosed with neuroblastoma could be stratified based on RNA-seq and CNV: one high-risk patient with MYCN amplification, LOH at 1p and 11q, gain of 17q, and MYCN overexpression; one high-risk patient with MYCN amplification, LOH at 1p, gain of 17q, and MYCN overexpression; one high-risk patient without MYCN amplification or LOH at 1p and 11q, and no evidence of MYCN overexpression; and one intermediate-risk patient without MYCN amplification or LOH at 1p and 11q and no evidence of MYCN overexpression. Medulloblastoma subgrouping was supported by overexpression and CNV in two patients. Poor prognostic features were found in two other patients: low expression of *PAX8, FHIT, CASP10, CHD2*, with high expression

Oberg *et al. Genome Medicine* (2016) 8:133

Page 9 of 19

**Table 2** Sub-classification of potentially targetable somatic mutations for treatment planning

| PIP ID | Diagnosis | Target alteration | Mutation (change) | Potential target therapy |
|---|---|---|---|---|
| Tier 1 (Data demonstrating benefit- same tumor type, same gene) | | | | |
| 14-85546 | CML | BCR-ABL1 | Fusion | TKI |
| 13-45348[a] | AML | IDH1 | c.394C > T (p.R132C) | IDH Inhibitor |
| 14-24794[a] | AML | c-KIT; TET2; FLT3 | c.2446G > C (p.D816H); c.3663delT (p.C1221Wfs); c.2505 T > G (p.D835E) | TKI; Hypomethylating agent; TKI |
| 14-53198 | AML | TET2 | c.1156G > T (p.V386L) | Hypomethylating agent |
| 13-77086 | AML | c-KIT | c.1965 T > G (p.Asn655Lys) | TKI[b] |
| Tier 2 (Data demonstrating benefit- different tumor type, same gene) | | | | |
| 15-18928 | Hepatic rhabdoid tumor | SMARCA4 | c.3574C > T (p.R1192C) | EZH2 Inhibitor |
| 14-13487 | Osteosarcoma | TSC1 | c.2503-1G > C (p.?) | mTOR Inhibitor |
| 14-47205 | Nephroblastomatosis | PIK3CA | c.1035 T > A (p.N345K) | PI3K/AKT/mTOR Inhibitor |
| 15-40141 | Pleomorphic xanthoastrocytoma | TMEM106B-BRAF | Fusion | MEK Inhibitor |
| Tier 3 (Published, presented, in press pre-clinical data demonstrating benefit- same tumor type, same gene) | | | | |
| 15-64793 | ALL | FOXP1-ABL1 | Fusion | TKI[b] |
| 15-26188 | ALL | NUP214-ABL1 | Fusion | TKI[b] |
| 15-79700 | ALL | NRAS | c.183A > T (p.Q61H) | MEK Inhibitor |
| 14-20062 | ALL | KRAS | c.183A > T (p.Q61H) | MEK Inhibitor |
| 14-24794[a] | AML | NRAS | c.183A > C (p.Q61H) | MEK Inhibitor |
| 15-29224 | AML | JAK3 | c.1718C > T (p.A573V) | JAK Inhibitor |
| 13-45348[a] | AML | NRAS | c.38G > C (p.G13A) | MEK Inhibitor |
| 13-95124 | AML | NRAS; MLL-AFF1 (KMT2A-AFF1) | c.182A > G (p.Q61R); Fusion | MEK Inhibitor; DOT1L Inhibitor |
| 14-45760 | AML | NRAS | c.38G > A (p.G13D) | MEK Inhibitor |
| 13-50662 | AML | PTPN11 | c.1508G > T (p.G503V) | MEK Inhibitor |
| 14-15491 | AML | NUP98-NSD1 | Fusion | DOT1L Inhibitor |
| 14-27243 | Neuroblastoma | NRAS | c.181C > A (p.Q61K) | MEK Inhibitor |
| 14-70449 | Rhabdomyosarcoma | NRAS | c.181C > A (p.Q61K) | MEK Inhibitor |
| 14-42817 | Neuroblastoma | KRAS | c.34G > T (p.G12C) | MEK Inhibitor |
| 15-11925[a] | Osteosarcoma | MYC | Overexpression | BET Inhibitor |
| 16-74654 | Rhabdomyosarcoma | FGFR4 | c.1582G > T (p.G528C); c.1648G > C (p.V550L) | FGFR4 Inhibitor |
| 15-23518 | Rhabdomyosarcoma | FGFR4 | c.1648G > C (p.V550L); c.1949G > T (p.R650L); Overexpression | FGFR4 Inhibitor |
| 14-37237 | Glioblastoma multiforme | Gain 12q.14.1 involving CDK2 | Copy number | CDK4/6 Inhibitor |
| 15-44470 | Medulloblastoma | PTCH1, SUFU, ZIC3 | Overexpression | SMO Inhibitor |
| 15-10838 | Glioma | H3F3A; FGFR1 | c.83A > T (p.K28M); c.1731C > A (p.N577K) | HDAC Inhibitor |
| 15-27992 | Hepatic rhabdoid tumor | Homozygous deletion of chr 22q11.23 with homozygous deletion of SMARCB1; Loss of expression of SMARCB1 | Copy number; Loss of expression with biallelic deletion | EZH2 Inhibitor |
| Tier 4 (Published, presented, in press pre-clinical data demonstrating benefit- different tumor type, same gene) | | | | |
| 15-36388 | AML | MLL3 (KMT2C) | c.2110G > T (p.E704X) | BET Inhibitor |
| 13-72282 | T-ALL | KRAS; JAK1; STAT5B | c.40G > A (p.V14I); c.3076A > G (p.K1026E); c.2110A > C (p.I704L) | MEK Inhibitor; JAK Inhibitor; BCL2/BCLX-L Inhibitor |
| 15-66870 | Adrenocortical carcinoma | ALK | c.3436C > A (p.Q1146K) | ALK Inhibitor[b] |

Oberg et al. Genome Medicine (2016) 8:133

Page 10 of 19

**Table 2** Sub-classification of potentially targetable somatic mutations for treatment planning (Continued)

| | | | | |
|---|---|---|---|---|
| Tier 5 (Anything else the molecular tumor board thought was sufficient to qualify for treatment planning) | | | | |
| 15-16072 | ALL | SMARCC2-PDGFRB | Fusion | TKI |
| 14-75899 | Neuroblastoma | CDK4, MDM2 | Overexpression | NEPENTHE trial [NCT02780128] |
| 15-11925[a] | Osteosarcoma | MCL1, CCNE1 | Overexpression | CDK4/6 Inhibitor |
| 15-35162 | Osteosarcoma | CUL4A | Overexpression | NAE Inhibitor |
| 14-71727 | Osteosarcoma | RAD51C | c.24 T > G (p.F8L) | PARP Inhibitor |
| 15-83826 | Osteosarcoma | PDGFRA, KDR (VEGFR2) | Overexpression | MTKI[b] |
| 13-21968 | Congenital fibrosarcoma | EML4-NTRK3 | Fusion | ALK Inhibitor |
| 14-84044 | Inflammatory myofibroblastic tumor | VCAN-IL23R | Fusion | JAK Inhibitor[b] |

[a]Same patient
[b]Targeted therapy received
ALL acute lymphoblastic leukemia, AML acute myeloid leukemia, CML chronic myeloid leukemia

of CHD11, FUS, and MTA1 in a patient with Ewing sarcoma [36], and gain of 1q and loss of 6q and overexpression of TNC, CALB1, PLAG1, ALDH1L1, and RELN in a patient with ependymoma [37]. Overexpression of CCND1 in a patient with hepatoblastoma was considered a good prognostic indicator. One patient with AML with a CBFB-MYH11 fusion could be assigned to risk-based therapy and the diagnosis of gamma-delta T-cell lymphoma [38, 39] was also corroborated by CNV with isochromosome 7q.

