Comparable detection of large tumor-derived vesicles (oncosomes) by known CTC platforms: potential new analyte for biomarker monitoring?

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SUMMARY

- Tumor-derived extracellular vesicles (tdEVs) are circulating membrane-enveloped particles that can be exploited to monitor tumor biology, including biomarker status. Large oncosomes (LO) are a unique group of tdEVs, characterized by larger size compared to other EVs (1-10 um diameter), and the presence of tumor markers. LOs carry tumor RNA, DNA, as well as plasma and membrane proteins. In many cancer types, including metastatic breast cancer, LOs are more abundant than CTCs.
- Our lab developed a RareCyte platform-based LO enumeration method (1).
- We analysed metastatic breast cancer patients' blood samples, comparing the RareCyte LO method with a published, CellSearch-based analysis method (ACCEPT software "tdEV" algorithm). On a sub-set of samples, the detection of HER2 on metastatic breast cancer large oncosomes was evaluated with the RareCyte platform.
- The RareCyte LO method detected more particles than CellSearch tdEV algorithm, especially in the HER2 negative group.
- LO HER2 status reflected tissue biopsy HER2 score.

METHODS

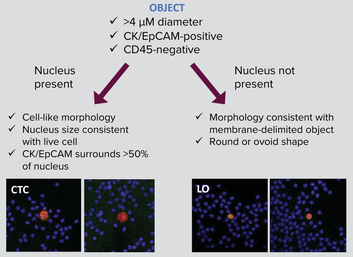
Mestastatic breast cancer patient (N=73) blood samples were collected into CellSave or AccuCyte tubes for CellSearch or RareCyte CTC analysis and processed according to manufacturer instructions, as previously described (2). Patient samples with no signs of lysis and sufficient blood volume (7.5 ml) were included in the analysis. Overall survival (OS) was defined as the time from baseline blood draw to death or last follow-up.

LO-like particle enumeration: CellSearch: LO-like particles (referred to as "tdEV") were enumerated along with CTCs with the ACCEPT algorithm with settings published earlier (3). RareCyte: Large oncosome-like object (referred to as "LO") detection criteria were developed as previously described (1) and enumerated independently from CTCs. A cutoff off 4000 was applied for high LO count samples. CTC counts generated by CellSearch and RareCyte were previously published (2).

HER2 analysis: A subset of patients (N=9) were selected to represent a range of HER2 tissue biopsy scores. HER2 overexpression in LOs and CTCs was determined using the RarePlex HER2/ER CTC panel, the cutoff for HER2 overexpression set to 350 MFI according to RareCyte guidelines.

Statistical analysis: Correlations were assessed using Spearman's Rho test. Comparability of particle counts between platforms was evaluated with the Wilcoxon Signed Ranks test. Median LO and tdEV counts were used as cutoff values for survival analysis. Survival curves were compared using the log-rank test. HER2 positive and negative subgroups were compared using Kruskal-Wallis test with Dunn post-test. Correlation of HER2+ analytes with tissue biopsy HER2 scores was also analyzed using Spearman's Rho test.

RareCyte LO detection

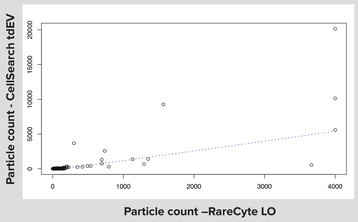


CellSearch tdEV and RareCyte LO identification criteria

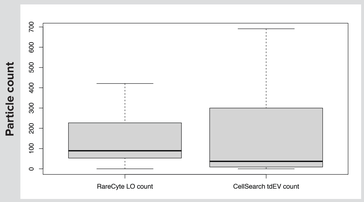
Large oncosome criterion	ACCEPT tdEV	RareCyte LO
Negative for WBC marker	Mean Intensity CD45 ≤5,	CD45 negative
Nucleus-free	Mean Intensity DNA ≤5,	Nuclear stain <10% of object
Positive for tumor-marker	Enriched by EpCAM Mean CK intensity >60	CK/EpCAM positive
Size 1 uM or above	CK signal <5 pixels	Minimum 4 µm major axis
Round or oval	Eccentricity CK ≤0.8	Visual confirmation
Membrane-delimited	Automated	

RESULTS

The RareCyte LO method captures significantly more particles than the CellSearch tdEV algorithm

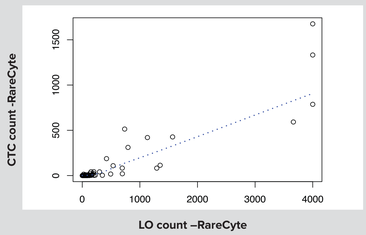


A strong positive correlation was observed between CellSearch tdEV and RareCyte LO counts (Spearman's rho = 0.79, p < 0.001).



