H18. Assessment of a High-Throughput Sequencing Assay for Measurable Residual Disease (MRD) Monitoring in Patients with T-Cell Malignancies

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Introduction: High-throughput sequencing of the T-cell receptor (TCR) beta and gamma loci is becoming more widely utilized due to its high sensitivity, specificity, and versatility in the diagnosis of T-cell malignancies. Application of these technologies for tracking disease burden can be valuable in detecting recurrence, determining response to therapy, guiding future management of patients, and establishing endpoints for clinical trials. In this study, we assessed the performance of a commercially available highthroughput sequencing assay for determining residual disease burden in patients with various T-cell malignancies receiving care at our institution. Methods: Peripheral blood samples from 56 patients previously diagnosed with various T-cell malignancies were sequenced with the commercially available LymphoTrack T-cell MRD assay (InvivoScribe, Inc), which includes the TRB and TRG assay kits, internal and low positive controls, and the LymphoTrack MRD analysis software. Samples were sequenced and analyzed according to the manufacturer's recommendations. Test performance characteristics including linearity, analytic sensitivity, specificity, and precision were assessed with both contrived and patient samples. The accuracy of the assay was further assessed by tracking 96 previously identified TRB clones and 130 previously identified TRG clones, and comparing results with concurrent MRD assessment by the clonoSEQ assay (Adaptive Biotechnologies). Results: The LymphoTrack T-cell MRD assay demonstrated excellent test performance characteristics for the DNA inputs tested. Assessment of intrarun and inter-run precision revealed coefficients of variation averaging less than 20% for samples containing 1,000 or more T-cell equivalents, whereas greater variances were seen for samples containing fewer than 100 T-cell equivalents. Both TRB and TRG assays reliably detected tracked clonotypes down to an order of 10 T-cell equivalents. Tracked clonotypes were also found to be highly specific to malignant cells when interrogated in pooled normal controls. Despite some notable differences with the clonoSEQ assay in the ability to detect certain rearrangements, MRD status was highly concordant with the LymphoTrack T-cell MRD assay at the sample level. Conclusions: High-throughput sequencing of the TCR beta and gamma loci is a highly sensitive, specific, precise, and accurate method for determining disease burden for various T-cell malignancies and can be readily implemented in molecular diagnostic laboratories capable of performing high-throughput sequencing.

ST50. Comprehensive Genomic Profiling of Different Subsets of Merkel Cell Carcinoma: Insights on Pathogenetic Pathways

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Introduction: Merkel cell carcinoma (MCC) is a rare aggressive cutaneous neuroendocrine (NE) carcinoma. It can arise from incorporation of Merkel cell polyomavirus (MCPyV) DNA into the genome of a host cell or from ultraviolet light-induced genetic damage. Tumors in the latter group include those with a "pure" small cell NE phenotype and those with "combined" NE and other morphological elements, most often foci of squamous cell carcinoma (SCC). We performed comprehensive genomic profiling of MCPyV+ and MCPyV- (pure and combined) tumors to better understand the mutational profiles of the different subsets and to shed light on their pathogenesis. Methods: The study protocol was approved by the hospital research ethics board. The cohort was comprised of 51 MCCs, consisting of 21 MCPyV+ tumors, 13 MCPyV- pure tumors, and 17 MCPyV- combined tumors which contained invasive or in situ SCC. Isolation and separate sequencing of the SCC component was attempted in 12 cases. All samples underwent comprehensive genomic profiling using the Illumina TSO500 DNA next-generation sequencing (NGS) panel. The panel interrogates all exons of 523 cancer-related genes, identifying small mutations in all genes, copy number gains in a subset of oncogenes, and global parameters of tumor mutation burden (TMB) and microsatellite instability (MSI). Data were processed using an in-house bioinformatics analysis pipeline, which included the CNVKit algorithm to provide copy number gains and losses for all 523 genes. Results: After eliminating samples failing to meet the minimum threshold for median read depth (>100x unique reads), 35 tumors remained (14 MCPyV+, 8 pure MCPyV-, 13 combined MCPyV-). The SCC component was successfully sequenced in 5 combined tumors. TMB was lower in MCPyV+ tumors than in MCPyV- ones (mean 1.66 versus 29.9/Mb, p <0.01). In combined tumors, no significant difference was observed in TMB between the NE and SCC components (41.6 versus 39.9/Mb, p = 0.90). MCPyV-

