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Analysis of αβ and γδ circulating T cells in the PHERGain randomized phase 2 trial for patients with HER2-positive early breast cancer receiving neoadjuvant trastuzumab and pertuzumab without chemotherapy: LINGain

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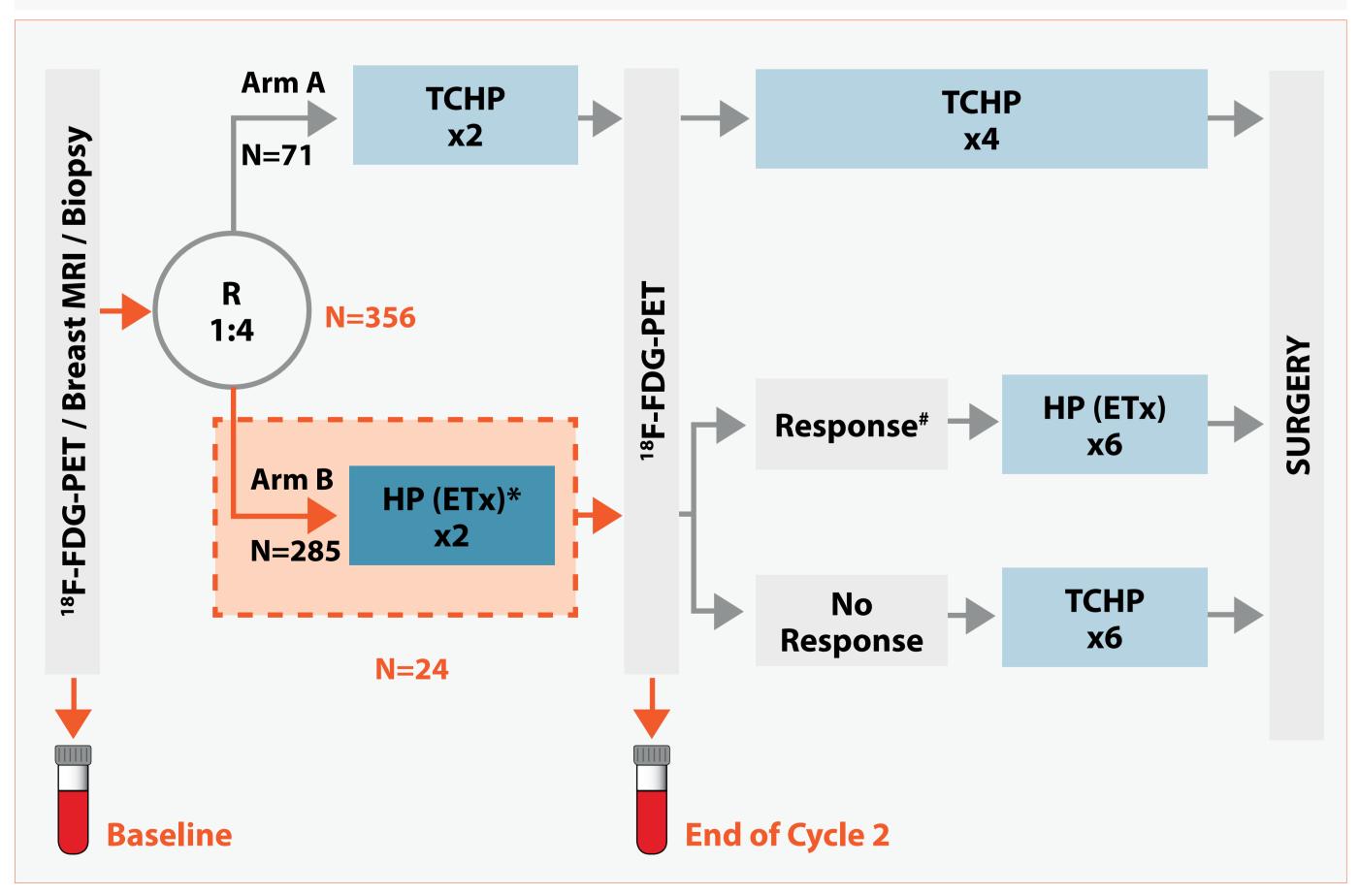
BACKGROUND

- The presence of tumor-infiltrating lymphocytes (TILs) at baseline is an independent, positive prognostic marker in HER2-positive (HER2^{+}) early-stage breast cancer (EBC) patients, treated with trastuzumab and pertuzumab (HP)–based neoadjuvant chemotherapy [1-2].

