Transcriptomic insights into lobular breast cancer biology: a retrospective analysis of the MINDACT clinical trial

Christine Desmedt[1,*], Ha-Linh Nguyen[1,*], François Richard[1], Sabine Linn[2,3], Otto Metzger Filho[4], Coralie Poncet[5], Jelle Wesseling[6], Florentine Hilbers[2], Kim Aalders[7], Mauro Delorenzi[8,9], Suzette Delaloge[10], Jean-Yves Pierga[11,12], Etienne Brain[11], Suzan Vrijaldenhoven[13], Peter A Neijenhuis[14], Karen Van Baelen[1], Marion Maetens[1], Emiel Rutgers[15], Martine Piccart[16], Laura Van 't Veer[17], Giuseppe Viale[18,19$], Fatima Cardoso[20,$]

1. Laboratory for Translational Breast Cancer Research, Department of Oncology, KU Leuven, Leuven, Belgium; 2. Depts of Molecular Pathology and Medical Oncology, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX, Amsterdam, the Netherlands. 3. Department of Pathology, University Medical Centre, Utrecht University, Huispost STR 6.131, P.O. Box 85500, 3508 GA, Utrecht, the Netherlands. 4. Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA. 5. European Organisation for Research and Treatment of Cancer (EORTC) Headquarters, Brussels, Belgium 6. Divisions of Diagnostic Oncology and Molecular Pathology, Netherlands Cancer Institute, Amsterdam; Department of Pathology, Leiden University Medical Center, Leiden, Netherlands. 7. Department of Surgery, Diakonessenhuis Utrecht, Utrecht, The Netherlands. 8. Department of Oncology, University of Lausanne, Epalinges, Switzerland. 9. SIB Swiss Institute of Bioinformatics, Bioinformatics Core Facility, Lausanne, Switzerland. 10. Gustave Roussy, Villejuif, France. 11. Medical Oncology Department, Institut Curie Paris & Saint-Cloud, France. 12. Université Paris Cité, Paris, France. 13. Noordwest Ziekenhuisgroep, Alkmaar, Netherlands.14. Alrijne Ziekenhuis, Leiderdorp, Netherlands. 15. Department of Surgical Oncology, Netherlands Cancer Institute, Amsterdam, the Netherlands 16. Institut Jules Bordet & l'Université Libre de Bruxelles, Brussels, Belgium. 17. Department of Laboratory Medicine, UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, USA. 18. Division of Pathology, IEO, European Institute of Oncology IRCCS, Milan, Italy 19. Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy. 20. Breast Unit, Champalimaud Clinical Center/Champalimaud Foundation, Lisbon, Portugal.

BACKGROUND & OBJECTIVES

- Invasive lobular breast cancer (ILC) represents the 2nd most common type of breast cancer and differs from invasive breast cancer of no special type (NST) at different levels, recently reviewed in [1],
- Most ILC are expressing the estrogen receptor (ER) and lacking *HER2* amplification (ER+/HER2-).
- It is crucial to better characterize biological features characterizing ILC from NST, those differing between classic and non-classic ILC as well as features associated with prognosis.

Here, using the **transcriptomic data from the MINDACT trial**, we aimed at identifying/refining the transcriptomic differences between:

Objective 1: ER+/HER2- NST versus ER+/HER2- ILC

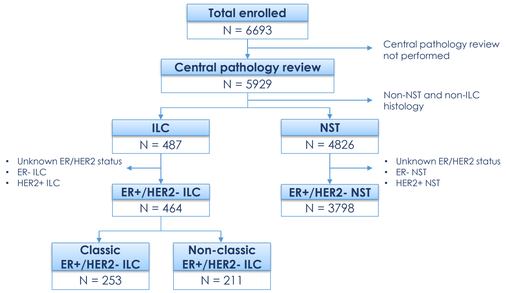
Objective 2: non-classic and classic ER+/HER2- ILC

