FUNCTIONAL ASSAYS OF DRUG-TARGET ENGAGEMENT ON CIRCULATING TUMOR CELLS CAPTURED FROM PATIENT BLOOD CORRELATE WITH PATIENT PROGRESSION

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ABSTRACT
Chemotherapeutic treatment is limited by the heterogeneous and short-lived efficacy of therapy. Functional assays of tubulin bundling on microchip-captured circulating tumor cells (CTCs) in late-stage castrate-resistance prostate cancer are presented for the first time, and results are interpreted in the context of patient progression. Anti-PSMA capture leads to high capture rates of cells that remain functionally responsive to chemotherapies. These results point to the potential for microfluidically captured CTCs to inform clinical care in this patient population.

KEYWORDS: Circulating tumor cell, CTC, clinical diagnostics, cancer, chemotherapy, microfluidics, antibody, J591, PSMA

INTRODUCTION
Clinical design of chemotherapeutic treatment is limited by the heterogeneous and short-lived efficacy of both hormonal therapy (for hormone-responsive cancers) and chemotherapy (for late-stage metastatic disease). While genetic profiling informs some treatment decisions, the efficacy of systemic chemotherapies cannot at present be predicted by molecular marker. Circulating tumor cells provide the opportunity to assay drug efficacy ex-vivo and inform clinical decision. This work shows the ability to monitor tubulin stabilization by taxane chemotherapeutics in microfluidically captured patient circulating tumor cells.

MATERIALS & METHODS
Circulating tumor cells in castrate-resistant prostate cancer patients were isolated from 1 mL of peripheral blood by use of geometrically enhanced differential immunocapture (GEDI) microdevices [1] functionalized with the J591 monoclonal anti-PSMA (prostate-specific membrane antigen) antibodies[2] and designed to preferentially capture cells larger than leukocytes. Same-day blood draws were also analyzed by Veridex CellSearch. Healthy donor blood samples were processed under blinded protocol as negative controls. Captured cells were stained for DAPI, PSMA, CD45, EpCAM, CD95, and tubulin. Circulating tumor cells (DAPI+/PSMA+/CD45−) were enumerated and functional engagement of paclitaxel and docetaxel on CTC microtubules was measured by scoring tubulin bundling in immunofluorescence images. Extracted RNA was processed and Sanger-sequenced for known mutations. ERG upregulation was used to identify TMPRSS2:ERG fusion status.

RESULTS & DISCUSSION
Microfluidic devices previously shown capable of capturing circulating tumor cells in patients [1] at high efficiency and purity are here (a) validated with comparisons to commercial devices with patient samples, (b) evaluated with healthy controls, (c) used to demonstrate for the first time characterization of drug-target engagement ex-vivo in patient cells, and (d) used to identify single-nucleotide polymorphisms and gene fusions in captured cell line models.

Staining and enumeration in patient samples show cell counts well above CellSearch [3] (n=18; Fig 1a); these cell counts are somewhat correlated but differ greatly, presumably owing to differences in EpCAM and PSMA expression in CTCs from patient to patient. Tests with healthy donor blood (n=12) give low cell counts, confirming low false positives (Fig 1b). Patient response to taxane chemotherapy, typically monitored by tumor burden or serum levels of prostate-specific antigen (PSA), can be correlated to single-cell assays on captured cells on chip (Fig 1c). Patients responding clinically to paclitaxel (measured by blood PSA levels) have CTCs that show tubulin bundling at clinically relevant dosages (Fig 1d; n=4). Response is drug-dependent: patients responding to paclitaxel but not to docetaxel show drug-specific tubulin bundling (Fig 2).

Sanger sequencing (Fig 3a) of captured cells from a sample with 50 C4-2 cells spiked into 1 ml of healthy whole blood demonstrates that the T857A (ACT-GCT; Thr-Ala) mutant AR form [4] is dominant among the cDNA from captured cells. Captured cells with TMPRSS2:ERG fusion [5] exhibit heterogeneous, but elevated ERG levels that can be measured with immunofluorescence (Fig 3b), consistent with TMPRSS2:ERG fusion in these cells. vCaP cells captured on chip were distinguished from fragments or leukocytes by DAPI+/CD45− staining and intact morphology.
The presented results show unprecedented capability to evaluate drug-target engagement and correlate with patient clinical response.

**Figure 1:** Circulating tumor cell assays in cells captured on chip: (a) staining and enumeration shows cell counts well above CellSearch and identifies correlations between PSMA and EpCAM capture; (b) GEDI counts are specific to disease state, (c) single-cell assays show cell-to-cell variation in tubulin response to taxanes: responsive CTC (magenta), non-responding CTC (white), responding leukocyte (yellow); (d) patients responding clinically to paclitaxel CTCs that show tubulin bundling at dosages matching serum drug levels 1hr and 24hrs after treatment.

**Figure 2:** Captured CTCs highlight drug-specific response: patient that has failed docetaxel shows diffuse, native tubulin (top) when cells are exposed ex-vivo to docetaxel but highly bundled tubulin (bottom) when cells are exposed ex-vivo to paclitaxel.
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[4] Han+ PNAS 2004

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Figure 3: Characterization of SNPs and fusion status in chip-captured cells. Sanger sequencing of captured cells. (b) immunofluorescence for TMPRSS2:ERG fusion status as indicated by ERG upregulation.