Characterization of immune microenvironment before and following anti-HER2 neoadjuvant therapy (NAT)

G. Griguolo[1], G. Serna[2], T. Pascual[3,4,5], R. Fasani[2], N. Chic[3], L. Paré[4], S. Pernas[6], M. Muñoz[3], M. Oliveira[7], M. Vidal[3], A. Llombart Cussac[8], J. Cortés[7,9], P. Galván[3], B. Bermejo[10], N. Martínez[11], R. López[12], S. Morales[13], P. Villagrasa[4], A. Prat[3,4], P. Nuciforo[2];

1 Department of Surgery, Oncology and Gastroenterology, University of Padova, Padova, IT; 2 Molecular oncology group, Vall d´Hebron Institute of Oncology, Barcelona, ES; 3 Medical Oncology, Hospital Clinic/IDIBAPS, Barcelona, ES; 4 SOLTI Breast Cancer Research Group, Barcelona, ES; 5 Department of Genetics - University of North Carolina, Chapel Hill, USA; 6Medical Oncology, Institut Catala d'Oncologia, Hospitalet De Llobregat, ES; 7 Medical Oncology, Vall d'Hebron University Hospital, Vall d'Hebron Institute of Oncology, Barcelona, ES; 8 Medical Oncology, Hospital Universitario Arnau de Vilanova, Universidad Catolica, Valencia, ES; 9 IOB - Institute of Oncology, Quironsalud Group, Madrid & Barcelona, ES; 10 Medical Oncology, Hospital Clínico Universitario de Valencia / INCLIVA / CIBERONC, Valencia, ES; 11 Medical Oncology, Hospital Universitario Ramón y Cajal, Madrid, ES;12 Medical Oncology, Hospital Clínico Universitario de Santiago / CIBERONC, Santiago De Compostela, ES; 13Breast Cancer Unit, Hospital Universitario Arnau de Vilanova, Lleida, ES.

Background

- Immune infiltration in HER2+ breast cancer (BC) predicts 1) prognosis in the early setting[1], 2) pathological complete response (pCR) following neoadjuvant antiHER2-based therapy[2]
- However, despite its well-recognized role, the complexity of the immune microenvironment, both in terms of composition and of interaction with tumor cells, remains largely unexplored.
- Multiplex immunohistochemistry (mIHC) holds opportunity to more comprehensively assess BC immunity, potentially providing information to improve immunotherapy.
- Here, we characterize the immune microenvironment of early HER2+ BC before and after treatment with lapatinib and trastuzumab without chemotherapy in the context of the PAMELA trial and its relationship with intrinsic subtyping and pathologic complete response (pCR).

Patients and Methods

Figure 1. PAMELA trial design

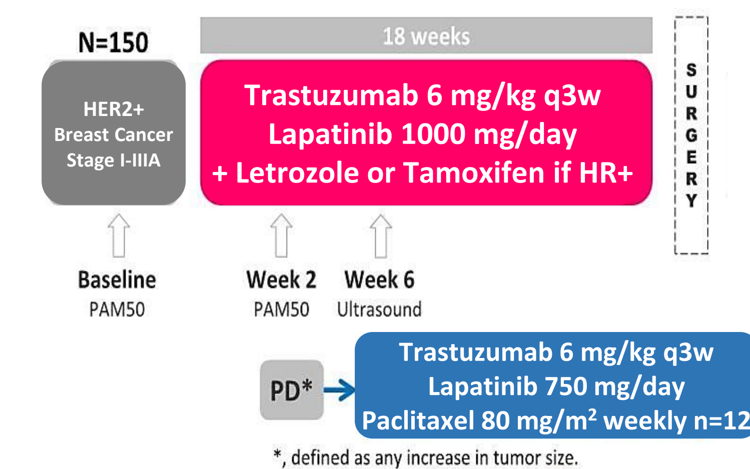
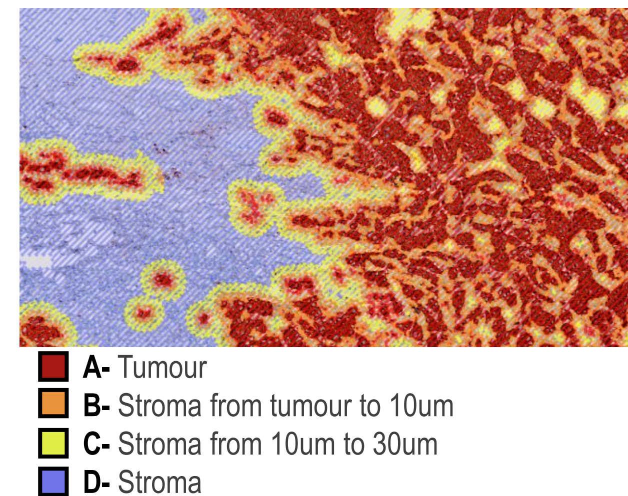


