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Tracking changes in circulating stromal cells and circulating tumor cells predicts responsiveness of new line induction in metastatic breast cancer after 1 cycle of therapy

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ABSTRACT

In metastatic Breast Cancer (mBC), Circulating Tumor Cells (CTCs) are established prognostic indicators of patients (pts) not responding to new lines of therapy and who have poor clinical outcomes. However, CTCs are typically found in <20% of mBC pts and many pts without CTCs will also progress. Recently an inflammatory pro-tumorigenic macrophage emanating from tumor stroma (i.e. Cancer Associated Macrophage-like Cell [CAML]) was found in >90% of mBC pts and were also indicative of poor clinical outcomes. As CTCs & CAMLs are isolated in conjunction from a single blood sample, and both are prognostic for therapy response, we evaluated CTCs & CAMLs before and after initiation of new therapies in mBC to determine their combined prognostic/predictive values.

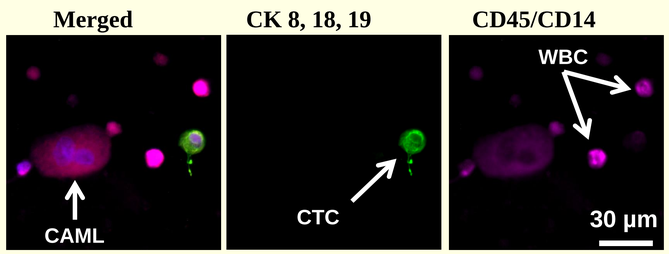

Figure 1. Example of CTC isolated with a CAML in a mBC patient. CTCs are Cytokeratin positive (green) & CD45/CD14 (purple) negative. In contrast, CAMLs are CD45/CD14 positive and may be weakly positive for Cytokeratin. White blood cells (WBCs) are normal sized CD45/CD14 positive.

INTRODUCTION

The discovery of CAMLs as independent prognostic indicators of survival has highlighted the need for more in depth analysis of blood based diagnostics. We have previously demonstrated CAMLs are cancer specific giant polyploid cells circulating in the blood of patients which appear prognostic for overall survival (OS). Further, it is well established that CTCs transit the circulatory system and are also prognostic for OS. As CTCs & CAMLs are isolated in parallel from a single blood sample and both are prognostic for therapy response, we hypothesized that monitoring CTCs & CAMLs before and after initiation of therapy might increase their prognostic value in a large array of cancer subtypes.

References

1. Adams DL, et al “Circulating giant macrophages as a potential biomarker of solid tumors.” PNAS, 2014, 111(9):3514-3519
2. Cristofanilli M, “Liquid Biopsies in Solid Tumors” *Springer Intl Publish.* 2017

MATERIALS & METHODS

A prospective two year single blind multi-institutional study was undertaken to evaluate CTCs and CAMLs changes before, and after, induction of a new line therapy in patients with mBC (n=101). A baseline (BL) blood sample was taken prior to induction of a new therapy and a 2nd sample (T1) was taken after initiation of systemic therapy (~30 days). LifeTracDx™ liquid biopsy test was performed to collect CTCs and CAMLs using CellSieve™ microfilters. The quantities and subtypes of CTCs and CAMLs were analyzed. RECIST v1.1 was used to define progression free survival (PFS) and overall survival (OS) for determining hazard ratios (HRs) by censored univariate & multivariate analysis at 2 years.

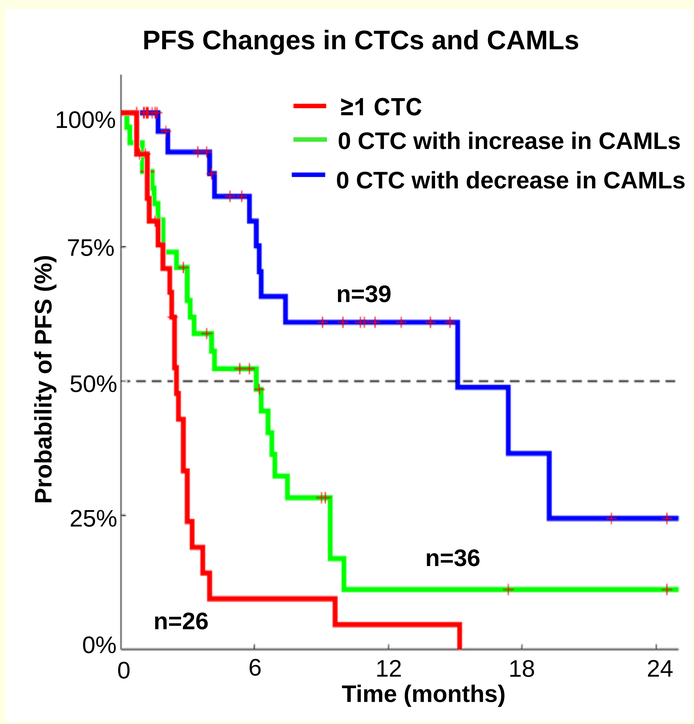

Figure 2A. PFS analysis based on changes of CTC and CAMLs between BL and T1.

