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# POSTER ABSTRACTS

## 618.ACUTE LYMPHOBLASTIC LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

### Cancer Genomic Profiling and Minimal Residual Disease Monitoring By Cell-Free DNA Sequencing in Pediatric Leukemia

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**Introduction**: Cell-free DNA (cfDNA) sequencing has been reported as a promising non-invasive tool for detecting cancer and monitoring treatment response. We sought to evaluate the potential of a cfDNA-based strategy for detecting diverse classes of somatic mutations relevant to pediatric leukemia and cancers.
**Methods:** Plasma samples collected at diagnosis from 135 acute lymphoblastic leukemia (ALL) and 34 acute myeloid leukemia (AML) patients were evaluated, including 114 B-ALL and 21 T-ALL. We also studied diagnostic plasma samples from 38 solid tumor and 19 brain tumor patients, and plasma collected from 12 patients with B-ALL at days 15, 22 and end of induction chemotherapy (EOI). Plasma cfDNA extraction and library preparation were performed with the NucleoSnap cfDNA kit and ThruPLEX Tag-Seq HV kit, respectively. Next-generation sequencing was performed using a capture panel covering 355 pediatric cancer genes (including noncoding regions covering hotspots of structural variation) and 8,103 heterozygous population polymorphisms. We developed analytic approaches for *de novo* detection of all types of somatic mutations, including focal copy number alterations, rearrangements, and large chromosomal abnormalities. Whole-genome sequencing (WGS) was also performed for 10 cfDNA samples from leukemia. Data were compared with corresponding whole-genome, exome and transcriptome tumor cell sequencing data at diagnosis.
**Results**: At diagnosis, plasma cfDNA yield was higher for leukemias than for solid and brain tumors (median, 142 ng/ml, 7.16 ng/ml, and 7.07 ng/ml, respectively. The average coverages of leukemia cfDNA sequencing were 790X by capture panel post error suppression and 69X by WGS. We found that 100% cases of leukemia at diagnosis had detectable circulating tumor DNA (ctDNA) in plasma cfDNA, and the median ctDNA purity was 77%, comparable to primary tumor purity from diagnostic sequencing. Overall, our panel covered 86% somatic variants present in the tumor samples. Of those covered events, capture-based cfDNA sequencing detected 98% SNVs/indels, 95% focal copy gains/losses, 100% internal tandem duplications, 95% chromosomal translocations, and 99% aneuploidy. In addition, capture-based sequencing identified cfDNA-specific variants such as NRAS/KRAS SNVs and a IKZF1 deletion that were subclonal in plasma cfDNA. Of those uncovered events, 85% were structural variants with their breakpoints in intronic/noncoding regions, and cfDNA WGS identified all somatic events present in the 10 sequenced tumor samples. To detect minimal residual disease (MRD) in B-ALL patients during induction, we increased cfDNA sequencing depth for low VAF events, and the detection limit was 0.4% for SNV/indel and structural variations. Compared to the MRD results from flow cytometry, somatic mutations were detected in 7/8 MRD-positive and 1/4 MRD-negative patients on day 15, 3/4 MRD-positive patients on day 22, and 0/2 MRD-positive patients at EOI. We also sequenced cfDNA from peripheral blood and bone marrow plasma for 2 B-ALL patients on day 0, day 15, and EOI, with comparable ctDNA purity and dynamics observed between the two compartments. In a patient with concurrent ALL and neuroblastoma (NBL) who experienced NBL relapse four months after the primary diagnosis, cfDNA genomic profiling identified ctDNA derived from ALL (52% of total plasma cfDNA), primary NBL (20%), and relapsed NBL (10%), suggesting that genetic variations of multiple tumors could be detected in a single cfDNA sample.
**Conclusion**: Our study has demonstrated the utility of cfDNA sequencing to identify both sequence and structural somatic mutations in childhood acute leukemia. This approach will be useful to monitor disease, as well as providing a non-invasive diagnostic approach.

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