Investigating differences in the composition of circulating tumor cells (CTCs) clusters in invasive lobular and ductal carcinoma to decipher lobular breast cancer metastasis

Eleonora Nicolò[1], Elisabetta Molteni[1], Lorenzo Foffano[2], Lorenzo Gerratana[2], Mara S. Serafini[1], Letizia Pontolillo[1], Caterina Gianni[1], Laura Munoz-Arcos[1], Nadia Bayou[1], Amanda K. Strickland[1], Hunter Gaudio[1], Brenno Pastò[2], Maroua Manai[1,3], Youbin Zhang[3], Paolo D'Amico[3], Andrew A. Davis[4], Jeannine Donahue[1,3], Huiping Liu[3], William J. Gradishar[3], Giuseppe Curigliano[5,6], Massimo Cristofanilli[1,3], Carolina Reduzzi[1,3]

1- Department of Medicine, Division of Hematology-Oncology, Weill Cornell Medicine, New York, NY, USA; 2- Department of Medical Oncology, Centro di Riferimento Oncologico di Aviano (CRO), IRCCS, Aviano, Italy; 3- Robert H. Lurie Comprehensive Cancer Center, Feinberg School of Medicine, Northwestern University, Chicago, IL, USA; 4- Washington University in St. Louis, St. Louis, MO, USA; 5- Division of New Drugs and Early Drug Development, European Institute of Oncology IRCCS, Milan, Italy; 6- Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy.

Background

- Invasive lobular carcinoma (ILC) has distinctive clinical and genomic features compared to invasive ductal carcinoma (IDC)[1]; however, for patients with ILC treatment is selected according to the same guidelines as IDC.
- A better characterization of ILC and development of specific approaches for ILC pts is an unmet need. Liquid biopsy (LB) is a useful tool to achieve this goal.
- Our group showed that, compared to IDC, ILC has specific circulating tumor DNA (ctDNA) alterations and higher CTC count[2,3].
- Another study reported higher detection of CTC clusters (CTC-CL), considered the main seed of metastasis, in ILC[4]. This finding is paradoxical since cell-cell adhesion (a key feature of CTC-CL) is impaired in ILC.
- Interestingly, an association between CTC-CL counts and ctDNA *CDH1* alterations (a hallmark of ILC) was reported, regardless of histology[5].

To investigate whether different mechanisms are responsible for CTC clustering in ILC and IDC, in this study we characterized CTC-CL according to the breast cancer histological type.

Methods

Blood samples were collected from 355 patients with metastatic BC (MBC) before starting a new line of therapy at Northwestern University (Chicago, IL) between 2016 and 2021 (NU16B06 trial).

At baseline, 7.5 mL of blood collected into CellSave preservative tubes were processed with the CellSearch® system (Menarini Silicon Biosystem, Bologna). CTC and CTC-CL were enumerated using the CellTracks Analyzer II® System (Menarini Silicon Biosystems) by a single expert operator. CTC-CL were defined as groups of ≥ 2 CTCs , or ≥ 1 CTC clustered with ≥ 2 white blood cells (WBCs). The number and size of CTC-CL, and the presence of WBCs in CTC-CL (heterotypic) were compared between ILC and IDC. Also, the association between CTC-CL and overall survival (OS) was tested.

To further explore mechanisms of CTC-CL formations, we assessed potential differences in the association between the presence of CTC-CL and ctDNA alterations in ILC vs IDC populations. For ctDNA analysis, matched plasma samples were analyzed using Guardant360™ (Redwood City, CA). Single-nucleotide variants (SNVs), insertions-deletions (indels), gene fusions/rearrangements, and copy number variants (CNVs) were evaluated. We tested the association between CDH1 and the top 10 alterations by frequency and the presence of clusters (Fisher's exact test).

Contact Information:
Eleonora Nicolò, MD
eln4006@med.cornell.edu
eleonora_nicolo

Carolina Reduzzi, PhD
car4012@med.cornell.edu
ReduzziCarol

References:
[1] Pramod et al, *Oncologist*, 2021
[2] Davis et al, *EBioMedicine* 2022
[3] Nicolo' et al, *AACR*, 2024
[4] Narbe et al, *Cells*, 2020
[5] Davis et al, *BCR,* 2019

Results

- Of the 355 pts included, 255 (73%) had IDC while 45 (13%) had ILC (**Table 1**).