## Clinically impactful germline alterations

A total of 90 patients had germline tissue sequenced. Cancer WES included germline analysis in 68/90 patients. Tumor sequencing plus constitutional WES was performed in eight patients and 14 patients had only germline tissue sequenced for a variety of indications including clinical suspicion of cancer predisposition or of an underlying immunologic defect responsible for the development of lymphoma or hemophagocytic lymphohistiocytosis (HLH).

Clinically impactful germline alterations (Table 4) were found in 18/90 patients (20%): 11/57 patients with solid tumors (19%) and 7/33 patients with hematologic conditions (21%). In the solid tumor category, two alterations in APC were diagnostic: one in a patient with hepatoblastoma and a family history consistent with familial adenomatous polyposis (FAP; p.R1114) and one associated with newly appreciated Gardner's syndrome (p.E1554fs) in a 14-year-old boy with pilomatricomas and epidermoid cysts prior to his carcinoma diagnosis. Two variants in ATM (p.R189K, p.K2756*) were found in a 16-year-old boy with medulloblastoma inferring an increased risk for developing other cancers. All were referred for genetic counseling and consideration for future cancer screening in the patient and family.

In patients with hematologic conditions, the incidence of germline alterations related to the primary diagnosis was observed in five patients (15%). A homozygous pathogenic variant in C1QA (p.Gln208Ter) diagnostic of C1Q deficiency was identified in a 2-year-old girl with HLH. A homozygous pathogenic variant in PMS2 (p.S459X) diagnostic of congenital mismatch repair deficiency was identified in one patient with T-cell lymphoblastic lymphoma and consanguineous parentage [40]. A likely pathogenic variant in XIAP (p.R443P) was identified in a 6-year-old girl with HLH, recurrent EBV infections, and suspected underlying immunodeficiency. Germline testing also revealed a heterozygous pathogenic splicing variant in RUNX1 (c.806-2A > G, r.Spl) in a patient with AML referred for transplantation for persistent thrombocytopenia following chemotherapy [41]. Both an HLA-matched sibling with borderline low platelets and the father were found to carry the same variant. An unrelated donor source was selected. A 2-month-old patient hospitalized for fulminant hemophagocytic syndrome was referred for evaluation of presumed familial HLH and was considered for hematopoietic stem cell transplantation. However, germline WES identified a pathogenic homozygous mutation in MLL2 (p.M3881Cfs*9) establishing the diagnosis of Kabuki syndrome [42] and familial HLH was ruled out due to the lack of alterations in any HLH-associated genes and subsequently plans for a bone marrow transplant were averted.

ACMG secondary findings were identified in six patients (Table 4) and were returned to the families by clinical genetics. A germline BRCA1 mutation was discovered in an 18-year-old boy with a rare hepatic tumor and a 17-year-old girl with ependymoma. A TP53 mutation was found in a 1-year-old girl with AML, a TNNT2 mutation associated with dilated cardiomyopathy was found in a 15-year-old boy with osteosarcoma, a RYR1 mutation associated with malignant hyperthermia was found in a 7-year-old girl with neuroblastoma, and a mutation in VHL was found in a 2-year-old boy with ependymoma.

Oberg *et al. Genome Medicine* (2016) 8:133

Page 11 of 19

**Table 3** Clinical utility beyond targetable somatic mutations

| PIP ID | Diagnosis | Alteration | Mutation (change) | Clinical utility | Implication |
|---|---|---|---|---|---|
| Sequence mutations | | | | | |
| 15-63375 | AML changed to JMML | PTPN11; SETB1 | c.181G > T (p.D61Y); c.2602G > A (p.D868N) | Diagnostic [31, 46] | JMML |
| 13-72282[a] | T-ALL | STAT5B | c.2110A > C (p.I704L) | Diagnostic [30, 47] | Gamma-delta T-cell lymphoma |
| 15-26188 | ALL | NT5C2 | c.1219G > T (p.D407Y) | Pharmacogenomic [32, 33] | Affects therapy |
| 13-45348 | AML | IDH1 | c.394C > T (p.R132C) | Diagnostic [48, 49] | Maffucci syndrome |
| 14-53198 | AML | CEBPA | c.939_940insAAG (p.K313_V314insK); c.326_327insC (p.P109fs) | Prognostic [50] | Improved prognosis |
| 15-10838 | Glioma | H3F3A | c.83A > T (p.K28M) | Prognostic [51] | Poor prognosis |
| 14-37237 | Glioblastoma multiforme | H3F3A | c.83A > T (p.K28M) | Prognostic [52, 53] | GBM subgroup, K27 |
| 14-35585 | Renal cell carcinoma | VHL | c.497 T > G (p.V166G) | Diagnostic [54] | Von Hippel Lindau |
| 14-47205 | Nephroblastomatosis | PIK3CA | c.1035 T > A (p.N345K) | Diagnostic [55] | Nephroblastomatosis |
| 14-78154[a] | Medulloblastoma | KDM6A | c.2989_2990dupAT (p.M997fs) | Prognostic [56] | Risk stratification, group 4 |
| 14-75899 | Neuroblastoma | ATRX | c.5239delA (p.T1747fs) | Prognostic [57] | Poor prognosis |
| 14-10141 | Pleuropulmonaryblastoma | DICER1 | c.5438A > G (p.E1813G) | Health Maintenance [58] | DICER syndrome |
| Transcriptome analysis and CNV | | | | | |
| 14-24794 | AML | CBFB-MYH11 | Fusion | Prognostic [59] | Low-risk stratification |
| 15-64793 | B-ALL | FOXP1-ABL1 | Fusion | Prognostic [60] | High-risk stratification |
| 15-84578 | AMKL | CBFA2T3-GLIS2 | Fusion | Diagnostic [61]; Prognostic [35] | AMKL; Poor prognosis |
| 14-85546 | CML | BCR-ABL1 | Fusion | Diagnostic [59] | CML |
| 13-72282[a] | T-ALL | Isochromosome 7q | Copy number change | Diagnostic [39] | Gamma-delta T-cell lymphoma |
| 15-46387 | Rhabdomyosarcoma | PAX7-FOXO1 | Fusion | Diagnostic [62, 63]; Prognostic [64] | Rhabdomyosarcoma; High-risk group |
| 13-81192 | Alveolar soft part sarcoma | ASPSCR1-TFE3 | Fusion | Diagnostic [65] | Alveolar soft part sarcoma |
| 13-65217 | Ewing sarcoma | EWSR1-FLI1 | Fusion | Diagnostic [66] | Ewing sarcoma |
| 15-47087 | Ewing sarcoma | EWSR1-FLI1; Low expression of PAX8, FHIT, CASP10, CHD2, with high expression of CHD11, FUS, and MTA1 | Fusion; Expression pattern | Diagnostic [66]; Prognostic [36] | Ewing sarcoma; Poor prognosis |
| 13-21968 | Undifferentiated sarcoma | EML4-NTRK3 | Fusion | Diagnostic [34] | Infantile fibrosarcoma |
| 16-88073 | Ependymoma | C11orf95-RELA; Alternating gains and losses on chr 11 and 22, consistent with a "chromthripsis-like" pattern | Fusion; Copy number change | Prognostic [67]; Diagnostic [68] | Poor prognosis; RELA-type supratentorial ependymoma |
| 14-27243 | Neuroblastoma | MYCN amplified, deletion at 1p and 11q, gain 17q; MYCN over expressed | Copy number change; Overexpression | Prognostic [69, 70] | Risk-based therapy |
| 14-42817 | Neuroblastoma | MYCN non-amplified, no LOH at 1p11q; MYCN not over expressed | Copy number change; No overexpression | Prognostic [69, 70] | Risk-based therapy |
| 15-39486 | Neuroblastoma | MYCN non-amplified, no LOH at 1p11q; MYCN not over expressed | Copy number change; No overexpression | Prognostic [69, 70] | Risk-based therapy |
| 14-44070 | Neuroblastoma | MYCN amplified, loss of 1p, gain of 1q and 17q; MYCN over expressed | Copy number change; Overexpression | Prognostic [69, 70] | Risk-based therapy |

