2025 Young Investigator Award Winning Abstracts

G010. **Identification of Hypomorphic and Pathogenic *ATM* Variants via High-Throughput Functional Screening**

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Introduction: *ATM* is a large gene (~10,000 bp of coding sequence) with a high number of variants of uncertain significance (VUSs), complicating clinical interpretation. Pathogenic *ATM* variants not only cause ataxia-telangiectasia but also increase cancer risk, particularly breast and prostate cancers. Functional characterization of VUSs is critical, and recent advances in genome editing enable parallel assessment of thousands of variants. Here, we used prime editing to introduce 27,513 single-nucleotide variants (SNVs) across the coding region of *ATM* and systematically evaluated their functional effects. By applying both natural selection and selective pressure via PARP inhibition, we assessed pathogenicity of the SNVs and identified hypomorphic variants – those with partial loss of function only under stress conditions. **Methods:** HCT116 cells were used to generate *ATM*-haploid (1 copy of *ATM*) cells using SpCas9 with sgRNAs flanking the 5' and 3' UTRs. Prime editor (PE2max) was inserted into the cells via lentiviral transduction. To generate SNVs, exon-specific epegRNA libraries covering all *ATM* coding exons (2 to 63) were designed and delivered via lentivirus. Next-generation sequencing was performed to quantify edited SNV frequencies on day 0 and after 10 days of culture. Function score was calculated as log2-fold changes in SNV abundance, indicating its functional effect. Missense variants classified as functional in untreated conditions but non-functional under the PARP inhibition were defined as hypomorphic. Variant classification was validated against ClinVar annotations and UK Biobank clinical data. **Results:** We successfully edited 23,092 exonic SNVs using prime editing. In the untreated condition, 3,760 SNVs (780 nonsense, 2,728 missense, 252 synonymous) were classified as non-functional. Under the PARP inhibition, 4,755 SNVs (1,048 nonsense, 3,455 missense, 252 synonymous) were non-functional. Function scores discriminated ClinVar-defined pathogenic and benign variants with AUC (area under the curve) values of 0.88 and 0.94 under each of the conditions, respectively. UK Biobank participants carrying the PARP-defined non-functional SNVs had a 1.64-fold increased risk of breast cancer (95% CI 1.19 to 2.25). Among 720 identified hypomorphic variants, 266 were VUSs and 451 were previously unreported variants. Carriers of hypomorphic variants had a 2.49-fold (95% CI 1.12 to 5.55) increased risk for breast cancer. **Conclusions:** High-throughput functional assay enables comprehensive and accurate classification of *ATM* SNVs, including VUSs, by leveraging genome editing tools. The findings were validated in the large-scale population datasets, highlighting the clinical relevance of hypomorphic variants and demonstrating a scalable framework for variant interpretation.

ID010. **Investigating the Role of Proviral Sequencing When Transitioning Virally Suppressed Adults with HIV to Long-Acting Injectable Cabotegravir-Rilpivirine**

