

**TO EVALUATE THE UTILITY OF CTCs COUNT and THE MOLECULAR CHARACTERIZATION OF CTCs IN THE TREATMENT OF ACASTRATION RESISTANT PROSTATE CANCER PATIENTS and ADVANCED CANCER WITH MOLECULARLY TARGETED DRUGS.**

**INTRODUCTION**

Over the last two decades, considerable progress has been achieved in the management of cancer with the implementation and the use of molecular targeted treatments (MTT). They have proven particularly efficient in the treatment of GIST, metastatic kidney, breast, colon, and lung carcinoma as well as chronic myeloid leukemia (1). More than 500 compounds are in development in phase 1 studies. Inherent to the development of molecularly targeted drugs is the selection of patients for treatment with these agents based on predictive biomarkers. New predictive biomarkers are clearly urgently necessary in early clinical drug development.

Prostate cancer is the commonest malignancy in western societies and the second most common cause of male cancer-related deaths in the UK, accounting for approximately 13% of deaths and over 10,000 deaths per year (2). Death from this disease is invariably due to resistance to androgen deprivation therapy in incurable metastatic disease. Poor preclinical models and difficulty acquiring CRPC tissue have hindered the study of the molecular aetiology of resistance. This has limited the development of novel therapies for this disease (3), with only docetaxel being shown to modestly improve survival since the identification of therapeutic castration by Charles Huggins in 1941 (4). This has limited the development of novel therapies for this disease.

Preliminary studies now indicate that CTC represent an extremely promising source of tumour tissue for biomarker studies and that CTC counts also have prognostic utility and have promise to potentially be an intermediate endpoint of outcome, with preliminary studies in breast cancer suggesting that this may even be superior to radiological evaluation, predicting response to treatment and outcome (5). This analysis is feasible due to advances in technology including the CellTracks® instrument from Immunicon/Veridex as well as filtration and microfluidic chip devices that allow the automated and high-throughput separation, visualisation and quantitation of cancer cells from blood (7.5 ml samples)(5). Importantly, these technologies allow molecular characterization of CTC by, for example, immunofluorescence (IF) for protein expression; fluorescent in-situ hybridization (FISH) for DNA amplification; and DNA/RNA sequencing of for example AR and the detection of TMPRSS2/ETS gene translocations (6).

## **CTC Counts in Phase I trials**

Phase I clinical trials play a crucial role for the development of innovative anticancer drugs. Although demonstration of a therapeutic effect is not a primary objective of phase I studies, most cancer patients and medical oncologists expect benefit from trial participation. In a recent meta-analysis, the authors reviewed phase I oncology trials which were sponsored by the Cancer Therapy Evaluation Program between 1991 and 2002. Interestingly, instead of restricting themselves to conventional criteria of objective response, they also assessed the data on stable disease. The overall response rate and the stable disease rate were 10.6% and 34.1% respectively, suggesting a higher benefit from phase I oncology trials than previously reported (7). In the era of targeted molecular therapy classical RECIST evaluation of response should no longer be sufficient in early clinical trials. Biomarkers of anticancer drug activity have been widely advocated in the literature as intermediate endpoints for these novel agents and provide a powerful approach to investigate various types of biomarkers during the early phase of drug development.

### Retrospective analysis of patients enrolled in phase I with CTC evaluation (2007-2009)

Using the CellSearch® System patients enrolled in Phase I trials at the Royal Marsden Hospital (more than 150 pts) have had CTC count evaluated during phase 1 trials.

I have played a major role in the design of a retrospective analysis of the prognostic utility of CTC counts in epithelial cancer patients participating in phase 1 trials. The aim of this project is to characterize patients enrolled in phase 1 (Clinical characteristics: age, sex, number of metastatic sites, LDH, PS...), define outcome in phase 1 (correlation with RECIST criteria, and PFS), and to correlate CTC evaluation and tumor types, prognosis and outcome in phase

## **CTC Isolation Technologies: Counting and Molecular Characterization of CTC**

### **Comparison of the CellSearch System and Ikonysis**

Different CTC isolation methods are available, each having different advantages and disadvantages. CTC detection can be based on slide-based methods (immunocytochemistry or FISH), molecular analysis (RT PCR) or flow-based cell sorting methods after immunofluorescent identification and immunomagnetic labelling of cells (anti-Ep-CAM and CK against CTC and antiCD45 against leukocytes)(8). At the present time, the CellSearch System (Veridex®) is the only method that has obtained approval for monitoring metastatic breast, colorectal and prostate cancer. Recent studies indicate that CTC counting is a biomarker predictive for clinical outcome in patients with disseminated and localized breast cancer as demonstrated for breast, colorectal and prostate cancers.

