Expression Profiling of Circulating Tumor Cells: a Prognostic and Predictive Biomarker in Cancer.

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ABSTRACT

We have developed a strategy to predict response to treatment of cancer patients based on expression profiling of **circulating tumour cells (CTCs)**. CTCs are collected from blood using AdnaGen immunomagnetic capture followed by expression profiling with TATAA Biocenter GrandPerformance assays via preamplification. Preliminary results from a pilot study on breast cancer (BC) patients demonstrates excellent sensitivity, technical reproducibility and identifies a set of genes that separates a group of non-responders. The approach shows great promise as liquid biopsy for the motoring of treatments and prediction of responses.

INTRODUCTION

The increasing number of treatment options for patients with metastatic cancer has created an accompanying need for biomarkers to determine if the tumour will be responsive to the intended therapy, to monitor drug efficacy and to anticipate emergent drug resistance. Ideally, these biomarkers would be obtained by minimally invasive means to allow serial sampling, to enable quantitative real-time molecular analyses of tumour heterogeneity and evolution as well as drug responsiveness. Identification and characterization of CTCs shed into the blood may satisfy this need and are commonly referred to as "liquid biopsies". The CTCs may have different characters and enumeration only is insufficient for reliable monitoring and prediction. The CTCs expression reveals their character and may potentially be used to predict treatment response.

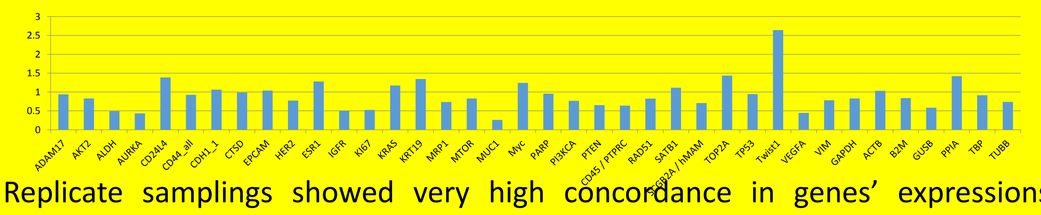
STUDY DESIGN

Blood samples were collected from:

- 39 breast cancer patients before and after treatment (paired study)
- 20 healthy controls

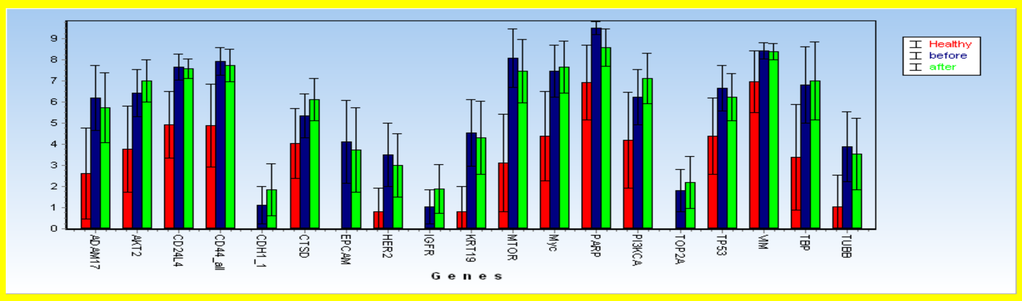
All samples were collected in duplicates to validate the technical reproducibility of our approach.

TECHNICAL PERFORMANCE

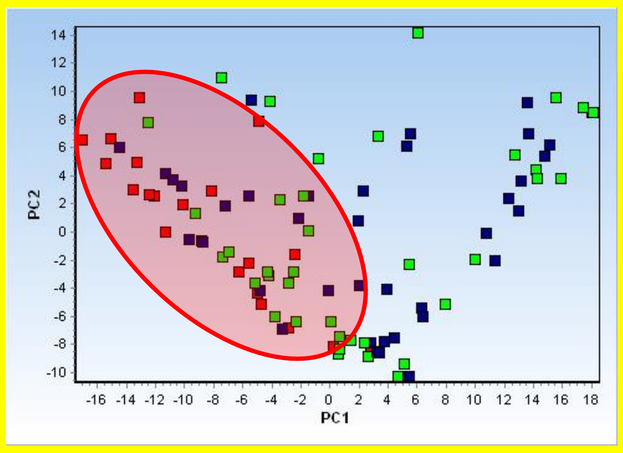


Replicate samplings showed very high concordance in genes' expressions evidencing exceedingly high reproducibility.