	Ductal BC (N = 255)	Lobular BC (N = 45)	P value
Age			
Median (IQR)	54 (44-63)	59 (53-64)	**0.029**
Subtype (IHC)			
HR+/HER2-	121 (48%)	37 (82%)	**< 0.001**
HER2+	56 (22%)	2 (4%)	
TNBC	78 (31%)	6 (13%)	
***De novo* metastatic disease**			
No	200 (78%)	35 (78%)	0.922
Yes	55 (22%)	10 (22%)	
Site of metastasis			
Visceral			
No	86 (34%)	18 (40%)	**0.002**
Yes	169 (66%)	25 (56%)	
Unknown	-	2 (4%)	
Non-visceral			
No	28 (11%)	6 (13%)	**0.003**
Yes	227 (89%)	37 (83%)	
Unknown	-	2 (4%)	
N. of metastatic sites			
< 3	148 (58%)	30 (67%)	0.277
≥ 3	107 (42%)	15 (33%)	
Line of therapy			
1	146 (57%)	21 (47%)	0.417
2	33 (13%)	7 (15%)	
≥ 3	76 (30%)	17 (38%)	
CTCs			
Median (IQR)	2 (0-20)	9 (2-47)	**< 0.001**
< 5	153 (60%)	17 (38%)	**0.006**
≥ 5	102 (40%)	28 (62%)	
CTC Clusters			
< 1	227 (89%)	33 (73%)	**0.004**
≥ 1	28 (11%)	12 (27%)	
Heterotypic CTC Clusters (in pts with CTC-CL)			
< 1	18 (64%)	6 (50%)	0.398
≥ 1	10 (36%)	6 (50%)	

Table 1 - Cohort characteristics according to subtype

- Overall, CTC-CL were identified in 45 (13%) patients and only in those with ≥ 5 CTCs.
- The association with outcome was thus tested only in patients with stage IV$_{aggressive}$ (i.e ≥ 5 CTCs). In the overall population, the presence of ≥ 4 CTC-CL was associated with shorter OS (6 vs 21 months, p = 0.001).
- **Figure 1** shows the association between overall survival and CTC-CL levels in the IDC and ILC populations.

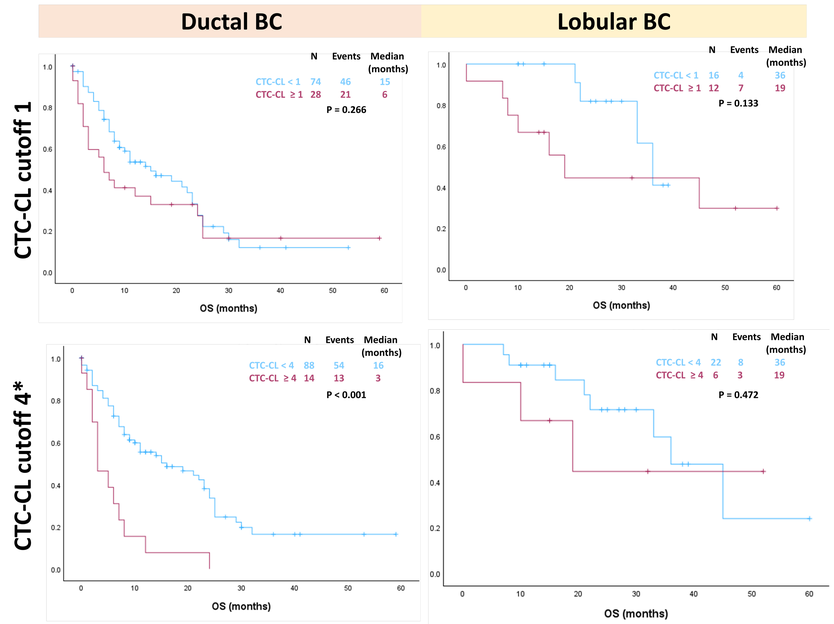

Figure 1 – Kaplan Meier in terms of OS based on CTC-CL levels for IDC and ILC patients
***Cutoff based on the median number of CTC-CL in patients with at least one cluster = 3.5**

