

NEXT GENERATION SEQUENCING OF DNA FROM URINE DETECTS MULTIPLE BLADDER TUMOR-DERIVED ALTERATIONS AND ADDITIONAL CHANGES THAT SUGGEST TUMOR HETEROGENEITY

Richard D. Abramson PhD¹, Gregory E. Alexander PhD¹, Ellen M. Beasley PhD¹, Francois Collin PhD¹, Michael Crager PhD¹, Andrew Dei Rossi BS¹, Joseph Dorado BS¹, Adam Friedman BS¹, Bill Gibb PhD¹, Jennie Jeong BS¹, Col Jones BS¹, Chin-Jen Ku PhD¹, Yan Ma PhD¹, John Morlan BS¹, Kunbin Qu PhD¹, Aibing Rao PhD¹, Aaron Scott BS¹, Haluk Tezcan MD¹, Neal Shore MD² and Phillip G. Febbo MD¹

¹Genomic Health, Inc., Redwood City, CA; ²Carolina Urologic Research Center, Myrtle Beach, SC

Introduction & Objectives: Noninvasive methods to detect circulating or urine tumor DNA (utDNA) are expected to transform the management of cancer patients. Given the importance of surveillance in management of non-muscle invasive bladder cancer (NMIBC), the ability of next generation sequencing (NGS) methods to identify the presence of utDNA was explored. A Genomic Health funded feasibility study using comprehensive NGS on 2 patients was performed to evaluate the value of global and individual markers for the detection of utDNA in clarified urine and urine sediment, focusing on detecting copy number aberrations (CNAs), differentially methylated regions (DMRs), and somatic single nucleotide variations (SNVs).

Methods: DNA was isolated from fixed paraffin embedded primary tumor, buffy coat cells, clarified urine and urine sediment from 2 patients diagnosed with Tis high grade NMIBC. One patient had a recurrence with multiple tumors 1.5 months after original resection and the other was newly diagnosed with a 4.4 cm lesion. CNAs and DMRs were detected using whole genome bisulfite sequencing (WGBS), and SNV by targeted-sequencing of 346 cancer-associated markers. Percent utDNA was estimated from the ratio (urine:tumor) of SNV, CNA or methylation sites compared to buffy coat.

Results: WGBS detected tumor specific CNA and methylation alterations in each primary tumor compared to buffy coat. By analyzing the genome using 100 Kb bins, we observed over 240 bins with detectable CNA signal. We observed over 75,000 CpG sites with differential methylation signal. Only one of the patient tumor samples had informative somatic SNV sites. CNA and DMR, and SNV for one patient, provided similar estimates of utDNA fraction within each patient, indicating around 50% in one patient and nearly 100% in the other. Estimates of tumor fraction were similar for clarified urine and sediment. Interestingly, measures for CNA and DMR allowed the detection of more aberrant regions than were detected in the sample obtained from the primary tumor.

Conclusions: Individual somatic SNVs in urine can be detected in recurrent disease, but one of two tumors was not informative for SNVs. Measuring and integrating CNAs or DMRs across the genome provided consistent estimates of utDNA fraction in urine. The ability to detect utDNA alterations not observed in primary tumor sample may ultimately provide insight into tumor heterogeneity, progression and response to therapy.