

PARSORTIX SYSTEM ENABLES ISOLATION OF VIABLE CTCs FROM LEUKAPHERESIS PRODUCT WITH SUBSEQUENT CULTURE

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Background

Solid tumors are constantly releasing cells into the circulatory system. They are genotypically and phenotypically different from the primary tumor. Most of these circulating tumor cells (CTCs) are either not proliferating or die. Thus, viable CTCs are of high interest to obtain therapeutically relevant information. Their extremely low frequency is one of the main limiting factors to obtain viable CTCs for further characterization. To overcome this challenge we tested to isolate viable CTCs from diagnostic leukapheresis (DLA) products obtained from breast cancer patients.

Methods

18 DLA samples and matched peripheral blood (PB) samples of metastasized breast cancer patients were collected and CTC numbers were determined by CellSearch® analysis. Viable cells were enriched from DLA product with Parsortix system. Genomic DNA of single cultured CTCs was isolated and amplified by whole genome amplification. Array-based comparative genomic hybridization of single cells was performed to analyze for genomic aberrations. Resulting profiles were compared to genomic aberration profiles of CTCs isolated before in vitro culture.

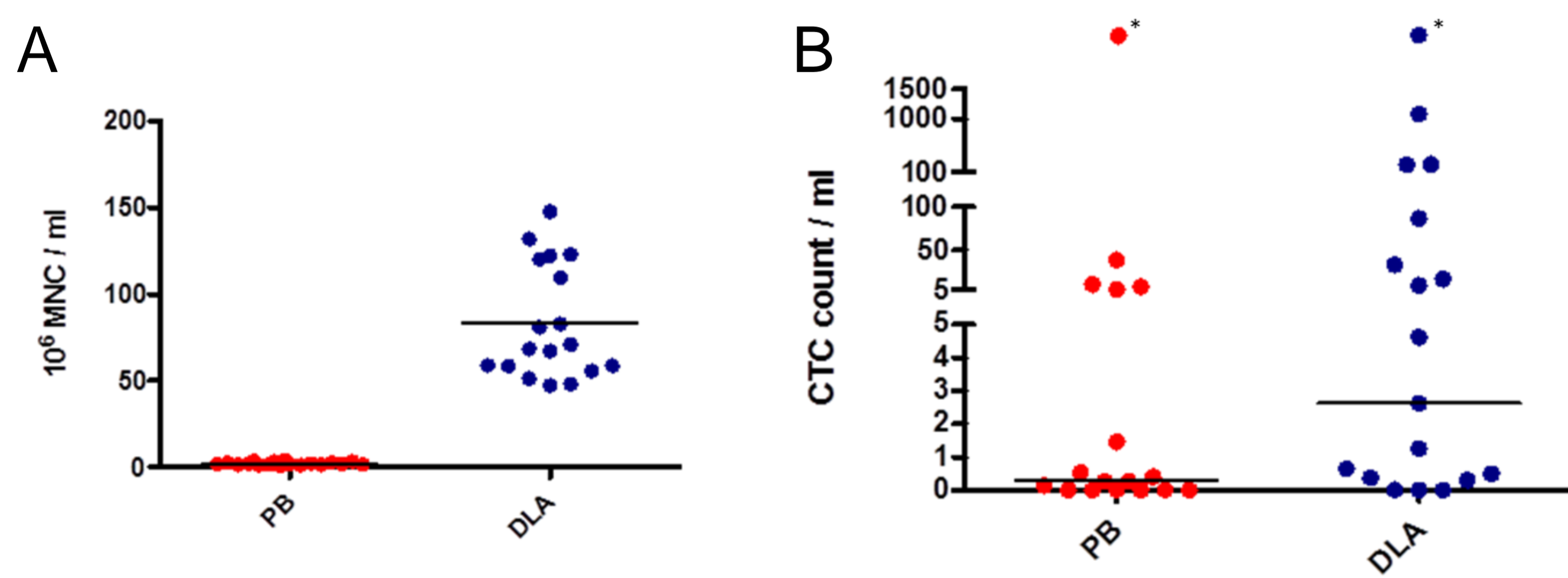


Figure 1: CTCs from leukapheresis samples

CTC count of blood samples and DLA of 18 breast cancer patients was determined by CellSearch®. For CellSearch® analysis 7.5 ml blood or 2×10^8 MNCs of DLA product were used. [A] MNCs per mL in PB samples versus DLA samples. Horizontal line indicates mean. [B] Number of CTCs detected via CellSearch per mL in DLA and PB samples. Horizontal line indicates median (*CTC number exceeded CellSearch® detection range).

