H06. Molecular Immunoglobulin/T-Cell Receptor Clonality Analysis by Bidirectional Amplicon Sequencing on MiSeq with a Phasing Primer Design Strategy

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Introduction: Compared to the capillary electrophoresis (CE)-based method, clonality analysis of immunoglobulin (*IG*) and T-Cell Receptor (*TCR*) genes by next-generation sequencing (NGS) can provide clonotype-specific sequence and count information to an unprecedented depth. The quality of sequencing data generated on the Miseq instrument (Illumina) relies heavily on the base complexity within each cycle that can vary significantly between multiplexed libraries. Fixed insert-DNA orientation in relation to sequencing adapters combined with a low number of BIOMED-2 consensus J primers can make the low-complexity problem particularly profound and result in compromised data quality. Spiking in a well-balanced library as a solution, however, inevitably comes at a price of consuming a considerable amount of reads on uninformative targets. We developed an approach to address the aforementioned challenges. **Methods:** A set of phasing primers were designed based on each individual BIOMED-2 V or J primer for *IGH*, *TCRG*, or *TCRB* by adding different numbers of random nucleotides at the 5' end. The amplicons generated with those modified primers were subject to library preparation with the Illumina TruSeq kit and ligated to sequencing adapters in random orientations. The libraries containing 6 to 25 indexed samples each were sequenced on MiSeq. The overlapped paired-end reads were joined using the publicly available software FLASH (Fast Length Adjustment of Short Reads). After removing primer sequences, the clonotypes were submitted to IMGT/HighV-Quest for *IG*/*TCR* rearrangement analysis. For method validation, sequencing of *IGH*, *TCRG* and *TCRB* was performed on 32, 12, and 30 patient and healthy control DNA samples, respectively. **Results:** Up to 22 million paired-end reads per run were generated with cluster passing filter rates of 88% to 96% at relatively high cluster densities. The Q30 scores with V2 and V3 kits were 91% to 96.5% for read 1 and 89% to 95% for read 2. Intra-run and inter-run precisions assessed with replicate samples showed no alignment mismatches. Sequencing paired samples (diagnostic and minimal residual disease) from CLL patients with known tumor clone sequences previously determined by Sanger sequencing, as a measure of assay accuracy, showed a 100% base matching rate with the tumor clone sequence. NGS-based analysis also demonstrated the heterogeneity of clonotypes at the same amplicon size that would contribute to the same peak on CE analysis. **Conclusions:** The phasing primer design combined with ligation-based library preparation is a robust strategy for clonality analysis by MiSeq. This approach effectively increases inherent library complexity and consistently leads to precise and accurate high quality sequencing data.

ST20. Activating *RAS-RAF* Mutations in Ameloblastoma

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Introduction: Ameloblastoma is a locally invasive odontogenic neoplasm whose overall mutational landscape has not been well characterized. We sought to characterize pathogenic mutations in ameloblastoma and their clinical and functional significance with an emphasis on *RAS-BRAF*. **Methods:** A total of 84 ameloblastomas and 40 non-ameloblastoma odontogenic tumors were evaluated with a combination of *BRAF* V600E allele-specific PCR, VE1 immunohistochemistry, the Ion AmpliSeq Cancer Hotspot Panel and Sanger sequencing. *In vitro* efficacy of a Braf inhibitor was evaluated in the ameloblastoma derived cell line AM-1. **Results:** Somatic, activating and mutually-exclusive *RAS-BRAF* mutations were identified in 82% of cases. *BRAF* V600E was the most common mutation, found in 62% of ameloblastomas as well as in ameloblastic fibromas/fibrodentinomas but not in other odontogenic tumors. This mutation was associated with a younger age of onset ($p<0.0001$) whereas *BRAF* wild-type cases arose more frequently in the maxilla ($p=0.0007$). In a multivariate analysis, *BRAF* wild-type status was also independently associated with earlier recurrences ($p=0.045$). 100% concordance was observed between VE1 immunohistochemistry and molecular detection of *BRAF* V600E mutations. Activating *RAS* mutations were present in 58% of *BRAF* wild-type cases. Somatic mutations in *SMO* (16%), *CTNNB1* (4%), *PIK3CA* (6%) and *SMARCB1* (6%) were also identified. Ameloblastoma cells demonstrated constitutive MAPK pathway activation *in vitro*. Proliferation and MAPK activation were potently inhibited by the Braf inhibitor vemurafenib. **Conclusions:** Our findings suggest that activating *RAS-BRAF* mutations play a critical role in the pathogenesis of most cases of ameloblastoma. Somatic mutations in *SMO*, *CTNNB1*, *PIK3CA* and *SMARCB1* may function as secondary mutations. The most common mutation – *BRAF* V600E – was not found in odontogenic neoplasms without ameloblastic epithelium suggesting a potential role as a diagnostic marker. VE1 immunohistochemistry was successfully

employed as a surrogate for molecular detection of these mutations. *BRAF* V600E mutations also have prognostic implications with *BRAF* wild-type cases arising more frequently in the maxilla and being independently associated with earlier recurrences. Finally, *in vitro* response of ameloblastoma to a Braf inhibitor suggests a potential role for targeted therapy.

ST38. Incidental Detection of Myelodysplastic Syndrome by Germline Next-Generation Sequencing Cancer-Risk Panel Testing

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Introduction: Recent attention has been drawn to the reporting of incidental findings that are discovered during germline testing for a separate indication. This discussion has gained prominence due to the expansion of panel, exome, transcriptome and whole genome testing facilitated by next-generation sequencing (NGS) technology. However, the possibility of uncovering a previously unidentified neoplastic process as an incidental finding in germline testing has not been well-addressed. **Methods**: NGS testing was performed using the 51-gene BROCA comprehensive cancer risk panel to sequence DNA isolated from peripheral blood of a 74-year-old woman with a personal history of ovarian cancer and a family history of breast, stomach, pancreatic, liver, and hematopoietic cancers. The sample tested was DNA extracted from whole peripheral blood. Targets of interest were enriched using the Agilent SureSelect custom BROCA array, and sequenced on an Illumina HiSeq to an average depth of 463x. The data analysis pipeline included detection of single nucleotide variants, small insertion/deletions, copy number variants (CNV), and structural variants. CNV calling was accomplished by determining copy number states for individual targets, then comparing read-depth for each exon against a reference sample. CNVs were called on an exon-by-exon basis and visualized using the R package ggplot2. **Results:** BROCA testing revealed a deleterious *TP53* mutation (p.R273H) at an allelic fraction of 64% (297/461 sequencing reads) and partial copy loss at six loci: *APC*, *RAD50*, and *CTNNA1* on chromosome 5q; *SMAD4* on chromosome18; and *POLD1* and *STK11* on chromosome19. This combination of findings was suspicious for a neoplastic hematopoietic process. Subsequent investigation confirmed a diagnosis of myelodysplastic syndrome (MDS) with cytogenetic results including 5q-, concordant with the findings from BROCA sequencing. **Conclusions:** To our knowledge this is the first description of MDS identified as an incidental finding in the setting of germline cancer risk assessment. Our findings demonstrate that cancer risk testing can reveal hematopoietic neoplasms, and that the possibility of somatic mutations should be considered in test interpretation, especially in older patients. Careful attention to variant allele fraction and CNV analysis can provide helpful clues. When a hematopoietic neoplasm is suspected, recommendations for appropriate diagnostic workup should be communicated by the laboratories providing these results.