Objective 3: recurring and non-recurring ER+/HER2- ILC in the subgroup of patients with a low clinical and low genomic (cL/gL) risk

PATIENTS & METHODS

- **Central pathology review** was performed for histological subtype, grade and Ki67 (G.V.) for 5929/6693 (88.6%) of the patients included in the MINDACT trial (NCT00433589).
- **Transcriptomic analyses:** Analysis of bulk transcriptomic data [1] was performed using the R/Bioconductor package 'limma' (version 3.46.0) to identify differentially expressed genes according to histologic subtype (NST vs ILC), subclassification of ILC (non-classic vs classic), genomic risk (high vs low), and relapse incidence (relapse vs no relapse). Linear models were adjusted for age (> 50 vs ≤ 50) and tumor grade (G3, G2 vs G1). False discovery rate (FDR) was controlled by p-value adjustment using the Benjamin-Hochberg method. Differentially expressed genes (DEGs) were determined as those having absolute log-fold change (logFC) ≥ 0.2 and FDR-adjusted p-value (q-value) < 0.05.
- In a corresponding manner, we performed gene set enrichment analyses using two independent approaches: the supervised population-based Gene Set Enrichment Analysis (GSEA – version 4.1.0)[2], and the unsupervised single sample-based method Gene Set Variation Analysis (R package 'GSVA' – version 1.40.1)[3]. The former method was executed using the complete list of genes pre-ranked by the logFC of the prior differential gene expression analysis. Hallmark gene sets available in the H collection of MSigDB (version 7.5.1) were used as references[4]. Hallmarks having an absolute normalized enrichment score (NES) ≥ 1 and q-value < 0.05 were considered differentially enriched.
- **Statistical analyses:** Statistical analyses were performed using R version 4.1.1. All statistical tests were two-sided and were considered statistically significant when p-value < 0.05. Fisher's exact test was used to assess the association of clinicopathological variables with histologic subtype (NST vs ILC), subclassification of ILC (non-classic vs classic) and genomic risk (high vs low). The evaluated clinicopathological variables include age (>50 vs ≤ 50), menopausal status (post- vs pre-menopausal), tumor grade (G3, G2 vs G1), tumor size (≥ 2 cm vs < 2 cm), nodal status (positive vs negative), receptor status (positive vs negative), Ki67 level (20% - 100%, 14% - < 20% vs < 14%), genomic risk (if appropriate, high vs low) and clinical risk. Univariable and multivariable Cox regression models were used to evaluate the association between GSVA scores of molecular hallmarks with disease-free survival (DFS).
- The clinical risk and genomic risk was defined by a modified version of Adjuvant Online! and the 70-gene signature, respectively.

FLOWCHART



RESULTS

Clinico-pathological comparison ILC vs NST

After central pathological review, 464 patients with ER+/HER2- ILC and 3798 patients with ER+/HER2- NST were identified.

Patients with ILC were significantly older at diagnosis, had larger tumors, less axillary nodal involvement, more grade 2 tumors than patients with NST (**Table 1**).

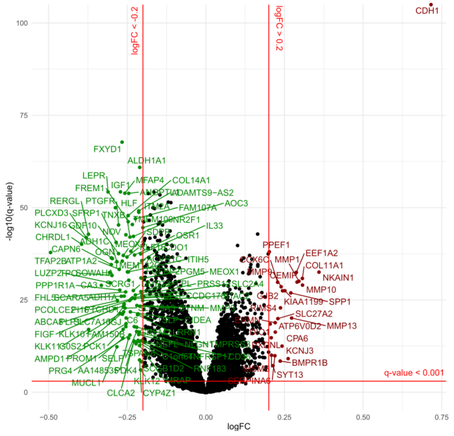
Table 1: Clinical and pathological characteristics of patients with ER+/HER2- ILC and NST tumors