**Table 3** Clinical utility beyond targetable somatic mutations *(Continued)*

| 15-88980 | Hepatoblastoma | *Amplification of 11q13.2 including CCND1; Over expression of CCND1* | Copy number change; Overexpression | Prognostic [71] | Good prognosis |
|---|---|---|---|---|---|
| 15-49177 | Medulloblastoma | *IMPG2, GABRA5, LAPTM4B, MAB21L2, NPR3, MFAP4, NRL, ZFPM2, TSHZ3, IGF2BP3, GALNT14, GPR98; Loss of 10q22.2-10qter involving PTEN and SUFU, loss of 17p, gain of 17q* | Overexpression; Copy number change | Prognostic [72] | Risk stratification, subgroup 3/4 |
| 15-70532 | Ependymoma | *Gain of 1q, loss of 6q* | Copy number change | Prognostic [73–75] | Poor prognosis |
| 14-78154[a] | Medulloblastoma | *KCNA1, RBM24, KLHL13, EN2, SNCAIP, PDE1C, GRM8, KCNIP4, EXPH5, UNC5D, NID2, ST18, GPR12, SH3GL3; i17q* | Overexpression; Copy number change | Prognostic [72] | Risk stratification, subgroup 4 |
| 15-40141 | Pleomorphic xanthoastrocytoma | *Gain of chromosome 7, LOH at 9p* | Copy number change | Diagnostic [76] | Pleomorphic xanthoastrocytoma |
| 15-97336 | Small round blue cell tumor | *EWSR1-WT1* | Fusion | Diagnostic [77] | DSRCT |
| 15-34296 | Ependymoma | *TNC, CALB1, PLAG1, ALDH1L1, RELN* | Overexpression | Prognostic [37] | Risk stratification, group A, poor prognosis |
| 15-80972 | ATRT | *LOH at 22q11.21qter, including SMARCB1; ASCL1* | Copy number change; Overexpression | Diagnostic [78]; Prognostic [79] | ATRT; Improved prognosis |

[a]Same patient

*ALL* acute lymphoblastic leukemia, *AMKL* acute megakaryoblastic leukemia, *AML* acute myeloid leukemia, *ATRT* atypical teratoid rhaboid tumor, *CML* chronic myeloid leukemia, *CNV* copy number variation, *JMML* juvenile myelomonocytic leukemia

Germline variants classified as VOUS (Additional file 5: Table S6) were not returned to patients except if they met the following criteria: (1) the variant was predicted to be destructive; (2) the variant was in a well validated cancer-associated gene; and (3) a second somatic alteration was identified or the variant was reduced to homozygosity in the tumor. Clinical genetics returned a VOUS to four patient families meeting these criteria, including an *ITK* (p.V175V) mutation in a 7-year-old girl with Hodgkin lymphoma and Epstein-Barr virus, an *SDHC* (p.G75D) mutation was found in a 12-year-old boy with ALL, a *DICER1* (p.D609Y) mutation in an 18-year-old boy with ALCL, and an *APC* (p.V1822D) mutation in a 7-year-old boy with Ewing sarcoma.

### Clinical impact of WES

To determine the overall clinical impact of NGS cancer analysis, we evaluated each case as to whether the sequencing data were of potential utility to the referring physician in a clinically meaningful manner. Overall, clinically impactful results were found in 67/101 cases (66%) (Fig. 4). Potentially actionable alterations were found in 38% of cases. In 23% of the cases, the data obtained provided diagnostic significance. Importantly, germline predisposition to cancer was identified in 14% of all cases.

WES and RNA-seq allows for significant additional analytical endpoints (CNV, fusions, gene expression) over targeted gene panels. Focusing on the 60 cases with full tumor/normal WES and RNA-seq (cWES),

the resulting data were clinically impactful in 45 cases (75%) (Fig. 5). A total of 72 potentially clinically impactful results were found with cWES accounting for 85% of the findings (tumor/normal WES: 45%, $n = 32$; RNA-seq: 40%, $n = 29$) followed by CNV (7%, $n = 5$) and RNA-seq and CNV together in 8% ($n = 6$). Of the 30 potentially targetable aberrations found, 14 were by tumor/normal WES, 15 by RNA-seq, and one by CNV (Fig. 5).

### Discussion

In this report, we reviewed the results of the first 101 patients evaluated in our precision cancer medicine program. While we used a variety of analytical approaches matched to the clinical indications, we primarily utilized a combination of tumor/normal WES and tumor RNA-seq. This platform provided several advantages over targeted cancer gene panels, including the ability to identify translocations, segmental chromosomal changes, and relative gene expression changes.

Similar to other sequencing efforts in pediatric oncology, we found that the overall mutational load in our patients was relatively low by comparison to adult cancers [38]. Of significance, we identified germline alterations that predispose to cancer in 14% of our patients. This is slightly higher than other studies that have demonstrated approximately 8.5–10% frequency of germline risk alleles in pediatric oncology patients and may reflect a selection bias to sequence patients with high-risk cancers [12, 43, 44]. These

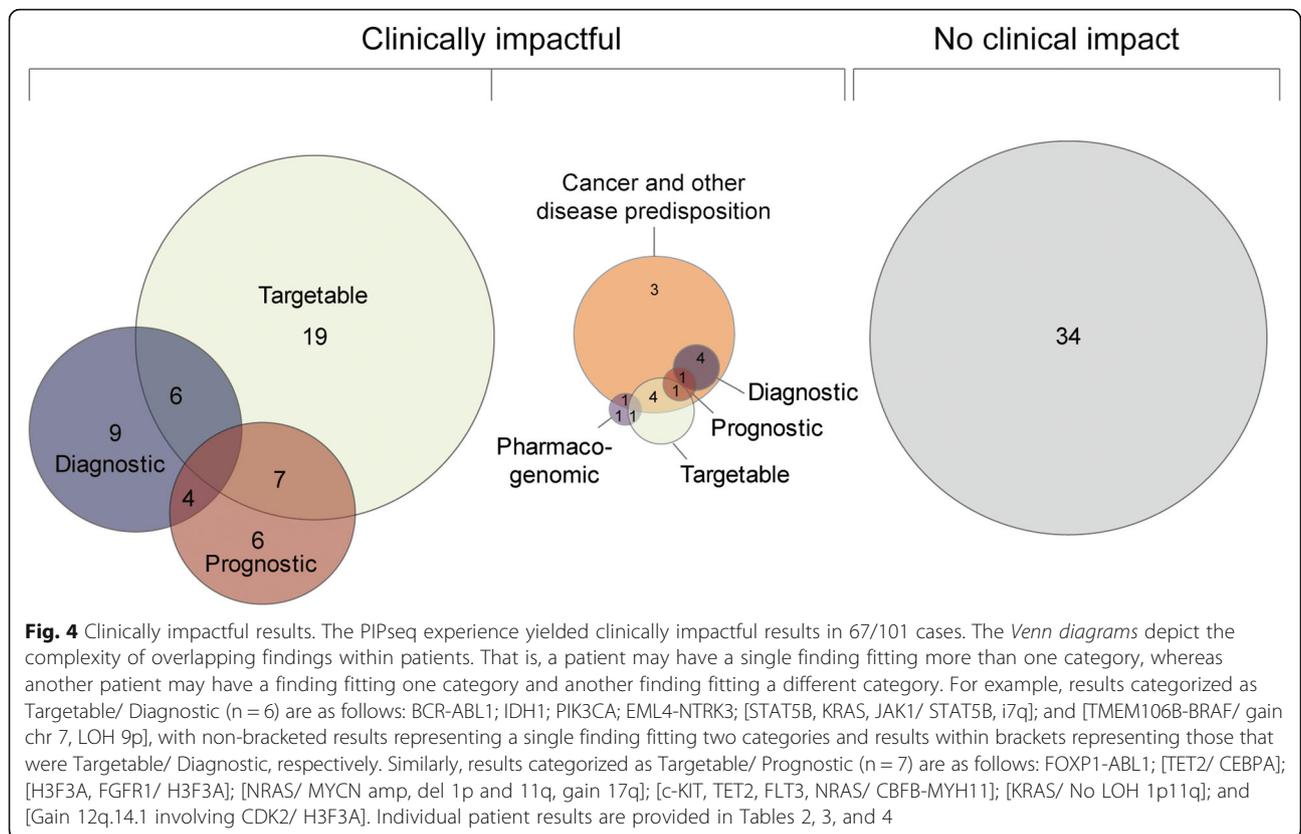**Table 4** Clinically impactful germline mutations

