Proof-of-concept study to enumerate large oncosomes using the RareCyte CTC platform

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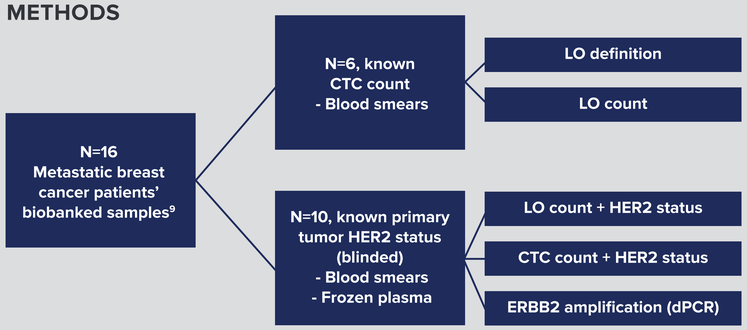
SUMMARY

We performed a pilot study to investigate if specimen collected for CTC detection with the RareCyte platform can form a basis of a multimodal liquid biopsy test combining CTC, large oncosome (LO) and cfDNA analysis to identify patients with HER2 overexpressing tumors in a metastatic breast cancer patient cohort. Large oncosome identification criteria were established and LOs were quantified in 15 patient specimens. LO count was higher and positively correlated with CTCs, while proportion of HER2 overexpression was comparable between LOs and CTCs. Plasma cfDNA was of suitable quality for dPCR and enabled ERBB2 copy number assessment. Multimodal HER2 detection (cfDNA+CTC+LO) is feasible and cost-efficient to perform utilizing existing workflows at CellCarta.

BACKGROUND

- Liquid biopsy enables non-invasive detection of tumor markers, e.g. HER2, complementing tumor biopsy.
  - Patients with HER2-positive CTCs benefit from anti-HER2 therapy even in the absence of tumor HER2 signal[1].
  - cfDNA ERBB2 amplification predicts tissue HER2 status[2].
  - HER2 positive large oncosomes have negative prognostic value[3].
- Low abundance of analytes often limit the sensitivity of liquid biopsies. Combining different analytes can increase biomarker detection sensitivity and improve predictive power[4].
- CellCarta has established workflows for RareCyte platform to enumerate and analyze CTCs.
- Large oncosomes are expected to co-isolate with CTCs due to similar density[5].
- cfDNA can be extracted from remaining plasma, sequencing results comparable to DNA Streck[6].
- Large oncosomes were found to complement CTC data in various oncology settings[7,8].

METHODS



HER2+ cutoff: MFI≥350

LO, CTC and cfDNA were analyzed independently in a blinded fashion. Primary tumor HER2/ERBB2 results were masked until final analysis.

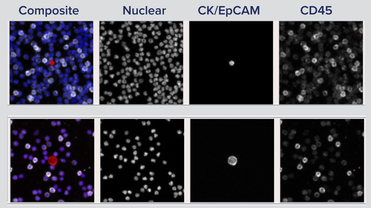
RESULTS

Large oncosome definition

Objects that were

- CK/EpCAM positive, nucleus free
- CD45 negative
- >4 µm in diameter
- Evidence of membrane at 40X

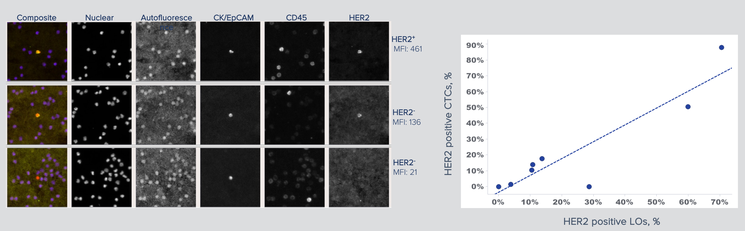
were categorized as large oncosomes.



Sample	HD	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
CTC count	0	0	0	0	1	4	4	41	42	69	83	99	181	311	737	1994
LO count	8	3	34	36	10	45	137	301	59	258	672	558	230	792	13587	10627

- LO count is higher than CTC count (Wilcoxon signed rank p=6.10E-5). There is a strong correlation between LO and CTC counts (Spearman r=0.91, p=8.11E-5).

HER2 detection in oncosomes



- HER2+ LOs detected in 8/9 (89%) blood smears.
- Significant correlation between the proportion of HER- overexpressing LOs and HER2+ CTCs (Spearman r=0.71, p=0.05).

Liquid biopsy HER2/ERBB2 concordance with tumor status

	Primary tumor		Large oncosome		CTC		cfDNA	
ID	Patient group (IHC score)	ERBB2 copy#	N	HER2+ LO rate	N	HER2+ CTC rate	Conc. (pg/ul)	ERBB2 copy# [95% CI]
1	HER2 Negative (0)	N/A	137	0%	4	0%	58	0.94 [0.77-1.11]
2	HER2 Negative (0)	N/A	258	4%	69	1%	461	1.02 [0.98-1.05]
3	HER2 Low (1-2+)	N/A	230	10%	181	10%	177	1.00 [0.92-1.09]
4	HER2 Low (1-2+)	1	13587	11%	737	14%	9280	0.91 [0.89-0.93]
5	HER2 Low (1-2+)	1	10627	14%	1994	18%	2170	1.02 [0.96-1.07]
6	HER2 Low (1-2+)	1	45	29%	4	0%	68	0.94 [0.71-1.16]
7	HER2 Low (1-2+)	1	558	60%	99	51%	122	0.93 [0.85-1.02]
8	HER2 Positive (3+)	10	59	70%	42	88%	108	7.50 [6.70-8.20]
9	HER2 Positive (3+)	3	10	80%	1	N/A - insufficient N	26	1.27 [0.94-1.60]
10	HER2 Positive (3+)	5.5	N/A – slides unavailable				86	4.50 [3.70-5.00]

- Proportions of HER2-overexpressing LO and CTC are higher in patients with strong IHC signal in the primary tumor.
- ERBB2 amplification status was correctly identified for 6/7 (85%) of patients with available tumor ERBB2 copy number data.

DISCUSSION

- A robust LO analysis method was established using preanalytical processes al ready in place for CTC analysis.
- The strong correlation between CTC count and LO count suggests that LOs detected with the RareCyte system are tumor derived and can complement CTC findings.
- The correlation of LO vs. CTC HER2 overexpression rate may be impacted by inaccuracy due to low CTC count[10], interconversion of CTCs[11], and other factors.
- The quality of cfDNA extracted from the plasma component of RareCyte biospecimens was suitable for downstream applications, although concentrations were variable. The lowest cfDNA concentration was observed in the only sample with discordant ERBB2 amplification results. The accompanying low CTC and LO counts suggest an overall absence of tumor signal.
- A limitation of our study is that while tissue IHC status was assessed from the primary tumor, liquid biopsy was performed at the time of relapse, potentially explaining the discrepancy seen between tissue and blood HER2 status.[12]
- cfDNA, CTCs and LOs analyses were complementary. Combining them increases the probability of detecting HER2 amplification or overexpression with liquid biopsy.
- A larger dataset will enable mathematical modelling of the relationship between multimodal liquid biopsy data and tumor HER2 status.

CONCLUSIONS

- Including large oncosomes in CTC analysis can increase accuracy and sensitivity, especially in low CTC settings.
- Plasma remaining after the RareCyte CTC analysis is suitable for cfDNA analysis.
- CellCarta's existing workflows support the development of a multimodal HER2 assay from blood, providing better insight into tumor biomarker status.

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