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