| PIP ID | Diagnosis | Alteration | Mutation | Change | Mutation type | Clinical utility | Implication | Previously known or suspected |
|---|---|---|---|---|---|---|---|---|
| Hematologic conditions | | | | | | | | |
| 14-19751 | HLH | MLL2 | c.11640_11640delG; c.1563IG>A | p.M3881Cfs*9 (de novo); p.E5211K | Compound heterozygous, frameshift | Diagnostic | Kabuki syndrome; Transplantation withheld | No |
| 15-90485 | HLH | C1QA | c.622C>T | p.Gln208Ter | Homozygous, nonsense | Diagnostic | C1Q deficiency | No |
| 15-33031 | MDS | GATA2 (de novo) | c.16delG | p.(E6fs) | Heterozygous, frameshift | Supports transplant recommendation; Genetic counseling for family | Increased risk for developing AML and MDS | No |
| 14-92247 | ALL | PMS2 | c.1376C>G | p.S459X | Homozygous, missense | Diagnostic; Health maintenance/ Genetic counseling | Constitutional mismatch repair deficiency syndrome; Lynch syndrome (parents) | No |
| 15-46877 | HLH | XIAP | c.1328G>C | p.R443P | Missense | Diagnostic | X-linked lymphoproliferative syndrome 2 (XLP2); Transplantation recommended | No |
| 14-19750 | AML | RUNX1 | c.806-2A>G | r.Spl? | Heterozygous splice site | Diagnostic | Familial platelet disorder; Transplantation donor changed from sibling to unrelated donor | No |
| Solid tumors | | | | | | | | |
| 14-56374 | Hepatoblastoma | APC | c.3340C>T | p.R1114 | Nonsense | Diagnostic; Health maintenance/ Genetic counseling | Familial adenomatous polyposis | Yes |
| 15-33544 | Poorly differentiated carcinoma with focal neuroendocrine differentiation | APC (de novo) | c.4660_4661insA | p.E1554fs | Frameshift | Diagnostic; Health maintenance/ Genetic counseling | Gardner syndrome; Familial adenomatous polyposis | No |
| 15-35162 | Osteosarcoma | RB1 | c.1216-3A>G | p.? | Splice site | Health maintenance/ Genetic counseling | Increased risk for developing other cancers | Yes |
| 15-44470 | Medulloblastoma | ATM | c.566G>A; c.8266A>T | p.R189K; p.K2756* | Missense, nonsense | Health maintenance/ Genetic counseling | Increased risk for developing other cancers | No |
| 15-78886[a] | Pineoblastoma | UGT1A1 | *28 allele ("(TA)7TAA") | | Homozygous | Pharmacogenomic | Drug sensitivity | No |
| 15-78886[a] | Pineoblastoma | DICER1 | c.4807dupC | p.L1603Pfs | Frameshift | Health maintenance/ Genetic counseling | Risk of ovarian Sertoli-Leydig cell tumor | No |
| 15-17264 | Hepatocellular carcinoma | UGT1A1 | *28 allele | | Heterozygous | Pharmacogenomic | Drug sensitivity | No |
| ACMG secondary findings | | | | | | | | |
| 15-29224 | AML | TP53 | c.644G>A | p.S215N | Missense | Affects therapy; Health maintenance/ Genetic counseling | Explains lack of response to conventional therapy; Increased risk for developing other cancers | No |

Oberg *et al. Genome Medicine* (2016) 8:133

Page 14 of 19

**Table 4** Clinically impactful germline mutations *(Continued)*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 14-59462 | Nested stromal epithelial tumor of the liver | BRCA1 | c.68._69delAG | p.Glu23Valfs | Frameshift | Health maintenance/ Genetic counseling | Breast cancer | No |
| 16-88073 | Ependymoma | BRCA1 | c.5587_5594delGTAGCACT | p.V1863Lfs*35 | Frameshift | Health maintenance/ Genetic counseling | Breast cancer | No |
| 14-75899 | Neuroblastoma | RYR1 | c.6838G > A | p.V2280I | Missense | Health maintenance/ Genetic counseling | Malignant hyperthermia | No |
| 14-13487 | Osteosarcoma | TNNT2 | c.422G > A | p.Arg141Gln | Heterozygous | Health maintenance/ Genetic counseling | Dilated cardiomyopathy | No |
| 15-34296 | Ependymoma | VHL | c.539 T > C | p.I180T | Missense | Health maintenance/ Genetic counseling | Von Hippel Lindau syndrome | No |

[a]Same patient
*ALL* acute lymphoblastic leukemia, *AMKL* acute megakaryoblastic leukemia, *AML* acute myeloid leukemia, *HLH* hemophagocytic lymphohistiocytosis, *JMML* juvenile myelomonocytic leukemia
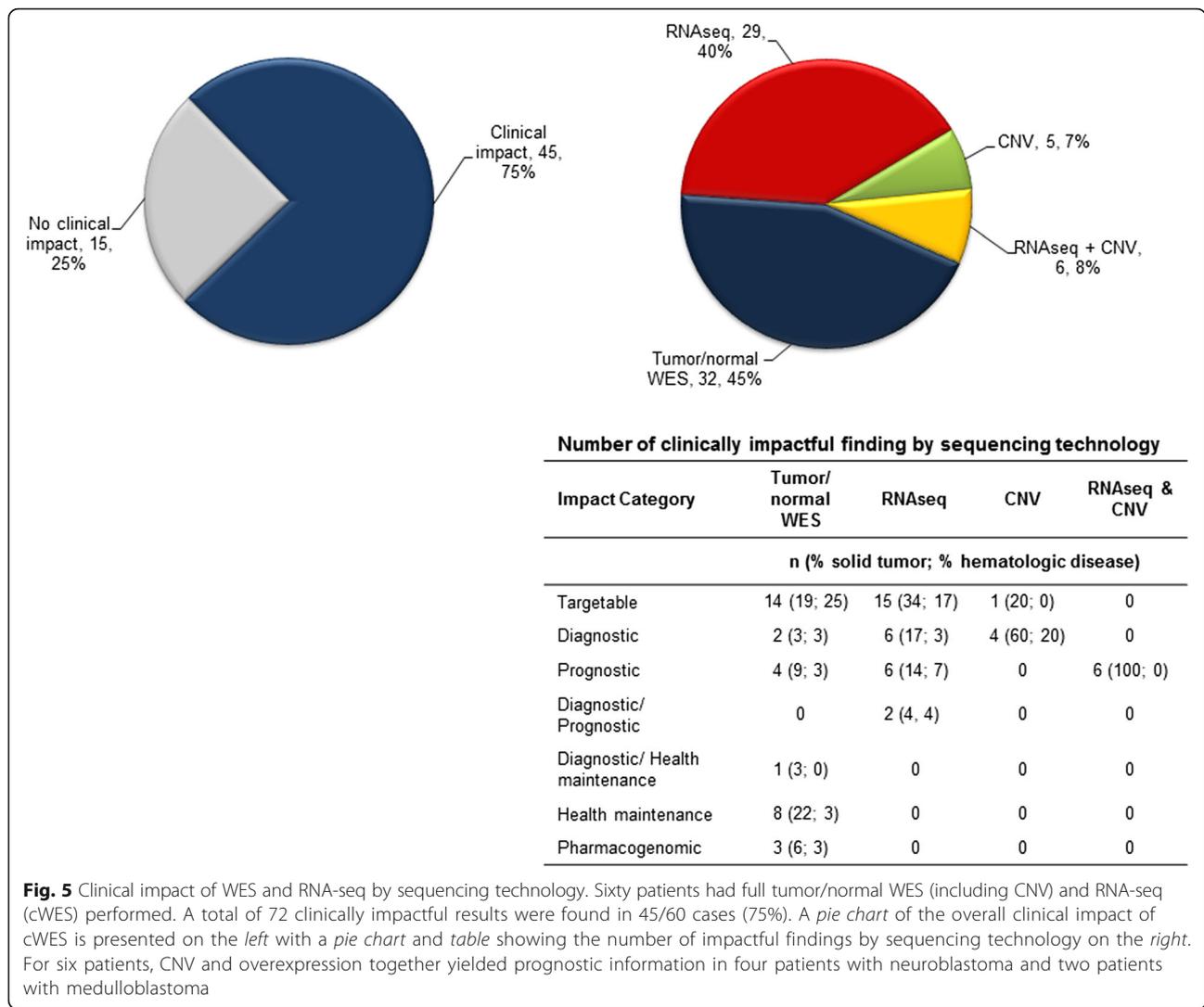
Oberg *et al. Genome Medicine* (2016) 8:133