tumors featured frequent mutations in *TP53* (95%), *RB1* (81%), and *NOTCH* family genes (95%). Mutations in MCPyV+ tumors were not consistently identified in any specific genes. The frequency of overlapping mutations in the NE and SCC components of combined tumors, ranged from 3.3% (5/151) to 99% (81/82). None of the tumors were MSI-high. **Conclusions:** Our results support existing evidence that MCPyV+ and MCPyV- MCCs are fundamentally distinct entities with different mutational profiles. Combined tumors with genetically related SCC and NE elements may arise via high grade transformation of the former, or due to a shared tumor stem cell precursor. The consistently recurrent mutations common to both MCPyV- subsets suggest that these pure and combined tumors arise via similar pathways, albeit with ultimate dominant expression of the NE phenotype in the former.

ST58. Genomic Profiling Uncovers Mutation Signatures That Differentiate Pediatric Rhabdomyosarcoma (RMS) Subgroups and Predict Clinical Outcomes

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¹Children's Hospital, Wilmington, DE; ²Children's Hospital of Philadelphia, Philadelphia, PA. Introduction: RMS is the most common soft-tissue sarcoma in childhood, and is a heterogeneous disease with variable clinical outcomes. We assessed the clinical utility of genomic profiling in informing diagnosis and prognosis of RMS. Methods: Fifty primary RMS tumors from 50 pediatric patients were subjected to next-generation sequencing testing using the CHOP comprehensive solid tumor panel. The panel interrogates 238 cancer genes for single nucleotide variants (SNVs), indels, and copy number variations, and 110 fusion gene partners for more than 600 fusions. Variants were classified according to the AMP/ASCO/CAP guidelines. Genomic changes of each subgroup were summarized, and their impact on overall (OS) and event-free survival (EFS) were evaluated by Kaplan-Meier curve with logrank test. Results: The cohort consists of 11 alveolar RMS (ARMS), 30 embryonal RMS (ERMS), 5 spindle/sclerosing RMS (SRMS), and 4 unspecified RMS (NOS) patients. All ARMS tumors were positive for either PAX3-FOXO1 or PAX7-FOXO1 fusion. Whereas SNVs were rare in ARMS, we observed amplification of MYCN in 3 tumors, gain of MYCN in 2 tumors, and gain of ALK or GNAS each in 3 tumors. ARMS patients with gain/amplification of MYCN had significantly worse OS (p = 0.01) and EFS (p = 0.02), and those with gain of GNAS had worse OS (p = 0.01). Loss of heterozygosity of 11p15 was found in 17 ERMS patients, and co-amplification of FGFR1 and MYC in 11, but neither alteration affected OS or EFS (p >0.05). Clinically significant mutations of DICER1 (8), NF1 (8), TP53 (6), BCOR (5), FBXW7 (3), ARID1A (3) or CTNNB1 (3) were frequently found in ERMS. DICER1 and CTNNB1 mutations were only found in non-anaplastic ERMS. Although NF1 mutations were dominantly (7 out of 8) seen in non-anaplastic ERMS, deletions of NF1 were often observed in anaplastic ERMS. Mutations and deletions of TP53 were largely associated with anaplastic ERMS. Ten ERMS with impaired cell cvcle/TP53 pathway (TP53/6, CDKN2A/2, MDM2/1, MDM4/2, CDK4/1) showed significantly worse OS (p <0.01) and EFS (p <0.0001); 4 with biallelic NF1 alterations and 5 with defects in WNT pathway (CTNNB1/3, CDC73/2) showed worse OS (p <0.03 and p <0.01, respectively). Three SRMS harbored a MYOD1 p.L122R mutation, and the other 2 had an infantile SRMS-associated fusion, FUS-TFCP2 or VGLL2-CITED2. Germline pathogenic variants in TP53, DICER1, NF1 or PTCH1 were confirmed in 5 ERMS and 1 NOS patients, and were associated with worse OS (p = 0.0001) in ERMS. Conclusions: Gain of MYCN or GNAS in ARMS, impaired cell cycle/TP53 pathways, biallelic loss of NF1, and WNT pathway defects in ERMS, as well as germline mutations are associated with poor survival in patients with RMS. Our data show that genomic alterations should be evaluated for incorporation into risk classifiers for pediatric RMS.