HEALTHY VS. BC

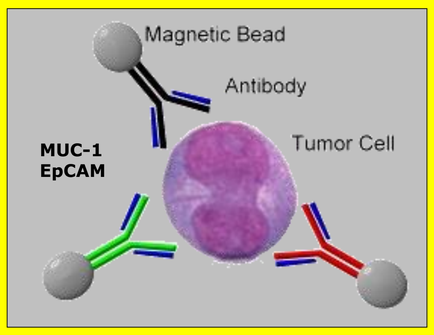


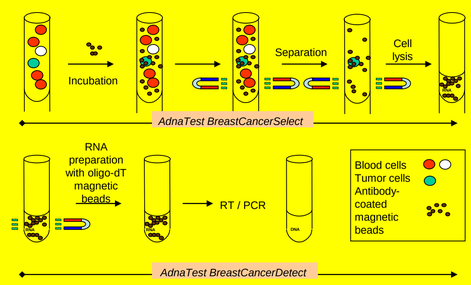
Selected markers are all more abundant in BC positive samples.



Negative samples cluster in Principal Component Analysis (PCA) using GenEx (MultiD Analysis) and differentiate from the majority of BC samples. Before treatment, After treatment, Healthy Subjects.

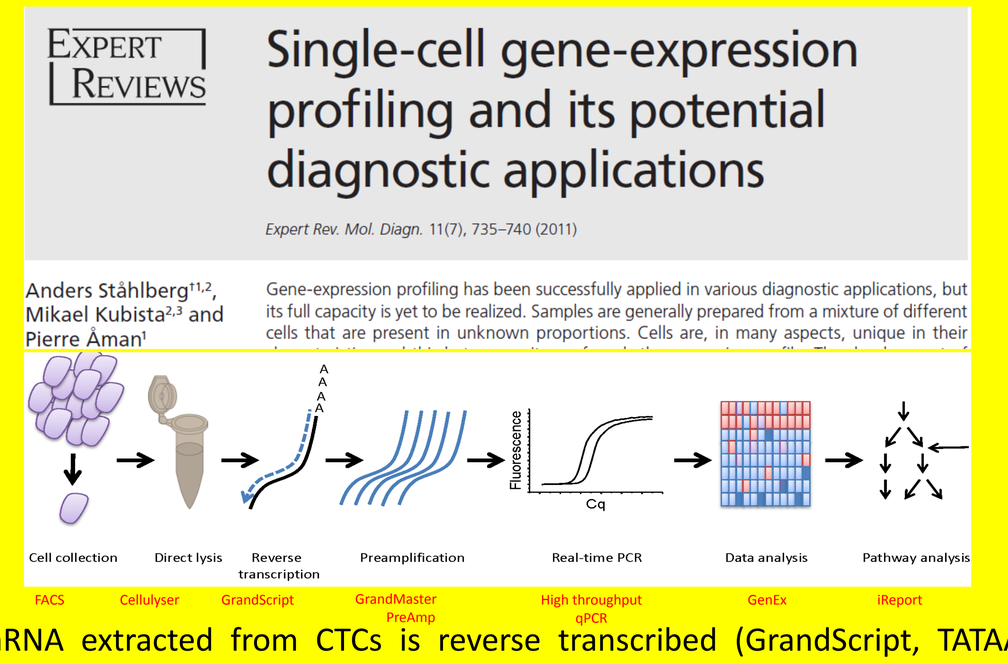
Immunomagnetic Enrichment of CTCs





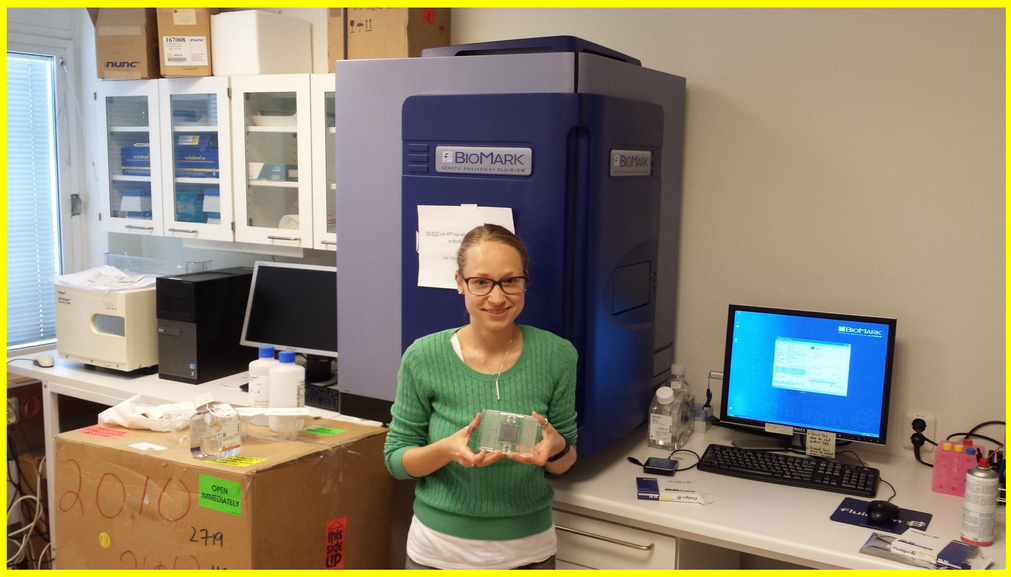
7 ml of blood is collected and enriched for CTCs using antibody-coated magnetic particles (AdnaTest Select, AdnaGen). Multiple antibodies are used that bind with high specificity and affinity the corresponding cancer cells. The enriched cells are purified and lysed so that the relevant tumor cell information exists in the form of mRNA.

