

Utilizing Circulating Tumor Cells To Identify Molecular Targets And To Optimize Drug Selection And Treatment In Patients With Hormone Refractory Prostate Cancer

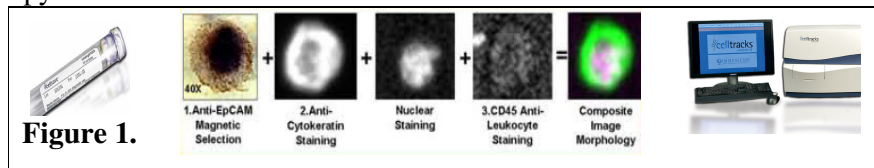
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Introduction

Prostate Cancer is now the most common cancer in men in Europe and the second most common cause of male cancer mortality, with a median survival of approximately 18 months for men with metastatic hormone resistant disease.¹ The molecular etiology of hormone resistance remains poorly understood² with preclinical models of prostate cancer proving to be poorly predictive of the behavior of this disease in patients. Rational drug development for this disease has therefore been hindered by difficulty in obtaining tumor tissue. The recent development of a new and emerging technology, allowing the isolation, separation and visualization of prostate cancer cells from the blood of HRPC patients, may support the molecular characterization of this disease and help in the identification of key molecular targets for drug treatment. Target evaluation on CTC can then assist in the optimization of dose and schedule selection for individual patients.

Background

The Study of CTC has proved to be very difficult until the development of the CellTracks® System, recently developed by Immunicon Corporation and available in our laboratories. This is a high-throughput and fully automated system that can isolate and enumerate CTCs as is described by Allard et al.³ In essence this involves the combination of immunocytochemistry, immunomagnetic enrichment, flow cytometry and automatized four-color immunofluorescence microscopy. CTC are not detected in healthy subjects or patients with non-malignant diseases³. CTC has been detected most frequently in patients with advanced prostate cancer. Studies in metastatic breast cancer (MBC) and prostate cancer have shown that CTC counts predict prognosis, and changes in CTC counts can predict response to therapy.⁴ In the same vein, preliminary studies suggest that CTC counts are predictive of survival in HRPC and can predict response to therapy for this disease.⁵



CTC count and dynamics

I have played a major role in the construction of a database documenting changes in CTC counts in 124 HRPC patients who have taken part in a range of clinical trials, alongside their baseline characteristics and other parameters including survival times

and well-defined response/progression as defined by RECIST and PSAWG criteria. We are currently analyzing these data and performing correlative analyses.

Pharmacodynamic (PD) markers in CTC

The conduct of pharmacodynamic studies on tumor cells in HRPC has been extremely difficult, due to limited access to easily and safely biopsiable metastatic disease. Nonetheless, these studies remain key in basing recommendations of optimal dose and schedule for the treatment of this disease, as well as the selection of patient subgroups that could derive clinical benefit from treatment. Further studies to evaluate the utility of CTC in patients with HRPC are now warranted. CellTracks® System permits the use of a fourth immunofluorescence antibody, which could be used for research purposes.

Thus, I am developing the use of PD markers established in other cancer cell lines in CTCs. In the development of each PD marker, I have used the following method: Qualitative and quantitative expression assessment of the target protein or phosphorylated protein, by immunofluorescence microscopy and flow cytometry respectively. This step serves a two-fold purpose: Firstly, a quantitative assessment of the basal and drug-induced changes in the target expression; and secondly, to find a guiding antibody concentration for subsequent use on CellTracks® System. A variable volume of suspension from the different studied, treated and non-treated, cancer cell lines is used to contaminate a healthy donor blood into a CellSave® tube. Subsequently, these samples are run on CellTracks® System, using different concentrations of the research antibody against the target marker. I have used this method for the following markers development with the different results:

(a) Phospho-Histone H3 as mitosis arrest marker for antimetabolic agents.