Figure 2. Classification according to localization



- In the PAMELA (NCT01973660) neoadjuvant phase II trial[3], 151 women with HER2+ breast cancer were treated with lapatinib and trastuzumab, and hormonal therapy if hormone receptor (HR) positive, for 18 weeks (**Fig.1**).
- Using the NGI (Next Generation Immunohistochemistry) mIHC workflow, a custom 6-plex panel, including immune subtyping (CD3, CD4, CD8, FOXP3), localization (keratin for tumor recognition), and activity (coexpression of Ki67 on immune cells to identify proliferating cells), was assessed on baseline (BSL, n=66) and day-15 (D15, n=52) biopsies from 76 patients treated in the PAMELA trial (**Fig.2**).
- Immune cell density (cells/mm2) and localization were determined by digital image analysis and classified in: intratumor (A), proximal peritumor (B - < 10um; C - 10 to 30um from tumor) and distal peritumor stroma (D).
- Intrinsic subtyping was determined at the same timepoints using PAM50 predictor (nCounter)[4].
- ANOVA was used to test differences in immune subpopulations across subgroups. Univariate logistic regression was used to test association with response. All statistical tests were two-sided and considered significant when $p<0.05$. R software was used for all analyses.

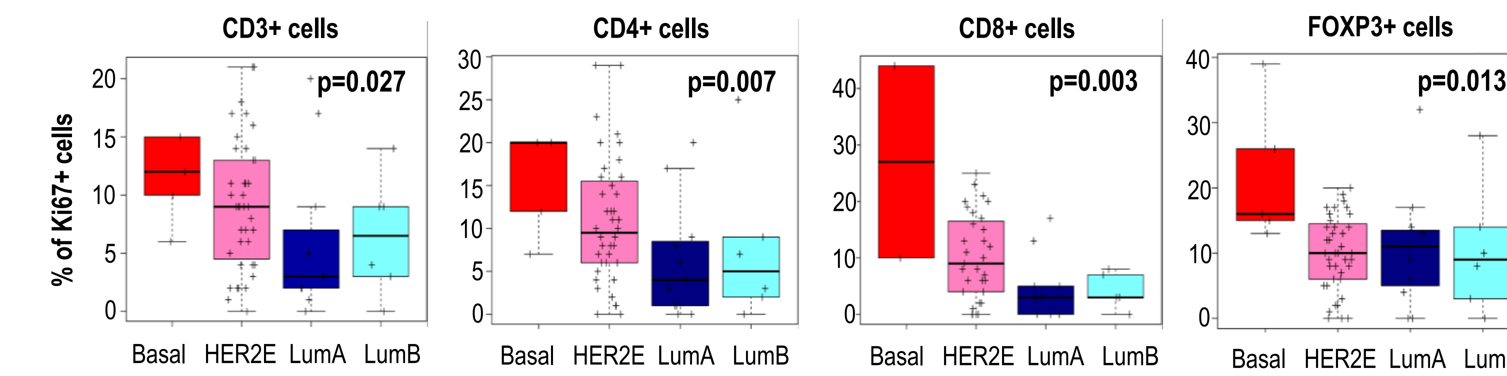
Results

Proliferation of immune cell subpopulations varies according to intrinsic subtype

At baseline, PAM50 subtype distribution was: Basal 8% (N=5), HER2-enriched (HER2E) 67% (N=44), Luminal A 17% (N=11), Luminal B 9% (N=6).