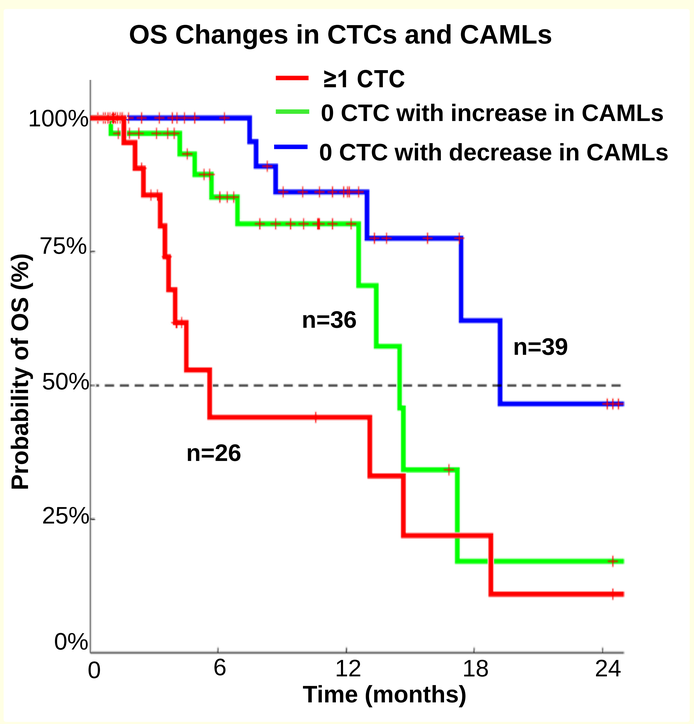

Figure 2B. OS analysis based on changes of CTC and CAMLs between BL and T1.

Table 1. Hazard ratio comparisons of CTCs Presence at T1 and changes in CAMLs

HR (95%CI) p value	≥1 CTCs T1 vs No CTCs T1	↑ CAMLs vs ↓CAMLs	≥1 CTCs T1 vs No CTCs+↑ CAMLs	≥1 CTCs T1 vs No CTCs+↓ CAMLs	No CTCs+↑ CAMLs vs No CTCs+↓ CAMLs
n value	26 vs 76	47 vs 54	26 vs 36	26 vs 39	36 vs 39
PFS	**6.5 (3.1-13.5) p<0.00001**	**2.8 (1.6-4.7) p=0.00029**	**2.4 (1.3-4.6) p=0.01395**	**11.7 (5.1-26.9) p<0.00001**	**3.0 (1.5-5.8) p=0.00232**
OS	**5.2 (2.0-13.7) p=0.00188**	**2.6 (1.3-6.0) p=0.01895**	2.5 (1.0-6.2) p=0.07788	**6.8 (2.4-19.2) p=0.00078**	2.9 (1.0-8.2) p=0.07516

Funding Sources

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RESULTS

- CTCs were identified in 35% (n=35/101) of pts at BL & 26% (n=26/101) at T1.
- A single CTC at T1 was highly prognostic for worse PFS HR=6.5, p<0.0001 and OS HR=5.2, p=0.00188 **(Table 1).**
- CAMLs were found in 93% (n=94/11) of pts as BL and 86% (n=87/101) of pts at T1.
- CAML decreases (ie size/number) were significantly prognostic for improved PFS (HR=2.8, p=0.00029 and OS HR=2.6, p=0.01895 when CTCs were absent **(Table 1).**
- Pts with ≥1 CTC at T1 (n=26) had median PFS=2.4 and mOS=4.7 months.
- Pts without CTCs plus increasing CAMLs (n=36) had mPFS=5.9 and mOS=14.1 months.
- Pts without CTCs plus decreasing CAMLs (n=39) had mPFS=15.0 and mOS=18.8 mos..

CONCLUSIONS

- Our data confirms that, though rare, pts with persistent CTCs have the worst clinical overall outcomes.
- Simultaneous CAML quantification provides a new dynamic predictive blood based biomarker in pts without detectable CTCs.
- Combining CTC and CAMLs may be useful to better individualize therapy and improve outcomes,
- Larger, prospective, interventional studies may evaluate the impact of early intervention using this novel biomarker.