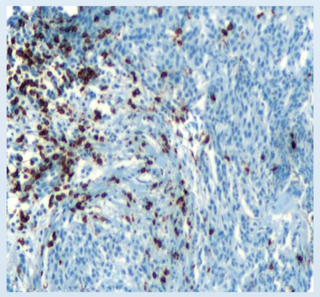
1067P- IMMUNOTHERAPY OF CANCER

Pathological complete response and changes related to T infiltrating lymphocytes and regulatory T cells in tissue and peripheral blood after neoadjuvant chemotherapy in breast carcinoma

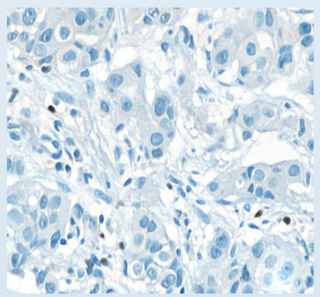
Luis de la Cruz Merino[1], Antonio Barco Sánchez[2], José Ibáñez Martínez[3], Javier Brugal Molina[3], Ana Vallejo Benítez[3], Mª Ángeles Lobo Acosta[1], Fernando Henao Carrasco[1], Esteban Nogales Fernández[1], Víctor Sánchez Margalet[2], Adoración Nieto García[4]

[1]Clinical Oncology Department. HUVM, Seville, Spain; [2]Biochemistry Department. HUVM, Seville, Spain; [3]Pathology Department. HUVM, Seville, Spain; [4]Public Health Department, Faculty of Medicine. Universidad de Sevilla, Sevilla, Spain.

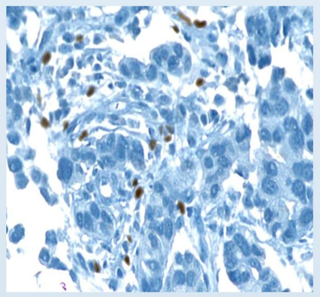
Background Some clinical trials in breast cancer have reported imppressive outcomes related to laboratory immune findings in neoadjuvant setting. In this context, tumor infiltrating lymphocytes (TILs) and regulatory T cells (Tregs) in tissue specimens and in peripheral blood are being tested as two emerging prognostic and predictive factors. Recently, Denkert et al. examined pretherapeutic core biopsies of 1058 patients showing that the presence of intratumoral lymphocytes and lymphocyte-predominant breast cancers were associated with a 31 and 41% pathological complete response (pCR) rates, respectively. On the opposite, pCR rates were only 2% in patients without any lymphocytic infiltration (1). Ladoire et al. reported another interesting study conducted on 56 patients with operable breast carcinoma where pCR patients had a significantly lower number of FOXP3 cells than nonresponders [2]. We designed a protocol to analyze specifically TILs before, during and after neoadjuvant chemotherapy (CT) in breast cancer in peripheral blood and tissue, and their eventual relationship with pathological complete response (pCR) following Miller and Payne criteria.



CD8+INTENSE



FOXP3+SLIGHT



FOXP3+INTENSE

Methods:

From march 2011 to january 2014, 47 patients with breast carcinoma treated with neoadjuvant CT in the Breast Cancer Unit of the Hospital Universitario Virgen Macarena, were included in the study protocol. CD3+, CD8+, CD8-16-56+ and Foxp3+ cell infiltrates were detected by immunohistochemistry before and after the end of neoadjuvant QT in tissue specimens. To evaluate the extent of lymphocytic infiltration, a grading system for semiquantitative scoring of lymphocytic infiltration based on the system established by Black et al. was used. In our system, grade 0 corresponded to absence of lymphocytes and grades 1 to 3 were related to increasing degrees of lymphocytic infiltration. Due to the scarse frequency of Foxp3+ cells in tumor microenvironment, a modified semiquantitative scoring system was used to assess these cells as Ladoire&Ghiringhelli group described (2). For all these labels, the levels of lymphocytic infiltration were evaluated by two independent pathologists.

Blood samples were collected in EDTA-K3 tubes before every cycle of QT to determine the immunophenotype and regulatory cell profile. Cell populations were determined by flow cytometry analysis of whole blood, including the study of CD3-CD4-CD25low and CD3-CD4-CD25high (Tregs).

Biostatistical analysis was performed comparing infiltrating TILs and circulating lymphocytes before and after neoadyuvant chemotherapy using the Wilcoxon matched pairs test. Circulating Tregs (%) was compared in subgroups of patients using Mann-Whitney U test (2 subgroups) or Kruskall-Wallis one-way analysis of variance test (>2 subgroups). Correlation of Tregs with CD8+T cells was measured using the Spearman correlation test. All the analysis was made on strata defined by Her2 status (positive or negative) and clinical outcomes (pCR or non-pCR).