No significant difference in immune subpopulation densities (CD3+, CD4+, CD8+, FOXP3+) was observed according to PAM50 subtype. Fraction of proliferating cells (coexpressing Ki67) of all 4 immune subpopulations differed significantly according to subtype (**Fig. 3**).

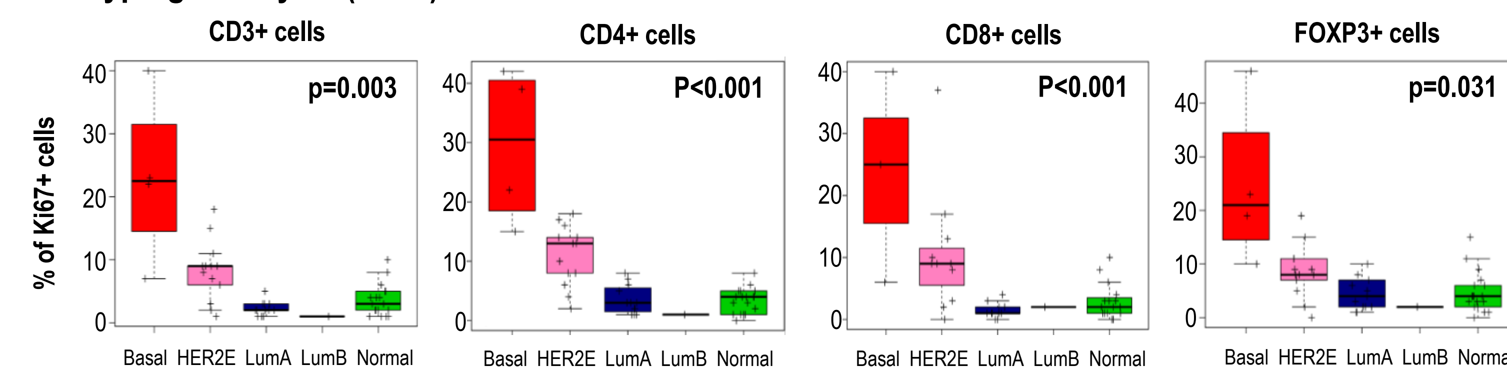
Figure 3. Percentage of proliferating (Ki67+) cells for each immune subpopulation according to PAM50 subtyping at baseline (N=66)



At D15, PAM50 subtype distribution was: Basal 8% (N=4), HER2-enriched (HER2E) 25% (N=13), Luminal A 29% (N=15), Luminal B 2% (N=1), Normal-like 37% (N=19).

No significant difference in immune subpopulation densities (CD3+, CD4+, CD8+, FOXP3+) was observed according to PAM50 subtype. Fraction of proliferating cells (coexpressing Ki67) of all 4 immune subpopulations differed significantly according to subtype (**Fig. 4**).

Figure 4. Percentage of proliferating (Ki67+) cells for each immune subpopulation according to PAM50 subtyping at Day 15 (N=52)



Association between immune subpopulation densities and response to anti-HER2 therapy

No significant difference in immune subpopulation densities (CD3+, CD4+, CD8+, FOXP3+) at baseline was observed according to response (pCR vs residual disease) to neoadjuvant treatment.

At D15, tumors achieving a pCR showed numerically higher densities of CD3+, CD8+, and FOXP3+ cells (significantly higher density of CD3+ cells and higher ratio in Ki67+CD8+ / Ki67+FOXP3+, **Table 1**).

