ID04. Hepatitis C Virus Genotyping of Organ Donor Samples to Aid in Transplantation of HCV-positive Organs

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Introduction: Kidneys from hepatitis C virus-positive (HCV+) organ donors are almost exclusively transplanted into HCV+ recipients. Because the number of HCV+ kidneys far exceeds the number of patients willing and able to accept such organs, nearly two-thirds of these kidneys are discarded every year. The development of new therapies for HCV that cure more than 95% of patients, coupled with the vast shortage of available kidneys, have led some transplant professionals to consider transplanting HCV+ kidneys into HCV-negative recipients. One potential barrier to doing so is the ability to rapidly genotype organ donors for HCV, since viral genotype guides therapy and no pan-genotypic antiviral regimens exist that can safely be used in patients with impaired kidney function. Also, it is not known whether donor stabilization procedures, such as fluid or drug infusions, affect genotyping ability (e.g., by reducing viremia or introducing PCR inhibitors into circulation). Our aim was to determine whether archived plasma samples from past organ donors could be accurately genotyped for HCV. **Methods:** A local organ procurement organization performed a retrospective review of their database to identify previous organ donors that were HCV+ on a qualitative nucleic acid screen. Archived frozen plasma samples were obtained for 17 such donors, along with donor demographics. RNA was extracted from the thawed samples using the QIAamp DSP Viral RNA kit on the QIAcube (Qiagen, Valencia, CA). HCV genotypes were detected using the eSensor HCVg *Direct* Test (RUO) and XT-8 System (GenMark Diagnostics, Carlsbad, CA). All samples were also genotyped by Sanger sequencing (Retrogen, San Diego, CA) and subjected to quantitative HCV viral load testing (COBAS AmpliPrep/TaqMan system, Roche Diagnostics, Indianapolis, IN). **Results:** The samples obtained were collected over a 10 month period primarily from Caucasian donors, both male and female, between 21 and 57 years old. All samples were successfully extracted and genotyped. The majority of samples (n=14) were HCV Genotype 1a with the remainder being Genotype 2b (n=1) or Genotype 3 (n=2). All genotyping results were concordant with those obtained via Sanger sequencing. The average HCV viral load across the samples was ~ 1.6 million IU/mL (range: ~16,000 IU/mL to 7 million IU/mL). **Conclusions:** Albeit a limited sample size, we found that viral RNA from plasma of organ donors can be successfully extracted and genotyped for HCV. Treatment provided to stabilize donors prior to organ procurement does not appear to interfere with the ability to extract or amplify viral RNA. The capacity to provide an accurate HCV genotype suggests that clinical trials designed to transplant HCV+ kidneys into HCV-negative recipients could be feasible.

H14. Bi-Allelic Amplification of *ATM* Gene in Blastoid Variant of Mantle Cell Lymphoma: A Novel Mechanism of Inactivation Due to Chromoanagenesis?

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Introduction: Mantle cell lymphoma (MCL) is a lymphoid tumor derived from naïve CD5+