An alternative method for CTC analysis uses filters with pores that retain the large tumour cells but not the smaller blood cells (The Metagenex® system, Paris, France or Ikonysis System). These techniques allow CTC counting and CTC isolation for cytomorphological and molecular characterisation (FISH). This method is independent of CTC epithelial marker expression and allows the enrichment of epithelial and non-epithelial circulating tumour cells. I am currently comparing the CellSearch System system which counts exclusively EpCAM+ CTC counts and the Ikonysis filtration system which counts EpCAM + and EpCAM- CTC.

### **Molecular characterization**

I am also playing an important role in the development of by, for example, immunofluorescence (IF) for protein expression; fluorescent in-situ hybridization (FISH) for DNA amplification in CTC isolated from new technologies (ISET and Ikonysis system).

We have collected sample from 50 patients who have just progressed on the novel prostate anti-cancer agent Abiraterone. These samples have been analyzed for CTC enumeration, but are also being evaluated by FISH for the presence of the TMPRSS2/ETS gene translocation, androgen receptor, PTEN loss and MYC amplification. All of them have been correlated with resistance to treatment and prognosis.

## **Androgen receptor and Oncogene sequencing**

Functional androgen receptor (AR) signaling is necessary for the development of prostate cancer. It has become clear that AR expression and signaling remains intact as the disease evolves from androgen-sensitive cancer to classically (but perhaps inaccurately) termed hormone refractory prostate cancer. AR gene amplification has been reported in 25–30% of patients with HRPC but is present at very low rates (1–2%) in those with primary prostate cancer, indicating that AR amplification is involved in the development of HRPC. AR gene amplification was associated with an increased mRNA expression and augmented levels of AR protein. Point mutation of the AR can result in altered ligand specificity such that mutated ARs can be activated by non-androgenic ligands such as anti-androgens. The first mutated AR was cloned from the LNCaP human prostate adenocarcinoma cell line (9). All AR mutations that have been associated with human diseases, including prostate cancer, have been recorded in a database (10). Moreover, several molecular biomarkers, such as oncogenic mutations, have been shown to be predictive and prognostic in response or survival in cancer patients.

A recent technology, The Sequenom MassARRAY is a Mass Spectrometry based system for quantitative and qualitative genomic analysis. Sequenom have recently released the OncoCarta™ panel: a set of multiplexed genotyping assays, which can analyze 238 mutations across 19 oncogenes (ABL-1, AKT-1,2, BRAF, CDK-4, EGFR, ERBB2, FGFR-1,3, FLT-3, JAK-2, KIT, MET, H-RAS, K-RAS, N-RAS, PDGFa, PIK3CA, RET). The mutation frequency cutoff is 10%, so we can detect rare mutations. We have design a specific assay for AR mutations.

I have played a major role in collecting and processing blood and tumor tissues from patients with CRPC. The aim of this study is to detect oncogenic mutations in plasma or circulating tumor cells, from patients enrolled to different clinical trials.

#### References

1-Krause DS et al, N Engl J Med 2005 ;353 :172-87.

2-<http://www.cancerresearchuk.org/aboutcancer/statistics/mortality>

3-Attard G et al, British Journal of Cancer, 95: 767-774, 2006.

4-Tannock I et al, New England Journal of Medicine, 351:1502-12, 2004.

5-Cristofanilli M et al. New England Journal of Medicine. 351, 781-791, 2004.

6-Attard G et al, Cancer Res. 2009 Apr 1;69(7):2912-8.

7-Horstmann E et al, N Engl J Med. 2005 Mar 3;352(9):895-904.

8-Pantel K et al, Nat Rev Clin Oncol. 2009 Jun;6(6):339-51. Review.

9-Taplin ME et al, Nat Clin Pract Oncol. 2007 Apr;4(4):236-44. Review

10-Gottlieb B et al, Hum Mutat. 2004 Jun;23(6):527-33.