Taxanes and other antimetabolic agents have been widely used in HRPC treatment, although to date, only docetaxel has been proven to improve patient survival in randomized trials. These drugs cause cell-cycle arrest during mitosis (M-phase). There has hitherto been no good marker available to detect cells in M-phase. Histone H3 becomes phosphorylated on serine 10 when the chromosomes condense during prophase and remains phosphorylated until telophase, at which time it becomes dephosphorylated by specific phosphatases.⁶⁻⁷ When we induced mitosis arrest, Histone H3 remains phosphorylated. Recently, phospho-Histone H3 staining has shown to be a simple and reliable method for quantifying proliferative potential⁸ and as a mitosis marker. Furthermore, inability to induce mitosis arrest has been recently described in cancer cell lines resistant to Taxanes.⁹ Therefore, phospho-Histone H3 in CTCs may be a useful pharmacodynamic marker following treatment with taxanes and other antimetabolics such as aurora kinase and polo-like kinase inhibitors. My preliminary laboratory studies with blood spiked with epithelial cancer cells indicate that phospho-histone H3 can be detected in CTC after cell exposure to antimetabolics. From preliminary work that I have done, the expression level of phospho-histone H3 has shown to be low in untreated HRPC cell lines (PC3 and NCI-660), but this increases up to 30-50% after exposition to docetaxel concentrations from IC₂₀ to IC₅₀ for 6-24 hours (unpublished data).

Recently, we have started to use this marker in the clinical setting. Firstly, we started an exploratory protocol, with a two-stage design, in HRPC patients who are to be treated with docetaxel. In this protocol we are looking for changes of phospho-Histone H3 expression in CTCs at different times between baseline (pre-1st dose) until 7 days post-1st dose. Our primary objective is to detect an increase greater than 30% in the levels of phospho histone H3 expression after drug-exposure. We also aim to correlate these changes with clinical response and outcome. Unfortunately, this exploratory protocol is in its initial phase of recruitment. Secondly, we have planned to use this marker in CTC from breast cancer and HRPC patients included in phase-I trials, which involve the use of small-molecules targeted against mitosis-related tyrosine and/or serine-threonine kinases.

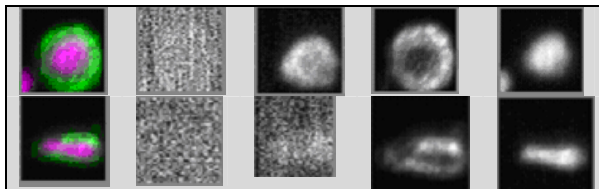


Figure 2. Positive and negative expression of phospho-Histone H3 on CTC from an HRPC patient. From left to right: Composite image, CD45 (negative for both), pHH3 (positive for first), pan-CK (positive for both), and DAPI (nucleus).

(b) Markers for PI3 kinase inhibitors. Activated phosphoinositide 3-kinase (PI3K) and its downstream target Akt/PKB are important signaling molecules and key survival factors involved in the control of cell proliferation, apoptosis and oncogenesis.¹⁰ I have developed the use of phospho-AKT and phospho-RAS40 as markers of PI3K inhibition in CTC, using different prostate, breast and ovarian cancer cell lines. However, the expression levels of these markers are very low and have limited roles as PD markers in this setting (unpublished data). Based on the expertise I have gained from the refinement of this technique, I am collaborating with other groups from the Institute of Cancer Research (in partnership with the Royal Marsden Hospital) to examine other promising PD markers.

Molecular characterization studies in isolated CTC

Molecular characterization studies on isolated CTC utilizing FISH and ICC are currently underway.¹¹⁻¹² I have collected samples from 30 patients who have just progressed to different treatments for HRPC disease. This sample has been analyzed for CTC enumeration, which I am also evaluating by FISH, for the presence of the TMPRSS2/ETS gene translocations (ERG, ETV1 and ETV4), androgen receptor (AR) amplification and pTEN loss. All of them have been correlated with resistance to treatment in HRPC setting. Feasibility studies to evaluate whether CTC DNA and mRNA can be amplified to evaluate mutated AR status will also be pursued in the future.

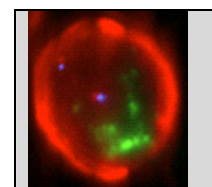


Figure 3. FISH on CTC. Blue probe against ERG and red probe against TMPRSS2 (2 copies each), green probe against AR (>2 copies amplification).

Increasing yield of isolated CTCs from individual patients

Another major problem in the evaluation of novel agents for the treatment of this disease is the lack of measurable and safely-biopsiable disease. Moreover, the CTC count isolated in these patients are often low. Hence, the lack of tumor tissue remains a major obstacle in the conducting of prostate cancer research.

We are currently developing the concept of **“liquid biopsy” on solid tumors** in HRPC patients. We have conducted preliminary experiments, combining density gradient separation of blood layers (using Lymph prep®) and Cell Tracks® System, which have shown that more than 70% of CTCs in HRPC patients could be isolated from the mononuclear layer. In light of this finding, we have designed an exploratory protocol to increase the yield of CTCs, at minimal clinical risks to these patients. We have submitted our proposal to the hospital ethics and research committees and the outcomes of their reviews are pending.

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