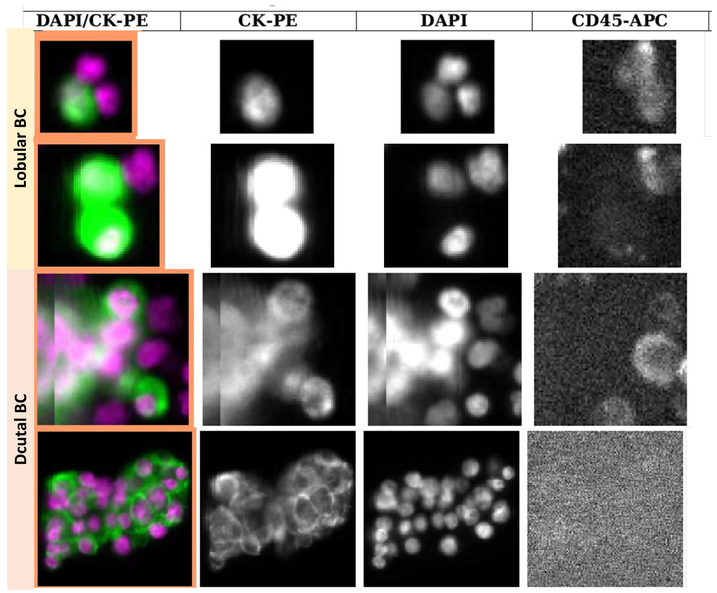

Figure 2 – Examples of CTC clusters in ILC and IDC patients. First row: 1 CTC and 2 WBCs; second row: 2 CTCs and 1 WBC; third row: 6 CTCs and 2 WBCs; fourth row: 30 CTCs and 0 WBCs.

- A matched plasma sample for ctDNA analysis was available for 129 IDC and 19 ILC patients.
- The 10 most frequent alterations in the study population were: *TP53* SNVs, *PIK3CA* SNVs, *GATA3* SNVs, *ESR1* SNVs, *BRAF* CNVs, *EGFR* CNVs, *PIK3CA* CNVs, *FGFR* CNVs, *MYC* CNVs, *CCND1* CNVs. In IDC only *EGFR* CNVs (p=0.029) and *MYC* CNVs (p=0.031) were significantly different, with a higher than expected frequency in patients with CTC-CL. No significant association between ctDNA alterations and CTC-CL was observed in ILC, possibly due to the small sample size.
- CDH1 alterations were detected in 2 IDC and 1 ILC patients and significantly associated with CTC-CL (p=0.015) only in IDC.

- The presence of CTC-CL was significantly higher among ILC patients (27% vs 11% in IDC, p=0.004) but the total number of clustered CTCs was significantly lower in ILC than IDC (median 4.5 vs 9.5, p=0.039), suggesting a smaller size of CTC-CL in ILC. Indeed, the maximum number of CTCs per CTC-CL was numerically lower in ILC than IDC (**Table 2 and Figure 2**).

	Ductal (n=28)	Lobular (n= 12)
Median # of CTCs in a cluster Median (IQR)	2 (2-2)	2 (1.12-2)
	P = 0.102	
Max # of CTCs in a cluster Median (IQR)	3 (2-5.75)	2 (2-2)
	P = 0.475	
Total number clustered CTCs Median (IQR)	9.5 (2.5-28)	4.5 (2.5-23.25)
	P = 0.039	

Table 2 – Composition in CTCs of CTC-CL in IDC and ILC

- Heterotypic CTC-CL were identified in 6 (50%) and 10 (36%) of ILC and IDC patients, respectively. Among these patients, there was a trend for a higher median (0 vs 2, p=0.37) and maximum number of WBCs (2.5 vs 3.5, p=0.26) in CTC-CL in ILC than IDC (**Table 3 and Figure 2**).

	Ductal (n=10)	Lobular (n=6)
Median # of WBCs in a cluster Median (IQR)	0 (0-2.25)	2 (0-3)
	0.368	
Max # of WBCs in a cluster Median (IQR)	2.5 (1-3.25)	3.5 (2.5-5)
	0.263	
Total number clusterized WBCs Median (IQR)	4 (1.75-22.75)	11 (2.75-25.75)
	0.492	
% of cluster with WBC Median (IQR)	32 (16-88)	79 (36-100)
	0.368	

Table 3 – Composition in WBCs of heterotypic CTC-CL in IDC and ILC

Conclusions

- ILC is characterized by a higher number of CTC-CL than IDC. CTC-CL in ILC appear to be different from IDC, being smaller but more frequently associated with WBCs. This suggests a possible different biology for CTC-CL formation in ILC related to the impaired cell-cell adhesion and a specific role played by the CTC-CL microenvironment. Indeed, the interaction with immune cells in ILC may promote the survival in the bloodstream of smaller CTC-CL thus enhancing metastatic efficacy.
- Further studies including a larger number of patients are needed to validate and better elucidate these findings. The study of CTC-CL could shed light on the distinct pattern of metastatic spread of ILC, potentially offering therapeutic opportunities, and serving as a useful prognostic factor.