

ST002. Analysis of Urinary Cell-free DNA for Early Detection and Surveillance of Bladder Cancer

J. Dudley¹, J. Schroers-Martin¹, D. Lazzareschi¹, W. Shi¹, S. Chen¹, J. Liao², A. Alizadeh¹, M. Diehn¹

¹Stanford University, Stanford, CA; ²VA Palo Alto Health Care System, Palo Alto, CA.

Introduction: Current regimens for the diagnosis and surveillance of bladder cancer (BLCA) include urine cytology, which has low sensitivity, and cystoscopy, which is invasive and relatively expensive. Several ancillary tests have been developed to address these challenges, but none are widely used due to limited performance characteristics. By contrast, cell-free DNA (cfDNA) profiling has demonstrated high performance characteristics in previous studies for the noninvasive detection and surveillance of multiple cancer types. **Methods:** We developed a high throughput sequencing method for urinary cfDNA and applied it to the detection and surveillance of BLCA. Our approach combines an extraction protocol compatible with large fluid volumes, hybrid-capture based target enrichment with a 311 kilobase panel designed for BLCA, and an informatic pipeline that accommodates the wide distribution of fragment sizes found in urine. We used this approach to profile cfDNA in urine samples from 54 patients with early-stage BLCA (74% pTa, 6% pTis, 9% pT1, 11% pT2), 64 patients undergoing surveillance after treatment of localized BLCA, and 67 healthy adults. **Results:** We detected a median of 7 mutations per patient with 73% concordance between urine and tumor. Across both cohorts, the two most commonly mutated regions were the *TERT* and *PLEKHS1* promoters (74% and 46% of cases, respectively). In the cohort of patients with early-stage BLCA, the sensitivity of our method was 93% with an approach leveraging prior knowledge of mutations in each patient's tumor and 83% when blinded to tumor mutation status, compared to 14% for cytology performed on the same cases ($p < 0.0001$). In a cohort of patients undergoing surveillance for recurrence, the sensitivities of tumor mutation informed and blinded approaches, respectively, were 91% and 84% while the sensitivity of cytology was 38% ($p = 0.0001$). Concurrent cystoscopy and UroVysion results were available for a subset of patients in the surveillance cohort who developed recurrent disease, and cfDNA profiling outperformed the sensitivity of cystoscopy (34%, $p < 0.0001$, $n = 32$), the combination of cystoscopy and cytology (53%, $p = 0.006$, $n = 32$), and UroVysion (43%, $p = 0.02$, $n = 7$). Across both cohorts and methods, cfDNA profiling maintained high specificity (96-100%) and detected tumor DNA in 21/21 (100%) of cases that were positive by cytology and 54/66 (82%) of cases that cytology missed. **Conclusions:** We present a workflow optimized for urinary cfDNA profiling and apply it to samples from patients with BLCA. Our method significantly improves on the sensitivity of existing diagnostic modalities while maintaining high specificity. It could therefore facilitate the early detection and surveillance of BLCA through entirely noninvasive means.

ST142. Differential Detection of *BRAF* V600E and V600K Using a Simple and Sensitive Droplet Digital PCR Assay

L. Landry^{1,4}, L. Bellizzi², J. Bruce¹, V. Rojas-Rudilla¹, S. Diegue¹, A. Duchesne¹, Y. Kuang³, C.P. Paweletz³, N. Lindeman¹, L. Sholl¹

¹Brigham and Women's Hospital, Boston, MA; ²University of Connecticut, Storrs, CT; ³Dana Farber Institute, Boston, MA; ⁴Harvard Medical School, Boston, MA.

Introduction: The *BRAF* V600 codon is a well-characterized oncogenic hotspot and certain mutations at this position predict therapeutic response to RAF and MEK inhibitors in a variety of tumor types. *BRAF* V600E is the most common somatic variant in *BRAF*, accounting for > 90% of variations at this codon and has been shown to have diagnostic, prognostic, and therapeutic relevance in glioma, melanoma, hematologic malignancies, lung cancer, and colon cancer, among others. *BRAF* V600K is less common and represents ~ 10% of codon 600 activating mutations in melanoma and is therapeutically relevant in that context. *BRAF*-mutated patients, particularly melanoma, can have rapidly progressive disease that can respond rapidly to treatment. As such, rapid standalone *BRAF* testing can be critical to support urgent clinical treatment decisions. We developed and validated a droplet digital PCR (ddPCR) assay for simultaneous, differential detection of V600E/K mutations from tumor tissue specimens. **Methods:** ddPCR primers and probe (Applied Biosystems) were designed against nucleotide sequences encoding for *BRAF* V600 wild type and V600E. The V600E probe binds both the E and K substitution sequences but generates distinct post-PCR mutant signal amplitude. The validation study included 55 tumor samples with known V600E ($n = 21$), V600K ($n = 11$), other exon 15 non-V600E/K variants ($n = 9$) and wild-type ($n = 14$) based on pyrosequencing or next generation sequencing. Specimen