Page 15 of 19



**Fig. 4** Clinically impactful results. The PIPseq experience yielded clinically impactful results in 67/101 cases. The *Venn diagrams* depict the complexity of overlapping findings within patients. That is, a patient may have a single finding fitting more than one category, whereas another patient may have a finding fitting one category and another finding fitting a different category. For example, results categorized as Targetable/ Diagnostic (n = 6) are as follows: BCR-ABL1; IDH1; PIK3CA; EML4-NTRK3; [STAT5B, KRAS, JAK1/ STAT5B, i7q]; and [TMEM106B-BRAF/ gain chr 7, LOH 9p], with non-bracketed results representing a single finding fitting two categories and results within brackets representing those that were Targetable/ Diagnostic, respectively. Similarly, results categorized as Targetable/ Prognostic (n = 7) are as follows: FOXP1-ABL1; [TET2/ CEBPA]; [H3F3A, FGFR1/ H3F3A]; [NRAS/ MYCN amp, del 1p and 11q, gain 17q]; [c-KIT, TET2, FLT3, NRAS/ CBFB-MYH11]; [KRAS/ No LOH 1p11q]; and [Gain 12q.14.1 involving CDK2/ H3F3A]. Individual patient results are provided in Tables 2, 3, and 4

results underscore the need to routinely incorporate germline analysis for pediatric oncology patients.

Although there is a paucity of Tier 1 actionable alterations in pediatric cancers, using a more lenient definition of actionable which includes same gene–different tumor type, likely pathogenic VOUS, and assessment of both clinical and preclinical data, resulted in the identification of potentially actionable alterations in 38% of all patients. This is comparable to other studies and may in itself be sufficient justification for comprehensive genomic analysis in cancer patients [10, 12, 15, 45]. Despite this finding, only 16% of patients received matched targeted therapy. The ability to intervene with targeted therapies is particularly challenging for pediatric patients. Many newer drugs lack efficacy data in pediatric diseases or safety data in children and are therefore not yet approved for administration. Additionally, insurance companies are not obligated to provide coverage for the off-label use of these high-cost agents. Compassionate use experimental therapies undergoing clinical testing or recently approved agents for adults are also rarely granted for pediatric patients. Finally, a number of targeted agents are not anticipated to have single-agent efficacy (e.g. MEK inhibition for *RAS* mutant tumors). Together, the lack of pediatric experience

and opportunities with combination therapy represent additional constraints in pediatric oncology.

Nevertheless, we believe that narrowing the definition of benefit to the identification of actionable targets and matched targeted therapy underestimates the potential clinical utility of comprehensive genomic analysis. We provide examples of genomic alterations that are not actionable per se, but which have significant clinical impact including for diagnostic, prognostic, or pharmacogenomics purposes. Taking a broad view of clinical impact, it is notable that the data from our sequencing platform impacted clinical decision-making in over two-thirds of all cases. With the increase in genomic medicine programs and the growing body of knowledge, the adoption of a more inclusive definition of clinical utility that does not narrowly focus on drug selection for patients with a specific biomarker is an important point to consider when incorporating NGS technologies into clinical practice.

Most cancer sequencing programs focus on interrogation of tumor DNA. It is notable that in our program the transcriptome data were responsible for a number of clinically impactful calls that were not evident from interrogating the DNA alone. In addition to verifying variants identified in the DNA analysis, the transcriptome was used to identify translocations and was mined to identify signaling pathway activity.

Oberg *et al. Genome Medicine* (2016) 8:133

Page 16 of 19



**Fig. 5** Clinical impact of WES and RNA-seq by sequencing technology. Sixty patients had full tumor/normal WES (including CNV) and RNA-seq (cWES) performed. A total of 72 clinically impactful results were found in 45/60 cases (75%). A *pie chart* of the overall clinical impact of cWES is presented on the *left* with a *pie chart* and *table* showing the number of impactful findings by sequencing technology on the *right*. For six patients, CNV and overexpression together yielded prognostic information in four patients with neuroblastoma and two patients with medulloblastoma

We generated a model from transcriptomes in our database, allowing us to identify expression outliers. We were also able to project the gene expression data into existing gene expression datasets for classification purposes, allowing us for example, to identify a *BCR-ABL1*-like gene expression pattern. Therefore, assessing tumor RNA is an important component of comprehensive genomic approaches and in our series samples interrogated with both WES and RNA-seq characterization resulted in clinically impactful data in 75% of cases.

The importance of assessing germline in addition to cancer DNA is evident from the 14% incidence of germline variants that may predispose to cancer. These findings clearly have broad implications that impact not only the patient but potentially the entire family. Moreover, the identification of germline risk offers opportunities for prevention and early screening and detection. It is

notable that given the opportunity to opt out of this knowledge, nearly all families actively choose for the return of these results, underscoring the fallacy of the paternalistic view that families need to be protected from learning these findings.

Finally, extending beyond a fuller appreciation for the potential clinical impact of sequencing technologies, it is important to consider that genomic approaches do not just provide incremental data, but may replace many conventional tests. Currently, many genetic alterations can be identified by standard approaches, such as karyotype and FISH, and with faster turnaround times. Similarly, existing NGS panels, which allow the detection of mutations and/or fusions of clear clinical relevance, may be adequate in certain clinical scenarios. Nevertheless, in an era where initial diagnostic biopsies are often performed through minimally invasive approaches, there is a compelling

Oberg *et al. Genome Medicine* (2016) 8:133

Page 17 of 19

argument to utilize comprehensive approaches with minimal tissue requirements. As the cost of NGS declines, the ability to comprehensively interrogate the genome may supersede the need for sequential, potentially tissue-exhausting directed testing, with the added benefit of uncovering rare targetable and potentially unexpected genomic drivers.

## Conclusions

Our results demonstrate the feasibility of incorporating clinical NGS into pediatric hematology-oncology practice. While the frequency of finding actionable alterations is consistent with reports of other pediatric oncology sequencing endeavors [10, 12, 15, 45], we feel this singular attribute grossly underestimates the potential clinical utility of these data. The ability to avoid ineffective/inappropriate therapies, to solidify a definitive diagnosis, and to identify pharmacogenomics modifiers all have clinical impact. Taking this more inclusive view, it is striking that the sequencing data were found to be clinically impactful in 66% of all cases tested through our program and in 75% of cases comprehensively assessed using cWES and RNA-seq. The value proposition for next generation diagnostics, therefore, should be measured both on the clinical impact of the data and the ability to replace multiple conventional single endpoint assays with a single comprehensive view of the genome.