		ILC ER+/HER2- (N = 464)		NST ER+/HER2- (N = 3798)		Fisher's exact p-value
		n	%	n	%	
Age	≤ 50	117	25.2	1221	32.1	0.0025
	> 50	347	74.8	2577	67.9	
Menopausal status	Pre-menopausal	139	31.3	1372	37.5	0.0123
	Post-menopausal	305	68.7	2291	62.5	
	Unknown	20		135		
Tumor grade	G1	81	17.5	953	25.2	0.0005
	G2	361	77.8	2310	61.0	
	G3	22	4.7	526	13.9	
	Unknown	0		9		
Tumor size	< 2cm	258	55.6	2614	68.8	< 0.0001
	≥ 2cm	206	44.4	1184	31.2	
Nodal status	Negative	378	81.5	2938	77.4	0.0443
	Positive	86	18.5	860	22.6	
PR status	Negative	37	8.0	306	8.1	1.0000
	Positive	424	92.0	3488	91.9	
	Missing	3		4		
Ki67	< 14%	255	55.3	1386	36.6	0.0005
	14% – < 20%	136	29.5	1090	28.8	
	20% - 100%	70	15.2	1315	34.7	
	Missing	3		7		
Clinical risk	Low (cL)	249	53.7	2170	57.1	0.1646
	High (cH)	215	46.3	1628	42.9	
Genomic risk	Low (gL)	394	84.9	2758	72.6	< 0.0001
	High (gH)	70	15.1	1040	27.4	
Corrected risk	cL/gL	221	47.6	1813	47.7	0.0005
	cL/gH	28	6.0	357	9.4	
	cH/gL	173	37.3	945	24.9	
	cH/gH	42	9.1	683	18.0	

OBJECTIVE 1

Transcriptomic comparison NST vs ILC

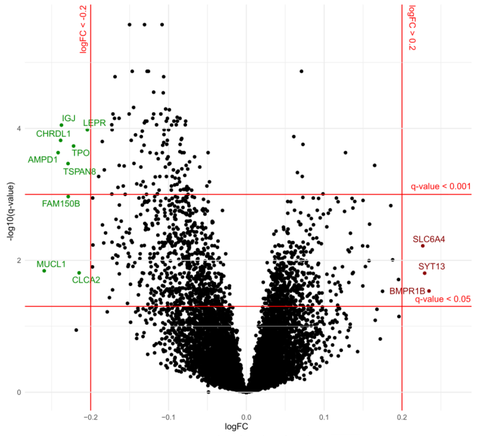
Figure 1. Transcriptomic differences ER+/HER2- NST and ILC. (A) Volcano plots of differential gene expression analysis by 'limma'. The x-axis represents the log2 fold change (logFC) of gene expression in NST tumours relative to ILC tumours. The y-axis represents the -log10 of FDR-adjusted p-value (q-value), the higher -log10(q-value) the smaller q-value. Genes with absolute logFC > 0.2 and q-value < 0.001 are highlighted and labelled (red: upregulated in NST, green: upregulated in ILC). **(B)** Lollipop plots displaying differentially enriched molecular hallmarks according to histological subtype detected by GSEA (q-value < 0.1) and heatmap showing their corresponding average enrichment scores computed by GSVA. The length of the lollipops represents the absolute value of the normalized enrichment score (NES) of a hallmark in NST tumours compared to in ILC tumours. The sign of the NES indicates the orientation of the differential enrichment (positive: enriched in NST, negative: enriched in ILC).



OBJECTIVE 2

Transcriptomic comparison non-classic vs classic ILC

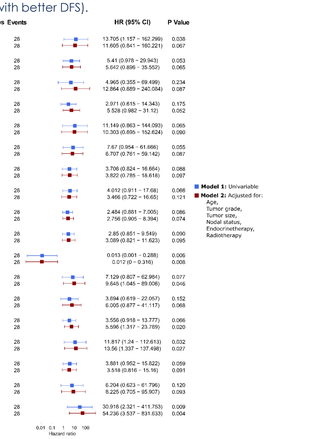
Figure 2: Transcriptomic differences between the non-classic and classic subclassifications of ILC ER+/HER2- tumours. (A) Volcano plots of differential gene expression analysis. Genes with absolute logFC > 0.2 and q-value < 0.05 are highlighted and labelled (red: upregulated in non-classic ILC, green: upregulated in classic ILC). (B) Lollipop plots displaying differentially enriched molecular hallmarks according to ILC subclassification detected by GSEA (q-value < 0.1) and heatmap showing their corresponding average enrichment scores computed by GSVA (positive NES: enriched in variant ILC (non-classic, NC), negative NES: enriched in classic ILC (C)).