types included formalin-fixed paraffin embedded tissue, aspirate smears, ThinPrep, and fresh or frozen tissue. The tumor types included glioma, melanoma, histiocytic, lymphoid and myeloid neoplasms, and carcinomas of the colon, lung, thyroid, and miscellaneous sites. Purchased engineered heterozygous cell lines were used as controls (Horizon Discovery). ddPCR results were compared to previous known results. **Results:** Average signal amplitude for V600E was 9300 [range 7500-11000] vs. V600K 3300 [2500-4000] fluorescence units. Samples generated between 500-4000 total amplified events per replicate. The ddPCR results were 100% concordant with prior results. The assay achieved perfect inter-run and inter-operator agreement and was linear from 50% - 1% mutant allele fraction. Limit of detection was empirically determined to be >1 mutant event, however A conservative cutoff of >3 mutant events was established for reporting. **Conclusion:** This novel assay design permits highly accurate, sensitive and simultaneous, differential detection of two clinically important *BRAF* variants with a single primer/probe design. This assay may complement other more time consuming and expensive molecular methods such as NGS, particularly for critically ill patients.

TT060. Stat *EGFR* Mutation Detection in Fresh Lung Cancer Tissue Specimens Using Touch Preparation and the Idylla System

M. Al-Turkmani, E.J. Rizzo, S.N. Schutz, G.J. Tsongalis

Dartmouth-Hitchcock Medical Center and Geisel School of Medicine, Lebanon, NH.

Introduction: Accurate and timely genomic assessment of tumor tissues has become imperative for optimal therapeutic strategies for cancer patients. The Idylla system (Biocartis, Mechelen, Belgium) is a fully integrated, cartridge-based platform that provides automated sample processing (deparaffinization, tissue digestion and DNA extraction) and real-time PCR-based mutation detection with all reagents included in a single-use cartridge. Here we describe an approach for rapid somatic mutation detection in lung cancer cases using touch preparation and the Idylla system. **Methods:** Touch preparation samples were obtained from fifteen lung cancer tissue specimens in the pathology gross room shortly after resection. This involved making a single incision into the tumor body at room temperature using a scalpel blade and touching one 10 mm filter paper on each of the two sides of the inner tumor surface and holding it in position for approximately 3 seconds. The two filter papers were placed in an Idylla *EGFR* Mutation Assay cartridge (Research Use Only) and the cartridge was subsequently placed in the Idylla instrument for automated *EGFR* mutation analysis. The tumor tissue specimen was subsequently processed using standard pathology protocols for fixation, embedding and sectioning. Idylla results were compared against those obtained by subsequent somatic mutation analysis by next-generation sequencing (NGS) using the Ion AmpliSeq 50-gene Cancer Hotspot Panel v2 (Thermo Fisher Scientific).

Results: Idylla testing gave valid results for all 15 samples tested and revealed an *EGFR* L858R mutation in one sample and a 15-base pair exon 19 deletion in another sample. The remaining 13 samples showed no mutations in *EGFR*. Subsequent NGS analysis of eight of the 15 cases that met the institutional criteria for NGS testing showed complete concordance with Idylla results and confirmed the mutated or wild type status of *EGFR* as determined by the Idylla, with no other clinically actionable mutations detected. The total time for performing touch preparation and subsequent analysis by the Idylla was less than 3 hours for all samples, compared to an average turnaround time of 20.75 days (range: 14 - 30 days) for NGS testing. **Conclusions:** Combining touch preparation with the simplicity and sensitivity of the Idylla instrument provides a simple means to rapidly detect actionable somatic mutations in lung cancer tissue specimens after resection while preserving tumor tissue for subsequent processing and analysis. This approach provides fast turnaround times that allow timely management decisions for time-sensitive cancer cases while awaiting more comprehensive tumor genome profiling.