EXPRESSION PROFILING WORKFLOW



mRNA extracted from CTCs is reverse transcribed (GrandScript, TATAA Biocenter), preamplified (GrandMaster, TATAA Biocenter) and profiled for selected markers using TATAA GrandPerformance assays.

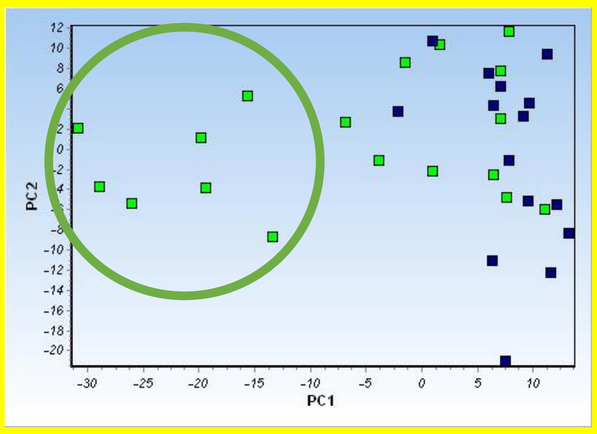
HIGH THROUGHPUT EXPRESSION PROFILING



Expression of up to 96 markers in up to 96 samples, including ValidPrime (TATAA Biocenter) to correct for genomic background and IPC (TATAA Biocenter) to normalize for variations between runs, were measured per run in the microfluidic BioMark qPCR platform (Fluidigm).

RESPONDERS VS. NON-RESPONDERS

A distinct group of non-responders separates, and can reliably be identified based on CTC expression profiles.



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