# Detection and molecular characterization of EpCAM positive and EpCAM negative circulating tumour cells isolated from SCLC patients using an epitope independent platform

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## Background

Personalised medicine relies on a detailed understanding of the molecular basis of disease in an individual patient that can subsequently be used to follow-up with a tailored course of treatment based on the presence of specific disease biomarkers


*Figure 1.* ***Stratification of patients in a personalised therapy approach (taken from Heertum et al, 2016)***

Small cell lung cancer (SCLC), an extremely aggressive disease, due to the small size and localisation of the tumors, as well as the co-morbidity of the methods of tissue collection, intralesional bioptic sampling is rarely tolerable and not feasible

Liquid biopsies, such as circulating tumour cells (CTCs), can be an alternative for standard procedures, and provide an option to determine the genetic profile of cancer patients


*Figure 2.* ***Isolation of CTCs***

The gold standard for enrichment of these rare cells is CellSearch®, an epitope dependent system that positively selects EpCAM expressing tumour cells

To fully realise the clinical potential of CTCs as a liquid biopsy there is a requirement to establish a robust pipeline of isolation and storage of CTCs that will facilitate retrospective as well as prospective analysis

The evaluation of the cells enriched by epitope independent devices, such as Parsortix (Chudziak, 2015), is important to identify and characterise the range of CTC phenotypes present in SCLC patients

## CTC Isolation and Storage

Blood samples from SCLC patients have been collected and CTC enriched by CellSearch® followed by isolation and/or storage

A pipeline has been developed to maximise the availability of CTCs for molecular analysis based on the CTC burden of patients

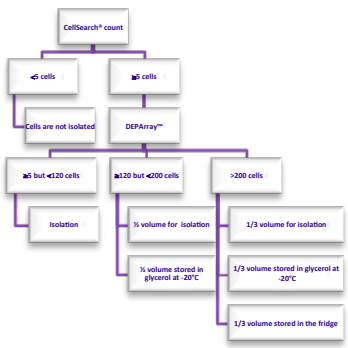


*Figure 3.* ***Workflow established for isolation of CellSearch® CTCs enriched samples***

Cells showing positive staining for pan-cytokeratin (CK), undetectable CD45 labelling and positive nuclear staining are classified as CTCs; corresponding white blood cells (WBCs), i.e cells staining as CK-/CD45+ and positive nuclear staining were also isolated as germline controls

## Molecular analysis of CTCs

Individual CTCs, pools of CTCs and control WBCs were subjected to whole genome amplification (WGA) and its efficacy was evaluated by multiplex PCR to determine the Genome Integrity Index (GII) of each sample

Low pass whole genome sequencing (WGS) was used to establish the genome wide copy number alteration (CNA) (0.1 to 0.2x coverage)

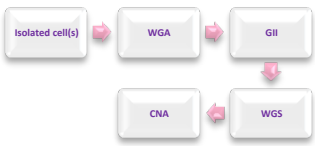


*Figure 4:* ***Protocol used for generation of low-resolution WGS of single CTCs from SCLC patient samples***

## Evaluation of Effects of Storage on CTCs

The effects of long term storage at -20°C in glycerol was evaluated with **CellSearch ® enriched CTC** samples from SCLC - no detrimental effect seen following WGA


*Figure 5:* ***Workflow to assess the effects of storage***

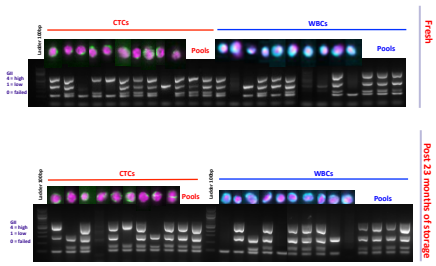


*Figure 6.* ***Comparison of genome integrity index of CTCs isolated from patient A after different storage conditions. A. DEPArray™ isolation 5 days post CellSearch® enrichment (cell count 307 cells). B. DEPArray™ isolation following 23 months of storage of enriched cells at -20°C in glycerol.***