Table 1. Immune cell densities at Day 15 according to pathologic response to neoadjuvant treatment

Immune Subpopulation	Immune cell density (D15) by response: Median (IQR)		
	pCR (N=12)	No pCR (N=40)	p-value
CD3+	1428 (569-1837)	608 (377-1213)	**0.026**
CD8+	943 (258-1157)	215 (102-396)	0.084
CD4+	824 (620-2029)	645 (191-1358)	0.946
FOXP3+	305 (106-514)	164 (87-325)	0.148
%Ki67+CD3+	4 (2-8)	3 (2-8)	0.983
%Ki67+CD4+	4 (1-8)	4 (2-8)	0.630
%Ki67+CD8+	2 (1-10)	2 (1-8)	0.215
%Ki67+FOXP3+	4 (2-8)	6 (2-9)	0.569
Ratio CD8/FOXP3	1.1 (0.2-1.5)	0.6 (0.2-1.1)	**0.029**
Ratio FOXP3/CD8	0.7 (0.5-2.3)	1.2 (0.9-2.9)	0.494

Immune cell localization influences association with response to anti-HER2 therapy

Localization of immune cells affects their association with response to anti-HER2 therapy. Association with pCR was stronger at D15 and for immune cells intratumor/more proximal to tumor (**Table 2**).

Table 2. Odds ratios (95% Confidence Interval) for pCR for 1000 cells/mm2 increases in immune cell density according to subpopulation and localization at baseline (a) and Day 15 (b).

Immune Subpopulation	Localisation (baseline)			
	A	B	C	D
CD3+ (N=62)	1.37 (0.97-1.94)	1.31 (0.95- 1.81)	1.35 (0.93-1.96)	1.02 (0.48- 2.17)
CD8+ (N=49)	1.51 (0.66-3.50)	1.33 (0.74- 2.40)	1.39 (0.69- 2.83	1.00 (0.26- 3.76)
FOXP3+ (N=62)	1.12 (0.92-1.36)	1.28 (0.88- 1.87)	1.43 (0.82- 2.48)	0.26 (0.01- 7.13)
CD4+ (N=62)	1.09 (0.71-1.68)	1.13 (0.78- 1.63)	1.19 (0.75- 1.90)	0.69 (0.27- 1.75)

Immune Subpopulation	Localization			
	A	B	C	D
CD3+ (N=49)	**1.38 (1.04- 1.82)**	**1.25 (1.01- 1.55)**	1.31 (0.98-1.73)	1.79 (0.87- 3.65)
CD8+ (N=42)	**1.61 (1.09- 2.39)**	**1.42 (1.01- 2.00)**	1.59 (0.97-2.61)	2.87 (0.73- 11.29)
FOXP3+ (N=49)	1.10 (0.99- 1.23)	**1.19 (1.01- 1.41)**	1.31 (0.99- 1.73)	4.61 (0.32- 66.13)
CD4+ (N=49)	1.09 (0.81- 1.47)	1.03 (0.76-1.41)	0.97 (0.62-1.51)	0.67 (0.19- 2.25)

Conclusions

- In early HER2+ BC, immune microenvironment shows differential activation according to tumor biology.
- Immune cells are significantly associated with pCR after priming by anti-HER2 treatment and when spatially interacting with tumor cells (proximal to tumour versus more distal regions)

References

1. Salgado R, et al. JAMA Oncol 2015
2. Solinas C, et al. Cancer Treat Rev 2017
3. Llombart-Cussac A, et al. Lancet Onc 2017
4. Parker JS, et al. J Clin Oncol. 2009

Acknowledgements

This study was funded, in part, by Instituto de Salud Carlos III - PI16/00904, The Breast Cancer Research Foundation, and Fundación Científica Asociación Española Contra el Cáncer (Ayuda Postdoctoral AECC 2017), Fundación Merck -- Immunología

This presentation is the intellectual property of the author/presenter.
Contact them at gaia.griguolo@unipd.it for permission to reprint and/or distribute.