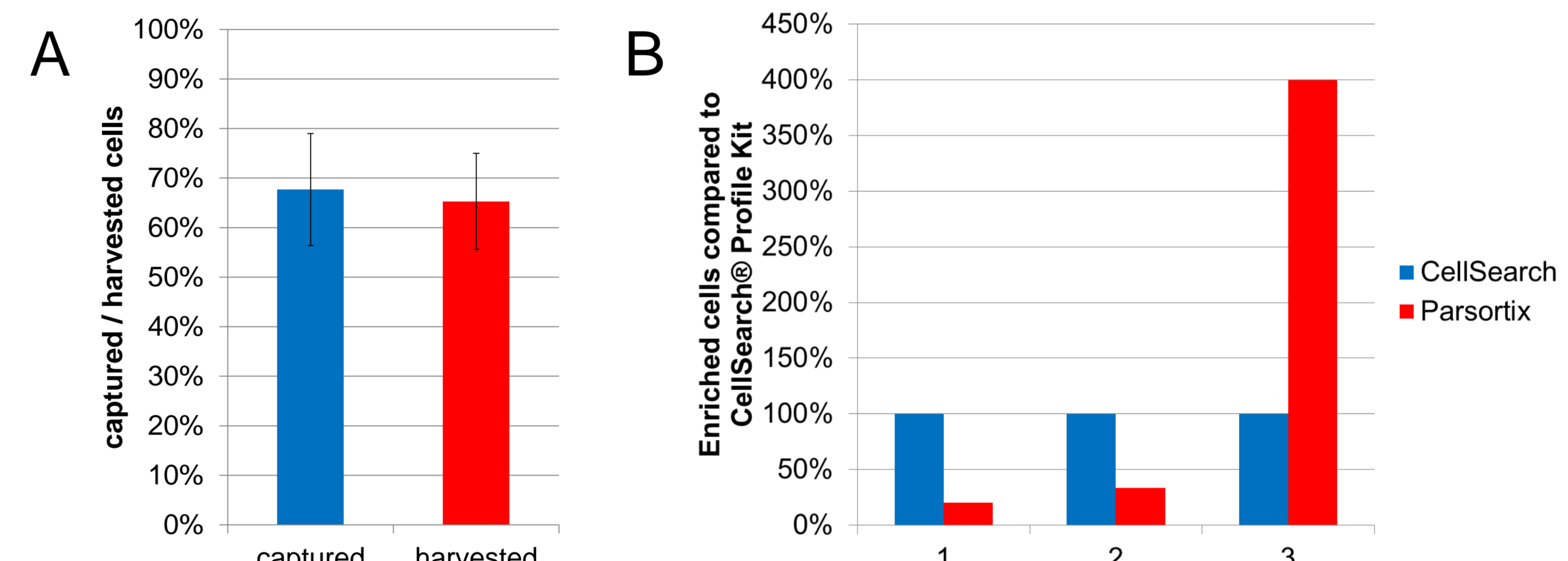


Figure 2: Parsortix enriches CTCs from blood

[A] Capturing rate of Parsortix was determined by spiking 100 stained MCF7 cells in DLA product of 2×10^8 MNCs. Captured and harvested MCF7 cells were counted. [B] CTCs were enriched from DLA product of three independent patients with CellSearch® Profile Kit or Parsortix system. Subsequently enriched cells were stained in parallel for DAPI, Cytokeratin and CD45 for identification.

Pat-ID	CTCs in 7,5 ml PB	cultured successfully?	CTCs in DLA product (per 2×10^8 MNCs)	cultured successfully?
#1	11	-	11	-
#2	34	-	320	+
#3	0	-	1	-
#4	~50000 *	+	~50000 *	+
#5	2	-	51	-
#6	0	-	0	-
#7	11	-	50	-
#8	2	-	11	-
#9			2913	+
#10			223	-
#11			296	-
#12			11	-
#13			6	-

Table 1: Sample overview

[A] CTCs could be successfully cultured from one out of eight PB and 3 out of 13 DLA samples (*CTC number exceeded CellSearch® detection range).