## Additional files

**Additional file 1:** A PDF file containing Supplementary Methods. Supplementary Methods DNA and RNA extraction; somatic variant calling strategy; CNV; transcriptome analysis. (PDF 82 kb)

**Additional file 2:** A PDF file containing Supplementary Tables S1–S5. **Table S1** Quality statistics for WES. **Table S2** Accession ID for all genes and fusions referenced in the manuscript. **Table S3** Individual diagnoses. **Table S4** Cancer WES consent preferences. **Table S5** Reported somatic alterations by tier and germline alterations by category. (PDF 240 kb)

**Additional file 3:** De-identified clinical cWES report. (PDF 157 kb)

**Additional file 4:** A TIFF file containing Supplementary Figure S1. **Figure S1** Total somatic mutation load across all patients. The total mutational load across patients was 19,308 variants (mean, 216.9; SD, 829.3; median, 69). By diagnostic category, the total mutational load for solid tumors was 5853 variants (mean, 97.5; SD, 111.7; median, 86; range, 15–881), and for hematologic conditions was 13,455 variants (mean, 463.9; SD, 1428.8; median, 52; range, 14–5950). (TIF 845 kb)

**Additional file 5:** An excel file containing Supplementary Table S6. **Table S6** Somatic and germline variants of unknown significance. (XLSX 20 kb)

## Abbreviations

ACMG: American College of Medical Genetics; CNV: copy number variation; cWES: cancer whole exome sequencing; EMR: electronic medical record; FFPE: formalin fixed paraffin embedded; HLA: human leukocyte antigen; HLH: hemophagocytic lymphohistiocytosis; VOUS: variants of uncertain significance; WES: whole exome sequencing

## Availability of data and materials
The dataset supporting the conclusions of this article can be obtained from the cBioPortal for Cancer Genomics (http://cbioportal.org) by selecting the DATA SETS tab and clicking on the citation listed in the Reference column. In accordance with CUMC IRB approval, vcf files, translocations, and gene expression data are available through the cBioPortal.

## Authors' contributions
JAO, JGB, MLS, and ALK conceived, designed, and supervised all phases of the project. DP, ANS, and RZ provided coordination of clinical samples and testing. MMM and PLN directed the clinical laboratory and oversaw sample preparation, data processing, and computational analysis. HH and HR provided interpretation of clinical analysis. MMM, SJH, and AT carried out clinical analysis, interpretation, and reporting to the EMR. JGB, MLS, FSDC, JHG, DJY, and ALK provided clinical expertise and interpretation of clinical sequencing data at the multi-disciplinary tumor board and were responsible for overseeing consent and return of results. WKC provided critical advice on the return of germline findings and provided oversight of genetic counseling. CK provided genetic counseling. SJA and JP carried out data processing and computational analysis. DD provided bioinformatics support. JAO, JGB, SGE, and ALK provided funding acquisition. JAO, JGB, MLS, and ALK wrote the paper. JAO, JGB, MLS, JHG, DY, FDC, DD, WKC, KC, ANS, MMM, and ALK revised the paper. All authors contributed to data interpretation, discussions, and editing of the paper. All authors read and approved the final manuscript.

## Competing interests
The authors declare that they have no competing interests.

## Consent for publication
This retrospective review and publication of results from all consenting participants was approved by the CUMC IRB (IRB# AAAQ8170 and AAAP1200).

## Ethics approval and consent to participate
All participants provided written informed consent for tumor and/or germline sequencing analysis. Participants signed either a Columbia University Medical Center (CUMC) Institutional Review Board (IRB) approved consent (IRB# AAAB7109 or AAAJ5811) or clinical consent. Our research was carried out in accordance with the Declaration of Helsinki and Good Clinical Practice (GCP).

## Author details
[1]Department of Pediatrics, Columbia University Medical Center, New York, NY 10032, USA. [2]Department of Pathology and Cell Biology, Columbia University Medical Center, New York, NY 10032, USA. [3]Department of Clinical Genetics, Columbia University Medical Center, New York, NY 10032, USA. [4]Department of Medicine, Columbia University Medical Center, New York, NY 10032, USA. [5]Department of Microbiology and Immunology, Columbia University Medical Center, New York, NY 10032, USA. [6]Herbert Irving Comprehensive Cancer Center, Columbia University Medical Center, New York, NY 10032, USA. [7]Present address: Memorial Sloan Kettering Cancer Center, New York, NY 10065, USA. [8]Present address: MNG Laboratories, 5424 Glenridge Drive, Atlanta, GA 30342, USA.

