of the genetic risk of identified variants toward the development of MCC is warranted. Future studies will include similar workup and analysis in older MCC patients to determine the relative contribution of variants in known cancer predisposition genes to more typical presentations of MCC.

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eP048

Identifying cancer predisposition in a series of 1,521 pediatric oncology patients by tumor-only panel-based testing

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Introduction: Tumor-only profiling by next-generation sequencing (NGS) has been implemented in the pediatric oncology setting to enhance pathologic diagnosis, provide prognosis, and identify molecular targets for therapy. Clinically significant germline variants in cancer susceptibility genes covered by the assay will also be detected with this approach. Current guidelines regarding the threshold of triggering follow-up testing for germline pathogenic variants detected by tumor-only NGS are limited, given the challenge of distinguishing germline vs somatic variants as well as the need for follow-up germline confirmatory testing and genetic consultation. Here, we reviewed the yield and utility of targeted germline testing following tumor DNA sequencing in a diverse pediatric patient population at Children's Hospital Los Angeles.
Methods: We retrospectively analyzed a cohort of 1,521 consecutive patients receiving tumor-only OncoKids cancer panel testing, a comprehensive DNA- and RNA-based NGS assay. For each patient, clinical, pathology, and molecular results were discussed at a weekly or biweekly multidisciplinary tumor board. Recommendations for germline testing were made based on clinical and family history, and one of the following criteria: 1) presence of a Tier I or Tier II variant in a tumor-suppressor gene at a VAF of approximately 50% with no copy number variants or loss of heterozygosity (LOH) in the variant locus; 2) a Tier I or Tier II variant in a tumor-suppressor gene with LOH encompassing the locus in the tumor; 3) the presence of 2 Tier I or Tier II variants in a cancer predisposition gene in the tumor; 4) a Tier I or Tier II variant in the tumor sample that had previously been published in the literature in the germline setting; 5) Tier I or Tier II variant in a cancer predisposition gene for patients with a clinical or family history suggestive of a cancer predisposition syndrome. Targeted Sanger sequencing of a germline sample was performed for the variant(s) detected by the somatic cancer panel. Patients who underwent germline testing using a custom cancer predisposition panel, focused exome analysis, or single-gene testing (eg, *SMARCB1*, *RB1* and *TP53* for patients with rhabdoid tumor, retinoblastoma or Li-Fraumeni syndrome, respectively) were not included in this study.
Results: A total of 1,521 pediatric oncology patients, including 568 with hematological malignancies, 557 with soft tissue and bone tumors, and 396 with CNS tumors, received tumor-only OncoKids testing. Recommendations for targeted germline testing were made for 179 patients (12%) based on the somatic testing results in conjunction with clinical and/or family history. Of these, 102 patients underwent targeted germline testing for the variants detected by tumor sequencing. Germline pathogenic/likely pathogenic variants were identified in 38% (39/102) of patients tested: *TP53* (n=11), *NF1* (n=6), *WT1* (n=5), *DICER1* (n=4), *SMARCB1* (n=3), *PTEN* (n=2), *RET* (n=2), *CBL* (n=2), *KRAS* (n=1), *GATA2* and *MSH6* (n=1), *SMARCA4* (n=1), and *RASA1* (n=1). Three patients showed low-level mosaicism for a *KRAS*, *SMARCB1* or *PTEN* pathogenic variant in the peripheral blood sample. The remaining 63% (64/102) of patients were negative for the variants identified with tumor profiling. Of the 77 patients who did not undergo targeted germline testing, 14 (16%) had a clinical or family history highly suggestive of cancer predisposition. Examples included a patient with a hyper-mutated high-grade glioma suggestive of a mismatch repair disorder, a patient with clear-cell meningioma and LOH encompassing *SMARCE1*, two hemangioblastoma patients, one with a clinical diagnosis of Von Hippel-Lindau syndrome and the other with a loss of chromosome 3p that included *VHL*, and a child with choroid plexus carcinoma and likely germline *TP53* variant.
Conclusion: Targeted germline analysis for variants detected by tumor sequencing increases the percentage of pediatric patients diagnosed with cancer susceptibility which may impact therapy selection, clinical surveillance, and genetic counseling in families. The number of patients with germline susceptibility to cancer is underestimated in this study since 1) not all genes related to cancer susceptibility are included in the somatic panel, eg, *SMARCE1*, 2) due to technical limitations exon level copy number variants and/or complex structural variants may not be detected, and 3) germline testing is not performed for all patients in whom suspected germline pathogenic variant(s) are identified by tumor-sequencing. Both targeted germline follow-up testing and expanded germline analysis independent of tumor sequencing in appropriate patients are needed to comprehensively identify clinically significant germline variants in the pediatric population.

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eP049

Discordant fluorescence in situ hybridization and RNASeq results in the identification of fusion partners in recurring translocations in hematological malignancies