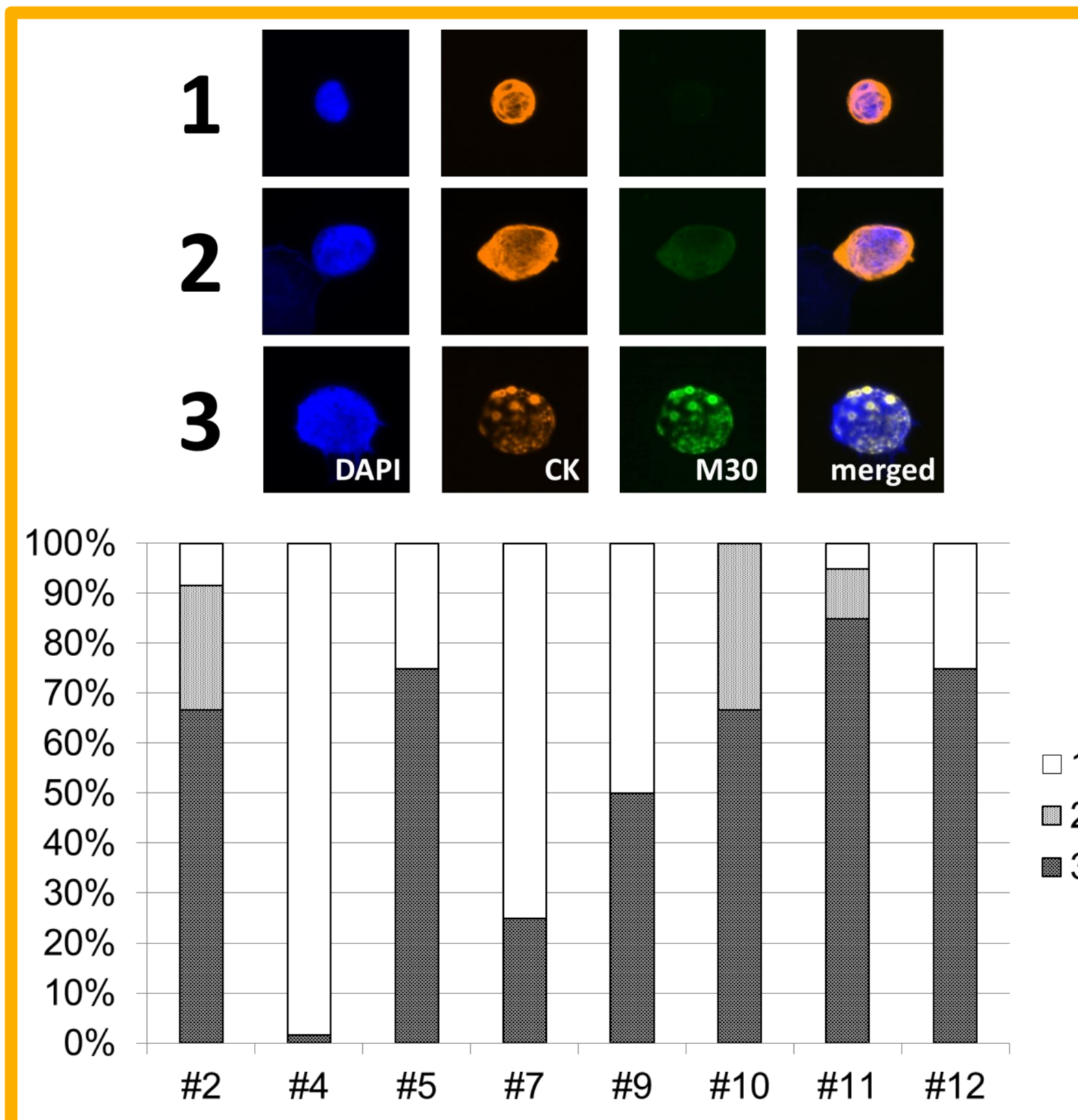


Figure 3: Rate of apoptotic CTCs

Cytosins of CellSearch® enriched CTCs were stained with M30 antibody and ratio of apoptotic CTCs (category 2 and 3) was determined.