Oberg *et al. Genome Medicine* (2016) 8:133

Page 18 of 19

## References

1. Murphy S, Xu J, Kochanek K. Deaths: Final Data for 2010. National vital statistics reports, vol. 61 no 4. Hyattsville, MD: National Center for Health Statistics; 2013.
2. Oeffinger KC, Mertens AC, Sklar CA, Kawashima T, Hudson MM, Meadows AT, et al. Chronic health conditions in adult survivors of childhood cancer. N Engl J Med. 2006;355(15):1572–82.
3. Hunger SP, Mullighan CG. Acute lymphoblastic leukemia in children. N Engl J Med. 2015;373(16):1541–52.
4. Pinto NR, Applebaum MA, Volchenboum SL, Matthay KK, London WB, Ambros PF, et al. Advances in risk classification and treatment strategies for neuroblastoma. J Clin Oncol. 2015;33(27):3008–17.
5. Beltran H, Eng K, Mosquera J, Sigaras A, Romanel A, Rennert H, et al. Whole-exome sequencing of metastatic cancer and biomarkers of treatment response. JAMA Oncol. 2015;1(4):466–74.
6. Frampton GM, Fichtenholtz A, Otto GA, Wang K, Downing SR, He J, et al. Development and validation of a clinical cancer genomic profiling test based on massively parallel DNA sequencing. Nat Biotech. 2013;31(11):1023–31.
7. Hovelson DH, McDaniel AS, Cani AK, Johnson B, Rhodes K, Williams PD, et al. Development and validation of a scalable next-generation sequencing system for assessing relevant somatic variants in solid tumors. Neoplasia. 2015;17(4):385–99.
8. Lanman R, Mortimer S, Zill O, Sebisanovic D, Lopez R, Blau S, et al. Analytical and clinical validation of a digital sequencing panel for quantitative, highly accurate evaluation of cell-free circulating tumor DNA. PLoS ONE. 2015;10(10):e0140712.
9. Meric-Bernstam F, Brusco L, Shaw K, Horombe C, Kopetz S, Davies MA, et al. Feasibility of large-scale genomic testing to facilitate enrollment onto genomically matched clinical trials. J Clin Oncol. 2015;33(25):2753–62.
10. Mody RJ, Wu Y, Lonigro RJ, Cao X, Roychowdhury S, Vats P, et al. Integrative clinical sequencing in the management of refractory or relapsed cancer in youth. JAMA. 2015;314(9):913–25.
11. Ortiz MV, Kobos R, Walsh M, Slotkin EK, Roberts S, Berger MF, et al. Integrating genomics into clinical pediatric oncology using the molecular tumor board at the Memorial Sloan Kettering Cancer Center. Pediatr Blood Cancer. 2016;63(8):1368–74.
12. Parsons D, Roy A, Yang Y, Wang T, Scollon S, Bergstrom K, et al. Diagnostic yield of clinical tumor and germline whole-exome sequencing for children with solid tumors. JAMA Oncol. 2016;2(5):616–24.
13. Shao D, Lin Y, Liu J, Wan L, Liu Z, Cheng S, et al. A targeted next-generation sequencing method for identifying clinically relevant mutation profiles in lung adenocarcinoma. Sci Rep. 2016;6:22338.
14. Uzilov AV, Ding W, Fink MY, Antipin Y, Brohl AS, Davis C, et al. Development and clinical application of an integrative genomic approach to personalized cancer therapy. Genome Med. 2016;8(1):1–20.
15. Harris M, DuBois S, Glade Bender JL, Kim A, Crompton BD, Parker E, et al. Multicenter feasibility study of tumor molecular profiling to inform therapeutic decisions in advanced pediatric solid tumors: The individualized cancer therapy (icat) study. JAMA Oncol. 2016;2(5):608–15.
16. ACMG Board of Directors. Points to consider in the clinical application of genomic sequencing. Genet Med. 2012;14(8):759–61.
17. Green RC, Berg JS, Grody WW, Kalia SS, Korf BR, Martin CL, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. Genet Med. 2013;15(7):565–74.
18. Magi A, Tattini L, Cifola I, D'Aurizio R, Benelli M, Mangano E, et al. EXCAVATOR: detecting copy number variants from whole-exome sequencing data. Genome Biol. 2013;14(10):R120.
19. Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, et al. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat Protoc. 2012;7(3):562–78.
20. Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, et al. Corrigendum: Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat Protoc. 2014;9(10):2513.
21. Ge H, Liu K, Juan T, Fang F, Newman M, Hoeck W. FusionMap: detecting fusion genes from next-generation sequencing data at base-pair resolution. Bioinformatics. 2011;27(14):1922–61.
22. Ramsköld D, Wang ET, Burge CB, Sandberg R. An abundance of ubiquitously expressed genes revealed by tissue transcriptome sequence data. PLoS Comput Biol. 2009;5(12):e1000598.
23. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2(5):401–4.
24. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal. 2013;6(269):l1.
25. Joseph L, Cankovic M, Caughron S, Chandra P, Emmadi R, Hagenkord J, et al. The spectrum of clinical utilities in molecular pathology testing procedures for inherited conditions and cancer. J Mol Diagn. 2016;18(5):605–19.
26. Wagle N, Berger MF, Davis MJ, Blumenstiel B, Defelice M, Pochanard P, et al. High-throughput detection of actionable genomic alterations in clinical tumor samples by targeted, massively parallel sequencing. Cancer Discov. 2012;2(1):82–93.
27. Kinoshita K, Hirota S, Isozaki K, Nishitani A, Tsutsui S, Watabe K, et al. Characterization of tyrosine kinase I domain c-kit gene mutation Asn655Lys newly found in primary jejunal gastrointestinal stromal tumor. Am J Gastroenterol. 2007;102(5):1134–6.
28. Mullighan CG, Su X, Zhang J, Radtke I, Phillips LA, Miller CB, et al. Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. N Engl J Med. 2009;360(5):470–80.
29. Roberts KG, Li Y, Payne-Turner D, Harvey RC, Yang YL, Pei D, et al. Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. N Engl J Med. 2014;371(11):1005–15.
30. Küçük C, Jiang B, Hu X, Zhang W, Chan JK, Xiao W, et al. Activating mutations of STAT5B and STAT3 in lymphomas derived from γδ-T or NK cells. Nat Commun. 2015;6:6025.
31. Tartaglia M, Niemeyer CM, Fragale A, Song X, Buechner J, Jung A, et al. Somatic mutations in PTPN11 in juvenile myelomonocytic leukemia, myelodysplastic syndromes and acute myeloid leukemia. Nat Genet. 2003;34(2):148–50.
32. Meyer JA, Wang J, Hogan LE, Yang JJ, Dandekar S, Patel JP, et al. Relapse-specific mutations in NT5C2 in childhood acute lymphoblastic leukemia. Nat Genet. 2013;45(3):290–4.
33. Tzoneva G, Perez-Garcia A, Carpenter Z, Khiabanian H, Tosello V, Allegretta M, et al. Activating mutations in the NT5C2 nucleotidase gene drive chemotherapy resistance in relapsed ALL. Nat Med. 2013;19(3):368–71.
34. Tannenbaum-Dvir S, Glade Bender JL, Church AJ, Janeway KA, Harris MH, Manuskhani MM, et al. Characterization of a novel fusion gene EML4-NTRK3 in a case of recurrent congenital fibrosarcoma. Cold Spring Harb Mol Case Stud. 2015;1(1):a000471.
35. Gruber Tanja A, Larson Gedman A, Zhang J, Koss CS, Marada S, Ta HQ, et al. An Inv(16)(p13.3q24.3)-encoded CBFA2T3-GLIS2 fusion protein defines an aggressive subtype of pediatric acute megakaryoblastic leukemia. Cancer Cell. 2012;22(5):683–97.
36. Ohali A, Avigad S, Zaizov R, Ophir R, Horn-Saban S, Cohen IJ, et al. Prediction of high risk Ewing's sarcoma by gene expression profiling. Oncogene. 2004;23(55):8997–9006.
37. Witt H, Mack SC, Ryzhova M, Bender S, Sill M, Isserlin R, et al. Delineation of two clinically and molecularly distinct subgroups of posterior fossa ependymoma. Cancer Cell. 2011;20(2):143–57.
38. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature. 2013;500(7463):415–21.
39. Tamaska J, Adam E, Kozma A, Gopcsa L, Andrikovics H, Tordai A, et al. Hepatosplenic γδ T-cell lymphoma with ring chromosome 7, an isochromosome 7q equivalent clonal chromosomal aberration. Virchows Arch. 2006;449(4):479–83.
40. Durno CA, Sherman PM, Aronson M, Malkin D, Hawkins C, Bakry D, et al. Phenotypic and genotypic characterisation of biallelic mismatch repair deficiency (BMMR-D) syndrome. Eur J Cancer. 2015;51(8):977–83.
41. Song WJ, Sullivan MG, Legare RD, Hutchings S, Tan X, Kufrin D, et al. Haploinsufficiency of CBFA2 causes familial thrombocytopenia with propensity to develop acute myelogenous leukaemia. Nat Genet. 1999;23(2):166–75.
42. Miyake N, Koshimizu E, Okamoto N, Mizuno S, Ogata T, Nagai T, et al. MLL2 and KDM6A mutations in patients with Kabuki syndrome. Am J Med Genet A. 2013;161A(9):2234–43.
43. Knapke S, Nagarajan R, Correll J, Kent D, Burns K. Hereditary cancer risk assessment in a pediatric oncology follow-up clinic. Pediatr Blood Cancer. 2012;58(1):85–9.
44. Zhang J, Walsh MF, Wu G, Edmonson MN, Gruber TA, Easton J, et al. Germline mutations in predisposition genes in pediatric cancer. N Engl J Med. 2015;373(24):2336–46.
45. Chang W, Brohl AS, Patidar R, Sindiri S, Shern JF, Wei JS, et al. Multidimensional ClinOmics for precision therapy of children and adolescent young adults with relapsed and refractory cancer: a report from the center for cancer research. Clin Cancer Res. 2016;22(15):3810–20.

Oberg *et al. Genome Medicine* (2016) 8:133

Page 19 of 19

46. Sakaguchi H, Okuno Y, Muramatsu H, Yoshida K, Shiraishi Y, Takahashi M, et al. Exome sequencing identifies secondary mutations of SETBP1 and JAK3 in juvenile myelomonocytic leukemia. Nat Genet. 2013;45(8):937–41.

47. Nicolae A, Xi L, Pittaluga S, Abdullaev Z, Pack SD, Chen J, et al. Frequent STAT5B mutations in [gamma][delta] hepatosplenic T-cell lymphomas. Leukemia. 2014;28(11):2244–8.

48. Amary MF, Damato S, Halai D, Eskandarpour M, Berisha F, Bonar F, et al. Ollier disease and Maffucci syndrome are caused by somatic mosaic mutations of IDH1 and IDH2. Nat Genet. 2011;43(12):1262–5.

49. Pansuriya TC, van Eijk R, d'Adamo P, van Ruler MA, Kuijjer ML, Oosting J, et al. Somatic mosaic IDH1 and IDH2 mutations are associated with enchondroma and spindle cell hemangioma in Ollier disease and Maffucci syndrome. Nat Genet. 2011;43(12):1256–61.

50. Ho PA, Alonzo TA, Gerbing RB, Pollard J, Stirewalt DL, Hurwitz C, et al. Prevalence and prognostic implications of CEBPA mutations in pediatric acute myeloid leukemia (AML): a report from the Children's Oncology Group. Blood. 2009;113(26):6558–66.

