Label free isolation of circulating tumor cells from castration-resistant prostate cancer patients discovers a novel androgen receptor transcriptional variant as a potential prognosis biomarker

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Zhigang Kang[1,3], Avani Shah[2], Yunkai Yu[1] , Ali Asgar Bhagat[4], Kyra Zhao[4], Andrew Wu[4], James Gao[1], Yuelin Zhu[1], Ravi Madan[2], James Gulley[2], Paul Meltzer[1], William Dahut[2], Liang Cao[1].

[1]Genetics Branch, [2]Medical Oncology Branch, Center for Cancer Research , National Cancer Institute, Bethesda, Maryland; [3]Basic Science Program, Leidos Biomedical Research, Inc, Frederick, Maryalnd; [4]Clearbridge Biomedics Pte Ltd, Singapore

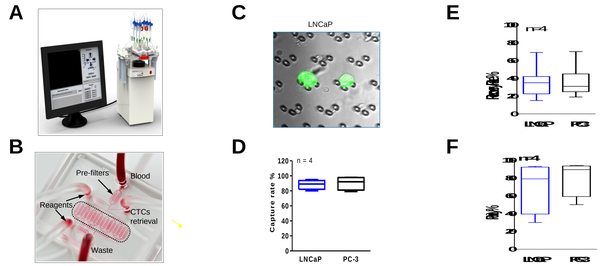
Background: Circulation tumor cells (CTCs) are critically important to understand tumor metastasis and may have a potential to noninvasively evaluate the cancer for clinical management. The isolation and characterization of CTCs represent a major technological challenge. While the CellSearch system by Janssen has shown that CTC enumeration can be an independent predictor of survival for breast, prostate and colon cancer, limited molecular information can be obtained from the CTC to have an impact on patient management or clinical outcome. Most of the hormone deprivation therapy responsive prostate cancers will eventually turn into castration resistant, many through the amplifications, mutations, or alternative transcripts of the androgen receptor (AR) gene. As multiple new AR pathway-targeted agents become available for this cancer and the difficulty of obtaining tumor material, there is an urgent need to be able to evaluate circulating biomarkers of CRPC for clinical management of the patients.

Objective: In order to assess biomarkers that may influence the management of CRPC, we worked on a novel antibody-free microfluidic chip CTC purification technology to isolate live CTCs from whole blood of CRPC patients for molecular marker analysis.

Results: Applying this novel microfluidic chip CTC technology, we are able to capture and isolate different types of spiked cancer cells with high recovery rate and purity from normal donor blood. We also designed and validated TaqMan assays include universal epithelial tumor molecular markers, normal blood cell markers and prostate cancer specific markers for CTC molecular profiling with single cell sensitivity. Our results of clinical study showed that 73% CRPC patients positive for EpCAM, and only about half of the EpCAM-positive CRPC cases were PSA or AR positive. Our results showed that positive detection of AR or PSA, but not EpCAM is statistically associated with poor prognosis of the CRPC patients. It is interesting that while there is a strong association between AR and PSA expression in CTC samples, there is no correlation between AR or PSA transcripts in CTCs and serum PSA protein levels in CRPC patients. Very intriguingly, we identified a novel androgen receptor transcript variant AR_v which is significantly associated with worse survival of CRPC patients ($p<0.0001$). This ligand-independent constitutive activity of AR_v suggest this AR variant might contribute to the late stage progression of the diseases and could be used as a potential indicator for alternative therapies.

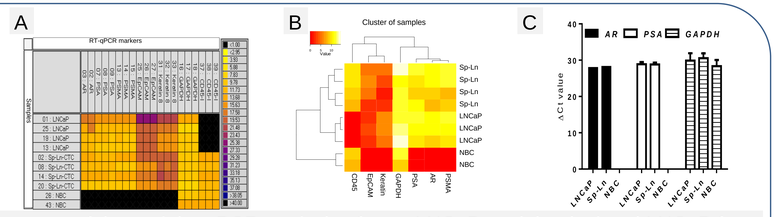
Conclusions: In summary, this new microfluidic device is capable of isolating CTC to enable molecular marker analysis, which may provide valuable information in prognosis and a potential marker for drug resistance of CRPC patients treated with AR targeted agents.

Microfluidics Instrument for Tumor Cell Isolation from Whole Blood



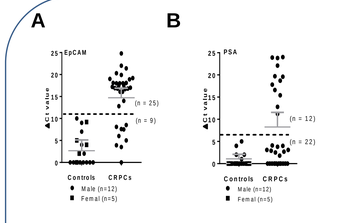
A. photograph of a computer-controlled micro-fluidics instrument for CTC isolation. B, a CTC isolation microchip has pre-filter and 5 ports. C, images of spiked LNCaP cells on CTChip. D. capture efficiency, E. recovery efficiency, F. purity of retrieval CTC, of the spiked prostate cancer cells in 4 independent experiments.