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Introduction: Reciprocal chromosomal translocations are implicated in neoplastic transformation in tumors. These can be identified by chromosome analysis, by fluorescence in situ hybridization (FISH) if cryptic, or by RNASeq/next generation sequencing. FISH probes are highly specific and sensitive, and have the advantage of faster turn-around-time, are less expensive, and can be used on a variety of specimen types. Therefore, several of these probes are commercially available, and are incorporated to screen diagnostic specimens. However, these probes may yield erroneous identification of fusion partners due to the proximity of genes covered by the FISH probe design. Here, we present two examples of this in hematological malignancies.
Methods: Clinical material, bone marrow (BM) or peripheral blood (PB) was evaluated for hematopathology (morphology, immunohistochemistry, and flow cytometry), cytogenetics (karyotype and FISH) and RNASeq (next generation sequencing) methods using standard protocols.
Results: Patient one is a 62-year-old man was diagnosed with plasma cell myeloma (PCM) after he presented with anemia, hypercalcemia, lytic bone lesions, and 80-90% involvement by CD38+ plasma cells in bone marrow (BM). Chromosome analysis of the BM aspirate showed a highly complex karyotype with multiple related clones. FISH analysis with probes for *FGFR3*, *MYC*, *CCND1*, *IGH*, *MAF* and *MAFB* detected *FGFR3* / *IGH* fusion. Therefore, a chromosomal

diagnosis of *FGFR3* rearranged PCM was rendered. In contrast, RNASeq data showed overexpression of *NSD2* due to fusion with *IGH*. Patient two is a 15-year-old girl who presented with fever, elevated leukocyte count, B-lineage blasts in peripheral blood (PB) and in cerebrospinal fluid and was diagnosed with B-lymphoblastic leukemia (B-ALL). Chromosome analysis of a PB specimen at diagnosis showed an abnormal karyotype including a t(1;5)(q23;q33). A high risk ALL FISH panel of probes (Cep4/Cep10, *ABL1*/*BCR*, *ETV6*/*RUNX1, ABL1*, *ABL2*, *PDGFRB*, *KMT2A*) detected rearrangement in *PDGFRB* in 69.5% cells and loss of one signal for *ETV6* in 19% cells. Therefore, a chromosomal diagnosis of *PDGFRB* rearranged B-ALL was rendered. Contrary to this, NGS analysis detected fusion between *MEF2D* (1q22) and *CSF1R* (5q33).

Conclusion: *FGFR3* and *NSD2* located 6.25kb apart on 4p16.3 are involved exclusively in the t(4;14) in PCM. Since *FGFR3* involvement is more frequent than *NSD2*, a commercial probe was designed to identify *FGFR3* rearrangement, but design of this probe included *NSD2*. *FGFR3*/*IGH* fusion is routinely interpreted as *FGFR3* positive PCM. Clinically patients positive for *NSD2*/*IGH* fusion have extremely poor prognosis compared to *FGFR3*/*IGH* positive patients. Moreover, the patients with t(4;14) with upregulation of *FGFR3* may be treated with the novel pan-TKI, dovitinib, on a clinical trial. Patients with high-risk B-ALL are routinely screened for rearrangement in *PDGFRB* using a FISH probe, and cases positive for *PDGFRB* are rendered a cytogenetic diagnosis of *PDGFRB* rearranged B-ALL. Another infrequent genetic marker of extreme poor prognosis in Ph-like B-ALL is translocation of *MEF2D* (at 1q22) with several partners including *CSF1R* which is about 500pb apart from *PDGFRB*. Since *PDGFRB* and *CSF1R* are closely linked, *PDGFRB* FISH positive patients can be erroneously classified as *PDGFRB* positive B-ALL. Similar to *FGFR3* positive PCM, some patients with *PDGFRB* rearranged B-ALL may benefit treatment with TKIs.

These two cases highlight the necessity to exercise caution in the interpretation of FISH results, and the necessity for performing fusion screening using RNAseq or NGS methods for prognostication and therapeutic management of patients.

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eP050

Cytogenomic profiling and clinical correlation of 21q22 amplification in acute myeloid leukemia reveal distinct cytogenomic features and poor outcomes

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Background: 21q22 amplification is a rare cytogenetic aberration in acute myeloid leukemia (AML). So far, the cytogenomic and molecular features and clinical correlation of 21q22 amplification in AML have not been well-characterized.

Case presentation: Here, we describe a case series of three AML patients with amplified 21q22 identified by fluorescence in situ hybridization (FISH) using a *RUNX1* probe. Two of these patients presented with therapy-related AML (t-AML) secondary to chemotherapy, while the third had de novo AML. There was one case each of FAB M0, M1 and M4. Morphologic evidence of dysplasia was identified in both t-AML cases. Phenotypic abnormalities of the myeloblasts were frequently observed. Extra copies of 21q22 were present on chromosome 21 and at least one other chromosome in two cases. Two showed a highly complex karyotype. Microarray analysis of 21q22 amplification in one case demonstrated alternating levels of high copy number gain split within the *RUNX1* locus at 21q22, a pattern distinct from the iAMP21 profile reported in B-cell precursor acute lymphoblastic leukemia (B-ALL). The same patient also had mutated *TP53*. Two patients died at 1.5 and 11 months post-treatment, while the third elected palliative care and died within 2 weeks.

Conclusion: Our results provide further evidence that 21q22 amplification in AML is associated with complex karyotypes, *TP53* aberrations, and poor outcomes. Furthermore, we demonstrate that 21q22 amplification is not always intrachromosomally localized to chromosome 21 and could be a result of structural aberrations involving 21q22 and other chromosomes.

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eP052

Coding defects in chromosomal segregation and protein targeting are central to TGCT predisposition

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Introduction: Testicular germ cell tumor (TGCT) is the most common cancer of young men of genetic European ancestry. Although rare, TGCT results in the most years of life lost of all adult cancers. Genome-wide association studies (GWAS) of TGCT have been highly successful, with over 66 independent hits, the highest OR = ~3.5 (*KITLG*). This reflects the high heritability of the disease. We and others identified the tumor-suppressor *CHEK2* as the first Mendelian gene associated with TGCT predisposition. Earlier exome studies yielded a variety of results, including association with *DNAAF1, PLEC*, *DNAH7*, *EXO5,* and ciliary genes. Follow-up studies indicated that the high heritable (HR) component is multigenic.

Methods: We performed exome sequencing and gene burden analysis on 293 individuals with HR-TGCT, representing 228 unique families, and 3157 cancer-free controls.