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.

Results: Single-variant association analysis identified the strongest association with a missense variant in the hinge domain of proto-oncogene *PIM1* (OR 8.3, 95% CI 3.24-21.3). Gene burden association identified association with several genes after multiple testing correction, including loss-of-function in *NIN* and *QRSL1I*, genes of centrosome assembly and oxidative phosphorylation. Previous results in this cohort showing an association with *CHEK2* were repeated. Gene-specific analysis showed a trend to association with *CFTR* (heterozygous 11% HR-TGCT, 5.9% controls, p=0.1362). Among other genes previously published by other groups, *STOML3* was associated to nominal significance ($p=9.42x10^{-3}$). Among genes associated with TGCT somatic changes, several genes were nominally associated, including *CDC27* and *SP8*. We identified no association with the sex and germ cell development pathways (p = 0.65 truncating variants, p = 0.47 all variants: hypergeometric overlap test), nor an association with any of the regions previously identified by GWAS. Finally, when considering all non-synonymous variation associated with HR-TGCT after multiple testing correction, together with genes associated with TGCT by GWAS and post-GWAS analysis, there were associations with three major pathways: mitosis/cell-cycle, cotranslational protein targeting, and sex differentiation (GO:1903047: log(o/e) 0.79, FDR $1.53x10^{-11}$; GO:0006613: log(o/e) 1.27, FDR $1.35x10^{-10}$; GO:0007548: log(o/e) 0.72, FDR $1.90x10^{-4}$).
Conclusion: The study presented here is the largest to-date of individuals with familial or bilateral (high risk; HR)-TGCT. Similar to previous studies, we identified association with a variety of genes, suggesting that the heritable component is multigenic. This work identified a new association with cotranslational protein targeting, along with previously reported mitosis/cell-cycle, as seen in a plethora of cancers, and sex differentiation, a feature unique to TGCT. Finally, this work suggests further potentially druggable targets for TGCT prevention or treatment, including *PIM1*, and pathways of cotranslational targeting.

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eP053

Detection of rare and novel fusions in pediatric B-Lymphoblastic Leukemia (B-ALL) by capture-based transcriptome sequencing (RNA-Seq)

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Introduction: Numerous newly recognized clinically significant genetic markers in B-lymphoblastic leukemia (B-ALL) that are being incorporated into the next edition of the World Health Organization (WHO) classification cannot be detected by routine karyotype and fluorescence in situ hybridization (FISH) analyses. For example, *EPOR* and *CRLF2* rearrangements associated with the *BCR-ABL1* (Philadelphia)-like subtype of B-ALL and abnormalities which define *DUX4*-upregulated subtype of the disease, remain undetected by standard cytogenetic evaluation. At Children's Hospital of Los Angeles (CHLA) we incorporate Chromosomal Microarray (CMA) and a custom next-generation sequencing (NGS) panel (OncoKids) into routine clinical assessment for hematologic malignancies. However, even after this comprehensive evaluation, the primary genetic driver remains unknown in up to 15% of our B-ALL cases. We hypothesized that capture-based transcriptome sequencing (RNA-Seq) and, in selected cases, confirmatory Optical Genome Mapping (OGM), could be used to identify clinically significant oncogenic fusions missed by our routine multi-modal B-ALL testing.
Methods: This study of archival clinical material included 53 pediatric B-ALL cases in which the genetic driver remained unknown after clinical karyotyping, FISH, CMA, and OncoKids analyses. RNA-Seq was conducted by enrichment with the Twist Comprehensive Exome capture probes set, followed by sequencing on the NextSeq or HiSeq instrument (Illumina, Inc., San Diego, CA). Data analysis for detection of abnormal gene fusions utilized a NGS analysis pipeline that incorporated Fusion Catcher, Dragen, STARfusion, and Arriba. Gene expression measurements were obtained using featureCounts. Normalized expression levels for the *DUX4* gene were evaluated by comparison with B-ALL samples that were known to contain or lack oncogenic *DUX4* rearrangements. Selected novel fusions detected by RNA-Seq were confirmed by OGM, a novel platform for high-resolution, genome-wide detection of copy number abnormalities and balanced chromosomal rearrangements, performed at the Bionano Genomics Service Laboratory (Bionano Genomics, San Diego, CA).
Results: RNA-Seq identified rare, previously described B-ALL fusions in 8/53 cases, and *IGH* rearrangements in 5/53 cases. In addition, RNA-Seq revealed novel fusions in 6/53 cases. Overall, confirmed or putative oncogenic drivers were identified in 19/53 (36%) B-ALLs. Recently described genetic subtypes identified in our cohort included *MEF2D* fusions with different partner genes (*BCL9*, *FOXJ2*, and *SS18*) in 4 cases, and *EP300-ZNF384*, *TCF3-PBX1*, *SPTBN1-PDGFRB*, and *ACIN1-NUTM1* fusions in one case each. The detected *IGH* rearrangements were *IGH-CEBPA* (1 case), *IGH-IL3* (1 case), and *IGH-DUX4* (3 cases). The *IGH-DUX4* rearrangements were confirmed by *DUX4* overexpression. Finally, the novel candidate fusions in 6 cases included two *JAK2* fusions with previously unreported partner genes (*RBM26* and *STRBP*), both of which were confirmed by OGM, and *PAX5-HIPK3, ETV6-MFN2, ZMYND8-PDGFRB* and *BACH2-ATRX* fusions for which confirmation is in progress.
Conclusion: In this retrospective study, RNA-Seq detected clinically significant gene fusions or *IGH* rearrangements in 13/53 (24.5%) of the cases which had remained negative after comprehensive clinical testing. These detected fusions define specific genetic subtypes of B-ALL and represent important prognostic markers. We also demonstrate the clinical utility of OGM, a novel technology, in confirming RNA-Seq findings. Furthermore, the assays allowed the discovery of 6 novel candidate fusions, including *JAK2* fusions, which are associated with the *BCR-ABL1* (Philadelphia)-like subtype of B-ALL and may predict response to treatment with JAK2 inhibitors. Our results support the diagnostic and research utility of RNA-Seq in pediatric B-ALL. Considering the heterogeneity of oncogenic fusions and rapid discovery of novel fusion partners, we propose that non-targeted transcriptome sequencing may be superior to targeted, fixed-content fusion panels in clinical B-ALL testing.

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