Topic: CTCs / CECs (circulating tumor cells/ circulating epithelial cells)

Title: Targeting VPAC Receptors on Cells Shed in Voided Urine to Image Prostate Cancer: A Novel Approach

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Introduction: Despite the advances in understanding of its genomic and molecular basis, early and accurate diagnosis of prostate cancer (PCa), remains challenging. Need is also unmet for screening PCa, staging it and to determine the effectiveness of its therapeutic interventions, noninvasively. Many cells shed from PCa lesions are excreted in voided urine. Robust literature demonstrates that VPAC receptors are expressed in high density on PCa cells but not on normal epithelial cells. Hypothesis was that PCa cells collected from voided urine can be detected optically by targeting VPAC receptors using a specific fluorophore, (TP4303), designed, synthesized and validated in our laboratory.

Method: Urine was collected as a standard of care from > 300 subjects presented to the Urology clinic with PCa, BPH (N=12) and with no history of PCa (N=40). With IRB “Exempt” status, discarded deidentified portion of urine was collected. In addition, with IRB approval, voided urine was collected from 18 patients with PCa (Gleason 3+3, 3+4) scheduled for PET scan followed by radical prostateectomy (Fig. 1). Cells from urine were collected on glass slides, stained with TP4303, and DAPI and observed under a confocal microscope. Normal cells appeared blue with nucleus and malignant cells had orange/red fluorescence around them.

Results: Sensitivity for detecting PCa was >98% and for BPH 100%. In 18 additional subjects, VPAC results were concordant with PET images and histology (Fig. 1). Blocking VPAC receptors on PC3 cells demonstrated VPAC receptor specificity. NFK3.1 immunohistochemistry assay confirmed prostate
origin for cells collected from voided urine. The PCa gene expression profiling studies depicted that the cells were malignant.

**Conclusion:** This simple, truly noninvasive and rapid assay can **i)** detect PCa with high sensitivity, **ii)** distinguish malignant from benign conditions, and **iii)** be developed for screening men for PCa, staging the disease and to determine noninvasively, the effectiveness of therapeutic interventions.