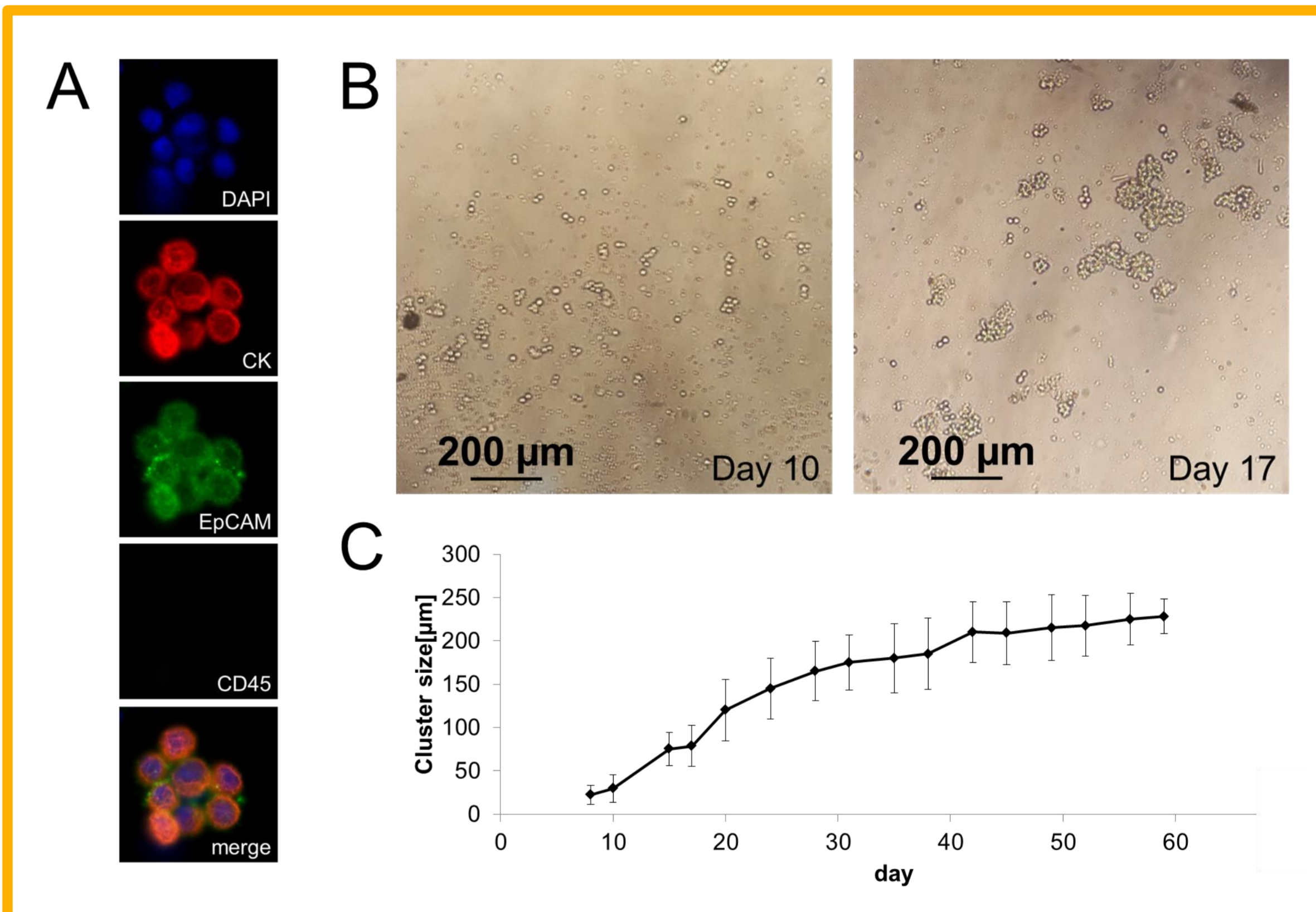


Figure 4: In vitro culture of CTCs

[A] cultured CTCs: Cells were stained for DAPI, Cytokeratin, EpCAM and CD45. [B] Cultured CTCs form clusters. [C] Proliferation in vitro: CTC clusters that have been isolated after seven days were followed for two months.

Results

- CTCs were detected in ten PB and matched DLA samples and in three more DLA samples whose corresponding blood samples were CTC-negative. CTC number per ml was increased by an average of 17.0 fold (Fig 1).
- Parsortix was optimized to enrich tumor cells from DLA product while keeping them best viable. An enrichment rate of 67.7% ± 11.3% and a harvesting rate of 65.3% ± 9.7% were determined with spiked MCF7 cells (Fig. 2A).
- Between 20% to 30% of CTCs could be enriched from different patient samples compared to CellSearch. One sample containing many EpCAM^{low} CTCs exceeded CTC numbers of CellSearch by fourfold (Fig 2B)
- We could grow viable CTCs from three out of 13 CTC positive DLA samples as determined by immune fluorescence analysis (Tab. 1). For samples with high CTC numbers where no CTC growth was observed high apoptosis rates were determined (Fig. 3).
- Cultured cells revealed DAPI and cytotkeratin positivity and CD45 negativity. CTCs form spheres within first weeks and could be cultured for several months (Fig. 4). Short tandem repeat analysis of amplified genomes of single cells confirm patient's origin of cultured CTCs.
- The growing cells harbor genomic anomalies confirming their malignant origin. Most aberrations are widely identical to the aberrations detected in uncultured CTCs (Fig. 5).