Results:

By january 2014, 47 patients (18 Her2+/ 29 Her2-) were operated. Pathological complete responses (pCR) or near pCR (grade 4/5 Myller&Payne) were attained in 20 patients. pCR was achieved in 66,66% (12/18) of tumors overexpressing Her2 treated with carboplatin-docetaxel or docetaxel plus herceptin in both schedules, but in only a 27,5% (8/29) of Her2 -ve tumors treated with TAC schedule (docetaxel, doxorrubicin and cyclophosphamide). Absence and/or disappearance of Tregs in tissue (Black grading system=0) was more frequent after neoadjuvant CT (58,9 vs 5,1%) (figure 1 and table 1). Using Wilcoxon test for non-parametric variables differences were statistically significant (p> 0,0005). Furthermore, average whole blood CD3-CD4-CD25high (regulatory) T cells were 126,36 before CT and 91,19 after neoadjuvant CT (p 0,006) (table 2 and figure 2). Overall Tregs diminished in the pCR and non pCR groups in tissue and blood but without statistically significant differences with respect to degree of pathological response. When applying Spearman rho correlation coefficient, no statistical association was found among Treg decrease in blood and tissue.

Infiltration by FoxP3 (Black modified)	Biopsy pre-neoadjuvant CT	%	Biopsy post-neoadjuvant CT	%
0 = Absence of Foxp3	2	5,1	23	58,9
1 = 1-4 Foxp3 in 5f/20x	21	53,8	14	35,9
2 = 5-14 Foxp3 in 5f/20x	12	30,7	2	5,1
3 => 15 Foxp3 in 5f/20x	4	10,2	0	0
Total	39		39	

Table 1. Evolution of Treg infiltrates before and after CT

	Basal	B4	Difference	LI IC95%	LS IC95%	p	n
Treg	126,36	91,19	35,17	8,89	61,46	0,01	43
NK	261,37	172,25	89,11	26,11	152,12	0,007	42
CD3	29,22	21,82	7,4	-0,54	15,34	0,067	44
CD8	99,28	62,13	37,15	14,34	59,95	0,002	42

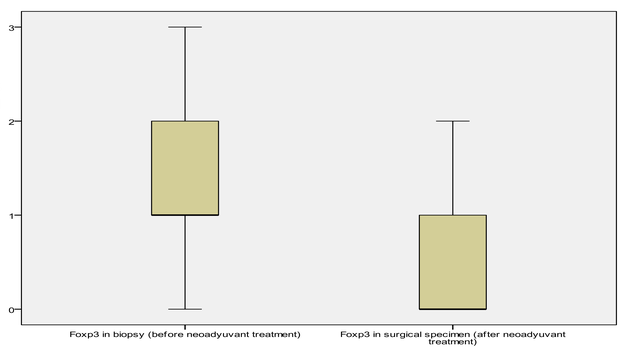
Table 2 Immunophenotype evolution in blood in all the population

CONCLUSIONS:

Our study confirms previous findings with respect to Treg decrease after neoadjuvant CT in surgical specimens. Furthermore neoadjuvant CT decreases CD3-CD4-CD 25high (regulatory) T cells in peripheral blood in all the population. Differences among predefined subgroups (pCR/non-pCR and her2+/her2-) were not statistically significant, however these data should be taken cautiously due to the small sample size (47 pts) of this study. Prospective and validation studies are needed to ascertain the value of Treg as a new therapeutical target in breast carcinoma

References: 1) C. Denkert, S. Loibl, A. Noske et al., "Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer," *Journal of Clinical Oncology*, vol. 28, no. 1, pp. 105–113, 2010.
2) S. Ladoire, L. Arnould, L. Apetoh et al., "Pathologic complete response to neoadjuvant chemotherapy of breast carcinoma is associated with the disappearance of tumor-infiltrating Foxp3+ regulatory T cells," *Clinical Cancer Research*, vol. 14, no. 8, pp. 2413–2420, 2008.

Fig2- Foxp3 infiltrates in biopsy and surgical specimen



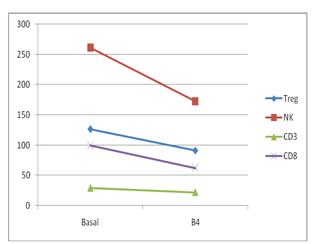


Fig 1- Evolution of lymphocytes means in peripheral blood pre and post CT in all the population.

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