OBJECTIVE 3

Recurring vs not-recurring cL/gL ILC

Figure 3. Association of enrichment of molecular hallmarks with DFS in the subgroup of ER+/HER2- patients with cL/gL. Forest plots presenting hazard ratios (HRs) of GSVA scores of molecular hallmarks with DFS estimated by univariable and multivariable Cox regression models. Hallmarks associated with DFS (p-value < 0.1) are shown. (HR > 0: higher enrichment is associated with worse DFS; HR < 1: higher enrichment is associated with better DFS).



CONCLUSIONS

1. Marked transcriptomic differences were identified between ER+/HER2- NST and ILC, with ILC presenting differences in lipid metabolism and in the extracellular matrix, a decreased ER-signaling and increased PI3K/Akt signaling.
2. Differences between classic and non-classic ER+/HER2- ILC were more subtle with enrichment of the hallmarks related to cell cycle in the non-classic ILC, and of the hallmarks related to epithelial-to-mesenchymal transition, hypoxia, adipogenesis and IL6/JAK/STAT3 signaling in classic ILC.
3. Enrichment of hallmarks related to apoptosis, inflammatory response, hypoxia and oncogenic signaling (PI3K/Akt, c-Myc) is associated with worse survival in patients with cL/gL ILC.

REFERENCES

1. Van Baelen, K. *et al.* Annals Oncol 2022 33(8):769-785; 2. Jacob, L. *et al. Commun Biol.* 2020 Jul 27;3(1):397. 3. Subramanian, A. *et al. Proc Natl Acad Sci U S A* 102, 15545–15550 (2005); 4. Hänzelmann, S., Castelo, R. & Guinney, J. *BMC Bioinformatics* 14, 1–15 (2013); 5. Liberzon, A. *et al. Cell Systems* 1, 417–425 (2015).

ACKNOWLEDGEMENTS

The MINDACT trial has received grants from the European Commission Framework Programme VI (FP6-LSHC-CT-2004-503426), the Breast Cancer Research Foundation, Novartis, F. Hoffman La Roche, Sanofi-Aventis, the National Cancer Institute (NCI), the EBCC-Breast Cancer Working Group (BCWG grant for the MINDACT biobank), the Jacqueline Seroussi Memorial Foundation (2006 JSMF award), Prix Mois du Cancer du Sein (2004 award), Susan G. Komen for the Cure (SG05-0922-02), Fondation Belge Contre le Cancer (SCIE 2005-27), Dutch Cancer Society (KWF), Association Le Cancer du Sein, Parlons-en!, Deutsche Krebshilfe, the Grant Simpson Trust and Cancer Research UK. This trial was also supported by the EORTC Cancer Research Fund. Whole genome analysis was provided in kind by Agendia. We are grateful to all women participating in this study; all the investigators, surgeons, pathologists, and research nurses; the National Coordinating Centers/BIG Groups (BOOG, GOIRC, NCRI-BCG, SOLTI, UNICANCER, WSG); World Courier; the pharma companies Novartis, Roche, and Sanofi- Aventis; and Agendia.. This study was financially supported by the Breast Cancer Research Foundation (BCRF).

RESEARCH POSTER PRESENTATION DESIGN © 2015
www.PosterPresentations.com

This presentation is the intellectual property of the authors. Contact them at christine.desmedt@kuleuven.be for permission to reprint and/or distribute.