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Introduction: Since its approval in 2021, long-acting injectable cabotegravir-rilpivirine (LAI CAB/RPV) has proven to be an effective antiretroviral therapy (ART) option for persons with human immunodeficiency virus type-1 (HIV). Promisingly, virologic failure (VF) on LAI CAB/RPV is rare, but if it occurs, it may severely limit a person's future ART options. A BMI ≥30 kg/m2, infection with HIV subtype A6, and the presence of resistance-associated mutations (RAMs) to RPV have been identified as potential risk factors for VF. These data are typically retrievable from the electronic medical record, but genotypic data may not always be accessible. For persons without available genotypic data and who are virally suppressed on oral ART, sequencing of integrated proviral HIV DNA from infected cells can identify RPV RAMs and, when relevant, subtype before starting LAI CAB/RPV. However, proviral sequencing is controversial largely due to concerns about assay sensitivity, mutation interpretation, and testing costs. Therefore, additional data on the real-world use of proviral sequencing in guiding LAI CAB/RPV initiation is needed. **Methods:** We performed a retrospective single-center review of all persons referred to the Madison Clinic at Harborview Medical Center in Seattle, WA, for consideration of LAI CAB/RPV treatment from May 2021 to December 2024. Manual chart review was performed to determine the clinical reasoning behind testing and treatment decisions. Mutation interpretation was based on lists of potential RAMs from the Ward 86 clinic protocols and the Stanford University HIV Drug Resistance Database. **Results:** We completed clinical eligibility review for 216 referrals and sent proviral sequencing in 33 (15%) cases. Of the 33 persons with proviral sequencing, 12 (36%) started LAI CAB/RPV, and 17 (52%) were deferred, at least in part due to the mutations identified by proviral sequencing. No one started LAI CAB/RPV with potential RPV RAMs. However, 10 of the 12 persons who started LAI CAB/RPV had potential CAB mutations identified by proviral sequencing – these were mostly polymorphic mutations but included 1 non-polymorphic G140R mutation. There has been no evidence of VF to date with a median follow-up of 9.2 months. **Conclusions:** Using our clinical eligibility review process, proviral sequencing was limited to 15% of LAI CAB/RPV referrals and, when obtained, provided clinically actionable information. Although interpretation of RPV RAMs was consistent, interpretation of CAB RAMs evolved during the study period and must continue to adapt as the use of integrase inhibitor-based regimens increases worldwide. Overall, our experience provides an important example of using patient risk factors to inform proviral sequencing when starting LAI CAB/RPV.

ST176. **Don't Judge a Tumor by Its Genome Only: Spatial Transcriptomics Uncovers Distinct Transcriptional Landscapes in ARID1A-Deficient Endometrial Cancers Dependent on Protein and Mutation Status**

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Introduction: Novel therapeutic options are available for targeting the SWI/SNF complex. Better therapeutic options are needed for endometrial cancer (EC), as its annual mortality is increasing, in contrast to other common cancer types. *ARID1A*, a gene encoding a key component of SWI/SNF, is most frequently mutated in EC. Little has been done to characterize loss of ARID1A protein in EC. Classically, loss of ARID1A protein is linked to presence of *ARID1A* mutation. In other malignancies ARID1A loss occurs regardless of mutation status, resulting in more aggressive tumors. We aimed to understand if loss of ARID1A occurs regardless of mutation status in EC and characterize the transcriptional landscape based on both mutation and protein status. Recent studies have highlighted ARID1A loss as a potential inclusion marker for immunotherapy and as a predictor of response to EZH2 and ATR inhibitors. This work will help in the identification of which EC patients are the best potential candidates for therapies targeting SWI/SNF. **Methods:** Immunohistochemistry (IHC) of ARID1A was performed on 74 formalin-fixed, paraffin-embedded endometrioid EC (38 *ARID1A* mutated, 36 *ARID1A* wild-type). In 16 EC, NanoString spatial transcriptomics was performed, with 95 regions of interest (ROIs) selected based on expression of ARID1A protein. Survival analysis was performed on endometrioid EC from the The Cancer Genome Atlas Uterine Corpus Endometrial Carcinoma (TCGA-UCEC) cohort based on genotype and median ARID1A protein expression. **Results:** TCGA (The Cancer Genome Atlas) data demonstrated the importance of ARID1A protein expression in endometrioid EC. In 128 *ARID1A*wild-type EC, lower median ARID1A protein predicted significantly worse overall survival ($p = 0.0054$). In our study set, 21% of wild-type and 44% of mutated *ARID1A* EC had ARID1A protein loss in at least 50% of the tumor. ROIs with ARID1A loss were defined by increased enrichment of hallmark pathways such as G2M checkpoint, E2F targets, and MYC targets V1 regardless of mutation status. Comparison of differential gene expression based on protein and/or mutation revealed that the presence of *ARID1A* mutation contributes to unique gene expression profiles in addition to those associated with protein status. **Conclusions:** Endometrioid EC is associated with a unique gene expression profile defined by the combination of ARID1A genotype and protein expression. Thus, clinical evaluation of ARID1A in EC patients may require both *ARID1A* genotyping and IHC assessment to more accurately guide therapeutic options and management dependent on prognosis. Failure to include both these assessments in the past may be one explanation of why this important and commonly altered gene in EC has not had more clinical impact to date.