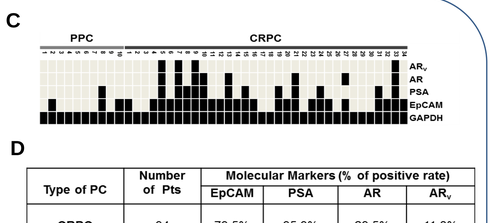
Results of Preclinical Validation Studies



A. Heatmap of the real time PCR analysis of pure LNCaP, and that isolated LNCaP from spiked (Sp-Ln-CTC) or non-spiked blood (NBC). B. Hierarchical clustering based on molecular profiling of the samples. C. Bar chart showing the average transcript levels of AR, PSA and GAPDH in these samples.

Detection of Gene Specific Transcripts from CTCs of Prostate Cancer Patients





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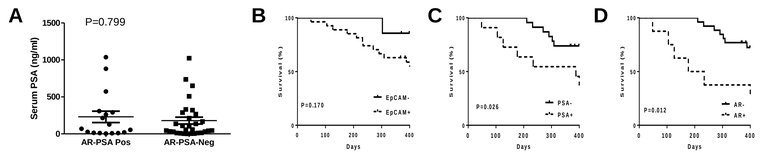
Type of PC	Number of Pts	Molecular Markers (% of positive rate)			
		EpCAM	PSA	AR	AR_v
CRPC	34	73.5%	35.3%	23.5%	11.8%

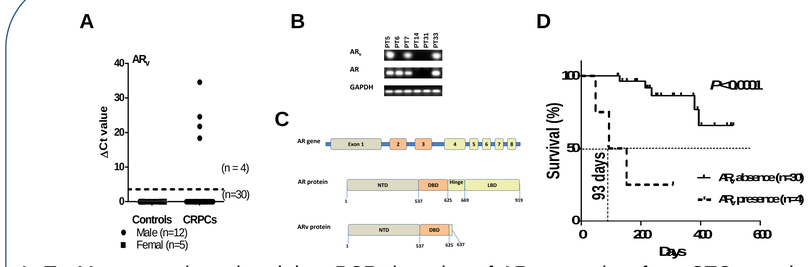
Transcript levels of EpCAM (A) and PSA (B) were quantified in healthy donors and CRPC patient CTCs. C. Summary of molecular profiling of CTCs from primary prostate cancer (PPC) and castration resistant prostate cancer (CRPC) patients. D, The positive rates of AR , PSA , EpCAM and ARv transcripts in the CRPC patients.

CTC Transcripts and Clinical Association



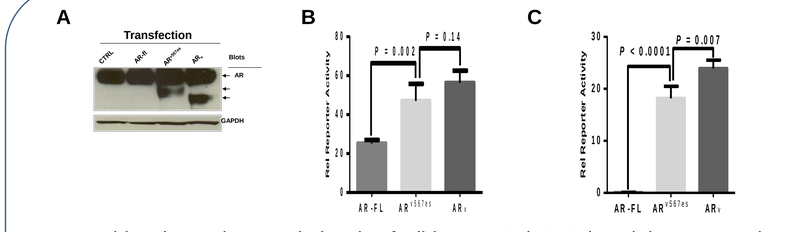
A. Lack of correlation between the transcript levels of AR and PSA of CTCs and PSA protein levels in the patient serum. B. Lack of association between EpCAM transcripts from CTCs and CRPC patients' survivals. C. Association of PSA transcripts from CTCs and survival. D. Association of AR transcripts from CTC and CRPC patient survivals.

Identification of an AR Variant (ARv) and Its Association with Poor Prognosis



A. TaqMan assay based real-time PCR detection of AR_v transcripts from CTC samples. B. AR_v is confirmed by PCR amplification C. Schematic diagram of exon structure and function domains of wild type androgen receptor in comparison with the truncated protein domains of the novel ARv (bottom). D. AR_v positive is associated with poor overall survive of CRPC patients ($p<0.0001$, by Log-rank test).

Constitutively Transcriptional Activity of ARv



A. Immunoblot shows the protein levels of wild type AR (AR-FL) and the truncated novel AR_v transiently expressed in LNCaP. AR^{v567es} is previously identified AR variant. B. Transactivation activity with an AR promoter reporter using luciferase assay in the presence of 10 nM of testosterone. C. Ligand-independent transactivation activity of AR_v in charcoal-stripped FBS medium that removed AR ligands.