cells with the cytogenetic hallmark t(11;14) resulting in over-expression of the CCND1 gene. The presence of multiple chromosome abnormalities in addition to t(11;14) is known to be associated with blastoid variants (BMCL) and poor prognosis in MCL. High proportion of these tumors also show deletion of chromosome 11q22-23 or loss of heterozygosity; a region where the ATM gene is located. MCL tumors with bi-allelic ATM inactivation show significantly higher chromosome imbalances compared to MCLs with wild type ATM alleles suggesting that loss of ATM alleles increases chromosomal instability of the tumor cells. **Methods:** We report a unique case of BMCL in a 52-year-old male who presented with worsening dyspnea, fever/chills, diffuse lymphadenopathy, splenomegaly and leukocytosis with blasts per differential. CT scan demonstrated extensive retroperitoneal, pelvic and inguinal lymphadenopathy with massive splenomegaly. Peripheral blood and bone marrow morphological, immunohistochemical and flow cytometry studies and bone marrow chromosome, FISH and high resolution microarray studies were carried out using standard methods. **Results:** The blood smear showed leukocytosis due to the presence of "blasts" appearing cells. Bone marrow aspirate showed about 40% abnormal “blast” appearing cells. Bone marrow biopsy revealed remarkable lymphoid infiltrate in interstitial pattern and large lymphoid aggregates, contributing to about 40% of entire cellularity. The abnormal cells were immunoreactive to CD20, PAX-5 and Cyclin D1. Scattered were a few CD3 T-cells. Flow cytometry from the bone marrow aspirate detected a population (about 50% of total events) of lambda monoclonal B-cell population expressing CD19, CD22, CD20, and FMC-7 with coexpression of CD5, but were negative for CD10, CD200, CD43, CD34 and CD38. Based on these results, the patient was diagnosed with BMCL. Conventional cytogenetic analysis showed multiple chromosome abnormalities including t(11;14) and, contrary to published reports, bi-allelic amplification of ATM by FISH and SNP microarray. Apart from several characteristic gains, losses and regions of LOH, SNP microarray also showed high complexity abnormalities suggestive of chromoanagenesis involving chromosomes 7 and 11, especially involving the ATM gene region. **Conclusions:** Although Bi-allelic ATM mutations are common, gene amplification has never been reported in MCL. Focal chromothripsis as a mechanism of inactivating tumor suppressor genes was postulated in Retinoblastoma and we propose that chromoanagenesis inactivated both ATM alleles in our patient leading to multiple chromosome abnormalities and development of BMCL.

TT76. Combining Data from Next Generation Sequencing using an Automated Platform with Two Chips Improves Laboratory Workflow and Reduces Rejection of Specimens Previously Judged Inadequate for Analysis

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Introduction: Manual processing of specimens for Next Generation Sequencing (NGS) is complex and time consuming. Automation can allow for better standardization and improved workflow. The Ion Torrent Chef requires 8 specimens be pooled on an Ion 318^{TM} chip, thus 16 specimens per 2 chip sequencing run. However, our lab volume is not sufficient to run two 318^{TM} chips per week. We describe an alternative NGS automated workflow with combined data from two 316^{TM} chips resulting in improved wet-bench turnaround time, reduced technician hands-on time, equivalent data and the ability to adequately analyze previously inadequate

specimens. **Methods**: 20 clinical specimens were previously sequenced using a manual workflow and the 50 gene Ion AmpliSeq™ Cancer Hotspot Panel, the Ion One Touch 2 and the Ion Torrent Personal Genome Machine (PGM). Eight additional specimens that previously failed or were excluded from clinical testing due to low quality or quantity DNA were also included in the study. Laboratory validation was then performed using the Ion AmpliSeq™ Kit for Chef DL8 and the Ion AmpliSeq™ Cancer Hotspot Panel v2 on the Ion Chef™. Combined libraries were prepared in duplicate (8 specimens per Ion 316 chip including control) using the Ion PGM Hi-Q Chef Kit and Ion 316™ Chip Kit v2 for clonal amplification, chip loading and sequencing. Aligned data from the same 8 specimens on two 316 chips were combined, using the Torrent Suite Software v5.0.4 with the variantCaller plugin v5.0.4.0, to achieve adequate coverage for detecting somatic mutations, rather than employing an Ion 318™ chip. This strategy has not been previously reported in the literature or by the vendor. **Results**: For specimens with previously known clinical results (n=20), all expected mutations were identified. Additionally the 8 specimens, deemed inadequate for the manual workflow by lab experience, were successfully amplified and sequenced using this method with a combined alignment average of 630,000 reads (almost 3000X average coverage). Five of the 8 "inadequate" specimens were compared with their alternatively tested specimens with adequate DNA and all sequence results matched. In one of 5 samples, a clinically actionable mutation (KRAS p.G12D) was identified. No mutations were detected in the 3 of 8 samples that had minimal DNA sufficient only for automated analysis. Hands-on technician time was reduced by 2.5 hours per sequencing run. **Conclusions**: Use of the Ion Chef™ for automated NGS processes generates data that is comparable to manual methods in a time-efficient manner. In addition, specimens that were deemed inadequate for manual library preparation, could be successfully analyzed using this two-chip combined data method.