The consequences of long storage of **DEPArray isolated cells** was also evaluated following storage at -80°C with no detrimental effect seen following WGA

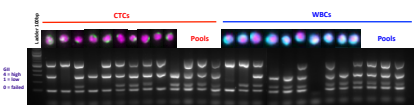


*Figure 7.* ***Genome integrity index evaluation post WGA of isolated cells kept at -80°C for 25 months. The DEPArray™ isolation was processed post 4 days of CellSearch® enrichment (cell count 2048 cells).***

## Molecular evaluation of CTCs following epitope independent enrichment

Blood samples from eight SCLC patients were enriched by Parsortix followed by DEPArray™ single cell isolation (poster presented by Chudziak et al, and Chudziak et al, 2015)

| Patient ID | Gender | Age at diagnosis | SCLC Stage | Timepoint analysed |
|---|---|---|---|---|
| Patient 1 | Female | 70 | Extensive | Baseline |
| Patient 2 | Male | 65 | Extensive | Baseline |
| Patient 3 | Male | 65 | Extensive | Baseline |
| *Patient 4 | Male | 81 | Extensive | Baseline |
| Patient 5 | Male | 78 | Extensive | Baseline |
| Patient 6 | Male | 53 | Limited | Relapse |
| Patient 7 | Male | 73 | Extensive | Baseline |
| Patient 8 | Female | 50 | Extensive | Relapse |
| Patient 9 | Male | 74 | Extensive | EndTx |
| Patient 10 | Female | 49 | Extensive | Relapse |
| Patient 11 | Female | 56 | Extensive | EndTx |
| Patient 12 | Female | 62 | Limited | Baseline |

*Table 1.* ***Clinicopathological characteristics of the patients with cell enrichment by Parsortix (EndTX: end of treatment)***

Sample stability was evaluated from enriched CTCs from patient 4 following storage at -20°C in glycerol for 6 months with no detrimental effect seen following WGA

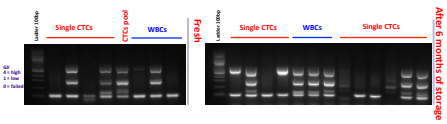


*Figure 8.* ***Comparison of genome integrity index of CTCs isolated from patient 4 following long-term storage post-Parsortix enrichment. A. DEPArray™ isolation post 8 days of enrichment. B. DEPArray™ isolation post 6 months of storing at -20°C in glycerol.***

Molecular analysis of single CTCs and WBCs was performed from cells enriched by Parsortix and isolated by DEPArray™ from patient 4 (*Table 1 and Figure 9*)

CNA profiles generated were consistent with tumour associated aberrations seen only in CTCs and a normal karyotype seen in WBC controls

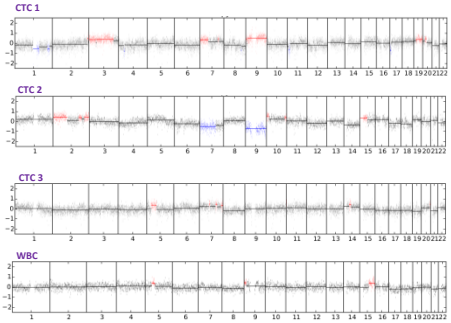


*Figure 9:* ***CNA profiles from CTCs and WBC***

## Conclusions

Development of a robust protocol for the isolation and storage of CTCs compatible with downstream molecular analysis

Demonstrated reliable whole genome amplification of single cells following storage of CellSearch® enriched samples for more than 2 years

DEPArray™ isolated CTCs can be stored for long periods (+2 years) and maintain a good genomic integrity index

Possible to isolate single cells from samples enriched with an epitope independent device (Parsortix) following long term storage

Preliminary results of tumour CNA profiles identified from cells isolated following Parsortix enriched samples

These results broaden the scope of SCLC analysis and describe a novel approach for isolating EpCAM positive and EpCAM negative CTCs in SCLC

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