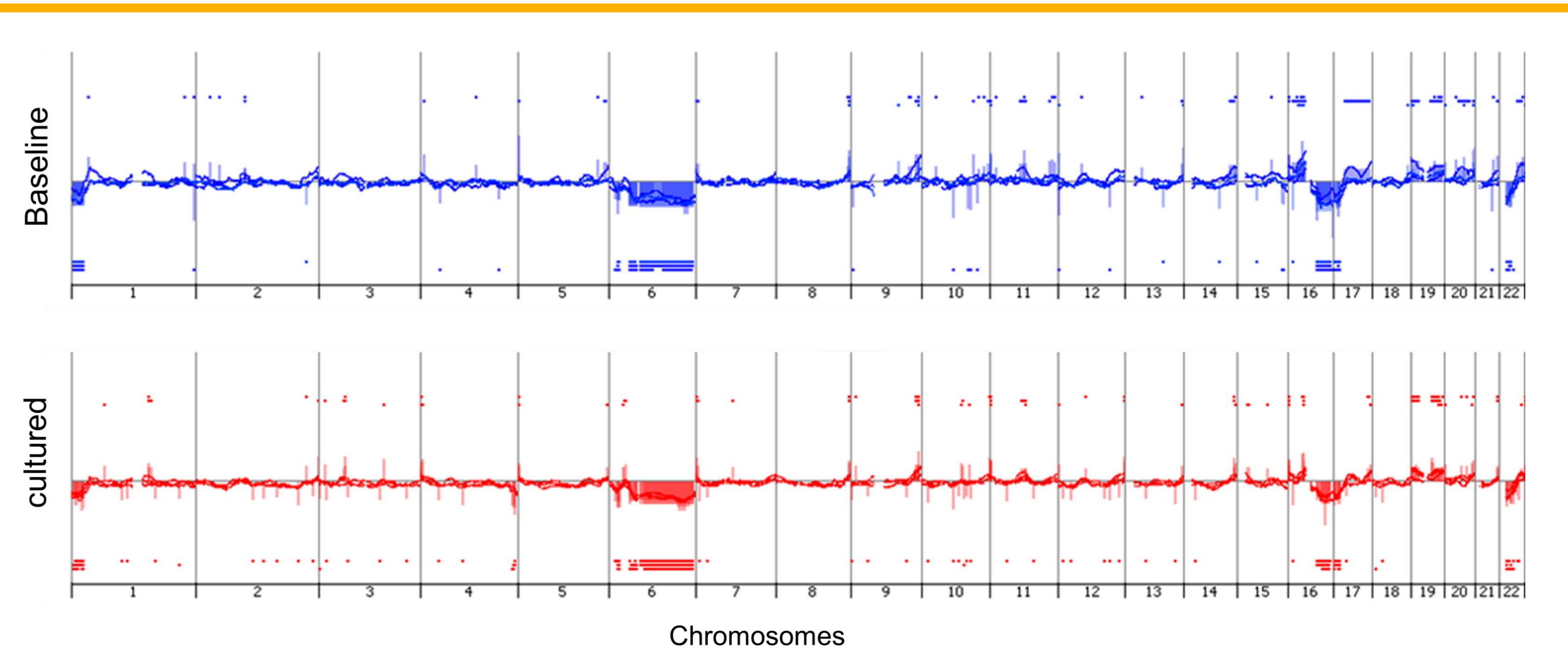


Figure 5: Genomic comparison of single cultured CTCs

aCGH analysis to detect chromosomal aberrations: Cultured cells (N=3) were compared to cells directly isolated from the CellSearch® (baseline, N=3).

Conclusion

DLA provides greater numbers of viable CTCs which can be enriched with Parsortix system in order to enable their in vitro cultivation. This workflow will allow functional studies.