51. Sturm D, Witt H, Hovestadt V, Khuong-Quang DA, Jones DT, Konermann C, et al. Hotspot mutations in H3F3A and IDH1 define distinct epigenetic and biological subgroups of glioblastoma. Cancer Cell. 2012;22(4):425–37.

52. Chan K-M, Fang D, Gan H, Hashizume R, Yu C, Schroeder M, et al. The histone H3.3K27M mutation in pediatric glioma reprograms H3K27 methylation and gene expression. Genes Dev. 2013;27(9):985–90.

53. Khuong-Quang D-A, Buczkowicz P, Rakopoulos P, Liu XY, Fontebasso AM, Bouffet E, et al. K27M mutation in histone H3.3 defines clinically and biologically distinct subgroups of pediatric diffuse intrinsic pontine gliomas. Acta Neuropathol. 2012;124(3):439–47.

54. Rao Q, Chen JY, Wang JD, Ma HH, Zhou HB, Lu ZF, et al. Renal cell carcinoma in children and young adults: clinicopathological, immunohistochemical, and VHL gene analysis of 46 cases with follow-up. Int J Surg Pathol. 2011;19(2):170–9.

55. Gripp K, Baker L, Kandula V, Conard K, Scavina M, Napoli JA, et al. Nephroblastomatosis or Wilms tumor in a fourth patient with a somatic PIK3CA mutation. Am J Med Genet. 2016;Part A 9999A:1–11.

56. Dubuc AM, Remke M, Korshunov A, Northcott PA, Zhan SH, Mendez-Lago M, et al. Aberrant patterns of H3K4 and H3K27 histone lysine methylation occur across subgroups in medulloblastoma. Acta Neuropathol. 2013;125(3):373–84.

57. Cheung NV, Zhang J, Lu C, Parker M, Bahrami A, Tickoo SK, et al. Association of age at diagnosis and genetic mutations in patients with neuroblastoma. JAMA. 2012;307(10):1062–71.

58. Brenneman M, Field A, Yang J, Williams G, Doros L, Rossi C, et al. Temporal order of RNase IIIb and loss-of-function mutations during development determines phenotype in DICER1 syndrome: a unique variant of the two-hit tumor suppression model. F1000Res. 2015;10(4):214.

59. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.

60. Ernst T, Score J, Deininger M, Hidalgo-Curtis C, Lackie P, Ershler WB, et al. Identification of FOXP1 and SNX2 as novel ABL1 fusion partners in acute lymphoblastic leukaemia. Br J Haematol. 2011;153(1):43–6.

61. Masetti R, Pigazzi M, Togni M, Astolfi A, Indio V, Manara E, et al. CBFA2T3-GLIS2 fusion transcript is a novel common feature in pediatric, cytogenetically normal AML, not restricted to FAB M7 subtype. Blood. 2013;121(17):3469–72.

62. Laé M, Ahn EH, Mercado GE, Chuai S, Edgar M, Pawel BR, et al. Global gene expression profiling of PAX-FKHR fusion-positive alveolar and PAX-FKHR fusion-negative embryonal rhabdomyosarcomas. J Pathol. 2007;212(2):143–51.

63. Williamson D, Missiaglia E, de Reyniès A, Pierron G, Thuille B, Palenzuela G, et al. Fusion gene–negative alveolar rhabdomyosarcoma is clinically and molecularly indistinguishable from embryonal rhabdomyosarcoma. J Clin Oncol. 2010;28(13):2151–8.

64. Skapek SX, Anderson J, Barr FG, Bridge JA, Gastier-Foster JM, Parham DM, et al. PAX-FOXO1 fusion status drives unfavorable outcome for children with rhabdomyosarcoma: a children's oncology group report. Pediatr Blood Cancer. 2013;60(9):1411–7.

65. Ladanyi M, Lui M, Antonescu C, Krause-Boehm A, Meindl A, Argani P, et al. The der(17)t(X;17)(p11;q25) of human alveolar soft part sarcoma fuses the TFE3 transcription factor gene to ASPL, a novel gene at 17q25. Oncogene. 2001;20(1):48–57.

66. Delattre O, Zucman J, Plougastel B, Desmaze C, Melot T, Peter M, et al. Gene fusion with an ETS DNA-binding domain caused by chromosome translocation in human tumours. Nature. 1992;359(6391):162–5.

67. Parker M, Mohankumar KM, Punchihewa C, Weinlich R, Dalton JD, Li Y, et al. C11orf95-RELA fusions drive oncogenic NF-[kgr]B signalling in ependymoma. Nature. 2014;506(7489):451–5.

68. Pajtler Kristian W, Witt H, Sill M, Jones DT, Hovestadt V, Kratochwil F, et al. Molecular classification of ependymal tumors across all CNS compartments, histopathological grades, and age groups. Cancer Cell. 2015;27(5):728–43.

69. Bosse K, Maris J. Advances in the translational genomics of neuroblastoma: From improving risk stratification and revealing novel biology to identifying actionable genomic alterations. Cancer. 2016;122(1):20–33.

70. Attiyeh EF, London WB, Mossé YP, Wang Q, Winter C, Khazi D, et al. Chromosome 1p and 11q deletions and outcome in neuroblastoma. N Engl J Med. 2005;353(21):2243–53.

71. López-Terrada D, Gunaratne PH, Adesina AM, Pulliam J, Hoang DM, Nguyen Y, et al. Histologic subtypes of hepatoblastoma are characterized by differential canonical Wnt and Notch pathway activation in DLK+ precursors. Hum Pathol. 2009;40(6):783–94.

72. Northcott PA, Korshunov A, Witt H, Hielscher T, Eberhart CG, Mack S, et al. Medulloblastoma comprises four distinct molecular variants. J Clin Oncol. 2011;29(11):1408–14.

73. Kilday J-P, Mitra B, Domerg C, Ward J, Andreiuolo F, Osteso-Ibanez T, et al. Copy number gain of 1q25 predicts poor progression-free survival for pediatric intracranial ependymomas and enables patient risk stratification: a prospective european clinical trial cohort analysis on behalf of the Children's Cancer Leukaemia Group (CCLG), Société Française d'Oncologie Pédiatrique (SFOP), and International Society for Pediatric Oncology (SIOP). Clin Cancer Res. 2012;18(7):2001–11.

74. Olsen T, Gorunova L, Meling TR, Micci F, Scheie D, Due-Tonnessen B, et al. Genomic characterization of ependymomas reveals 6q loss as the most common aberration. Oncol Rep. 2014;32(2):483–90.

75. Rousseau A, Idbaih A, Ducray F, Criniere E, Fevre-Montange M, Jouvet A, et al. Specific chromosomal imbalances as detected by array CGH in ependymomas in association with tumor location, histological subtype and grade. J Neurooncol. 2010;97(3):353–64.

76. Marko N, Weil R. The molecular biology of WHO Grade II gliomas. Neurosurg Focus. 2013;34(2):E1.

77. Gerald WL, Rosai J, Ladanyi M. Characterization of the genomic breakpoint and chimeric transcripts in the EWS-WT1 gene fusion of desmoplastic small round cell tumor. Proc Natl Acad Sci U S A. 1995;92(4):1028–32.

78. Jackson EM, Sievert AJ, Gai X, Hakonarson H, Judkins AR, Tooke L, et al. Genomic analysis using high-density single nucleotide polymorphism-based oligonucleotide arrays and multiplex ligation-dependent probe amplification provides a comprehensive analysis of INI1/SMARCB1 in malignant rhabdoid tumors. Clin Cancer Res. 2009;15(6):1923–30.

79. Torchia J, Picard D, Lafay-Cousin L, Hawkins CE, Kim SK, Letourneau L, et al. Molecular subgroups of atypical teratoid rhabdoid tumours in children: an integrated genomic and clinicopathological analysis. Lancet Oncol. 2015;16(5):569–82.