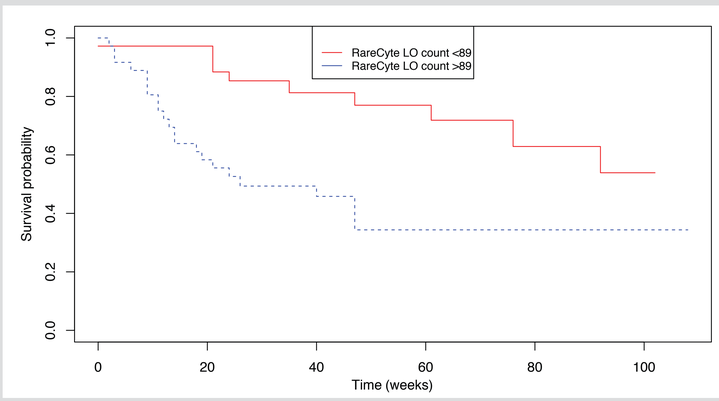
Notably, RareCyte LO count was higher han CellSearch tdEV count; median[IQR] was 89[53-208] and 37[9-288], respectively. This difference was statistically significant (p=0.007).

Consistent with earlier findings, vesicle counts also correlated with their respective CTC counts RareCyte LO vs. CTC: Spearman's rho = 0.75, p < 0.001. CellSearch tdEV vs. CTC: r = 0.88, p < 0.001; (data not shown).

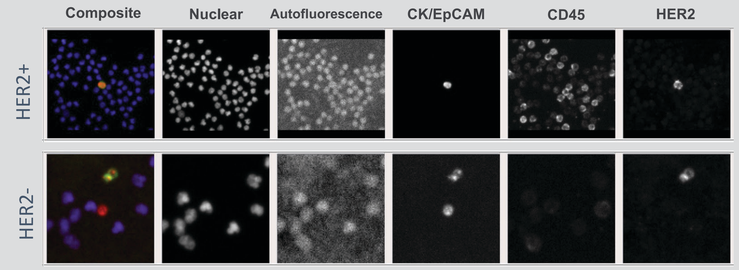


Particle counts generated with both the RareCyte LO and CellSearch tdEV methods predict overall survival

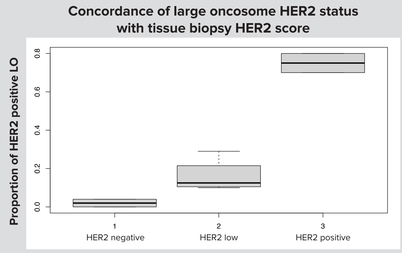
Vesicle counts above median were associated with worse survival outcomes
RareCyte LO: r = 0.39, p = 0.006, HR = 3.6, p = 0.02
CellSearch tdEV: r = 0.35, p < 0.001, HR = 3.5, p < 0.001 (data not shown).



RareCyte LO HER2 status correlates with tissue biopsy HER2 score



Large oncosomes displayed varying HER2 expression levels and could be categorized as HER2 positive or HER2 negative using the signal intensity cutoff established for CTCs (MFI=350).



LO HER2 status was generated by calculating the proportion of HER2+ LOs. There was a significant, positive correlation between LO HER2 status with tissue biopsy HER2 score (r=0.799, p=0.01).

In contrast, CTC HER2 status did not correlate with tissue biopsy (data not shown).

DISCUSSION

- The strong correlation between CellSearch tdEV and RareCyte LO counts indicates that the methods can reliably and comparably detect large oncosome-like particles.
- Large oncosomes represent a significant opportunity for liquid biopsy-based HER2 detection. This is especially valuable for patients with low or negative CTC count.
- Prospective studies are required to determine if LO HER2 status can predict HER2-targeted therapy response.

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