- Among distinct subsets of TILs, conventional CD8+ αβ T cells require TCR signaling as a part of adaptive immunity, while the unconventional γδ T cells display also innate-like activity via the NKG2D receptor [3].

- Human γδ T cells normally comprise only 1–5% of circulating T lymphocytes but undergo rapid expansion in response to tumor or inflammation [4]. Recently, specific γδ T cell subsets were associated with remission and improved overall survival of patients with triple-negative breast cancer [5]. However, very little is known about circulating αβ and γδ T cells subsets in the context of patients treated with HP.

- The randomized, phase 2 PHERGain study showed that early ^{18}F-FDG-PET assessment might be used to identify HER2^{+} EBC patients who achieved a pathological complete response through a neoadjuvant chemotherapy-free regimen with HP [6].

OBJECTIVE

- The aim of the prospective, case-control LINGain study was to investigate longitudinal changes in the proportion, activation, and apoptosis of circulating αβ and γδ T cell subsets in a cohort of patients with HER2^{+} EBC from PHERGain, who were treated with neoadjuvant HP.

STUDY DESIGN

PHERGain Study Design (NCT03161353)

* Patients with hormone receptor-positive received endocrine therapy concomitantly with trastuzumab and pertuzumab (except on chemotherapy).
# Patients who were responsers after cycle 2 with SUVmax reduction ≥40% as per adapted EORTC criteria.

Abbreviations: ETx, endocrine therapy (letrozole post-menopausal/tamoxifen pre-menopausal); EORTC, European Organization for the Research and Treatment of Cancer; HP, trastuzumab and pertuzumab; MRI, magnetic resonance imaging; FDG-PET, 18F-fluorodeoxyglucose positron emission tomography; R, randomization; SUVmax, the maximum standardised uptake value; TCHP, docetaxel, carboplatin, trastuzumab, and pertuzumab.

MATERIAL AND METHODS

Patients and Normal Controls

- Blood samples were obtained from 24 consecutive patients who were assigned to arm B of PHERGain trial at Arnau de Vilanova Hospital (Valencia, Spain). Patients initially received 2 cycles of neoadjuvant PH with endocrine therapy if had hormone receptor-positive status.

- Data cutoff date: September 15, 2021.

- Normal healthy donors were recruited among 48 volunteers with an intent to match controls and patients for age.

Blood Samples

- Blood samples from patients were collected before cycle 1 day 1 (BL) and at the end of the second cycle (cycle 2 day 21 [End C2]) during the outpatient visits and before any therapies.

- Before BL blood collection from normal healthy donors, subjects were interrogated for excluding any autoimmune, known immunodeficiency diseases, and treatment with antibiotics, immunosuppressive drugs or any kind of vaccine within 6 months prior to inclusion.

- Venous blood (10 mL) were collected into STRECK® tubes and immediately processed at central laboratory.

Flow Cytometry

- Flow cytometry was performed as previously described [7].

- Peripheral blood mononuclear cells were stained for expression of surface markers using the following anti-human monoclonal antibodies: TCR PAN αβ, TCR PAN γδ, CD19, CD56, CD4, CD3, CD8, CD45, CD45RA, and CD62L. Subset distribution of T cell differentiation within naïve (TN), central memory (TCM), effector memory (TEM), and terminally differentiated effector memory (TEMRA) cells was also determined.

- The apoptosis detection was performed with ANNEXIN V-FIT-C/7-AAD Kit (Beckman Coulter).

- Acquisition and analysis were done on a Beckman-Coulter multiparameter flow cytometry analyzer and later analyzed with Kaluza Software.

Statistical Method

- The changes in the number of T cells and the apoptotic levels across controls, patients, treatment periods, and T cell subsets were analyzed with paired and unpaired, 2-sided Wilcoxon tests. P values < 0.05 were considered statistically significant.

- Data were analyzed using the R software version 4.0.2. released on 2020-06-22.

RESULTS

1. Recruitment and Patient Disposition

- Between December 21, 2017, and December 10, 2018, 24 patients who were allocated in arm B of PHERGain and 48 normal healthy donors were recruited at a single-center in Spain.

- The median age of cancer patients was 50 (IQR 45–59) years vs. 52 (IQR 41–58.3) years in the controls (Table 1).

Table 1. Demographic and Disease Characteristics

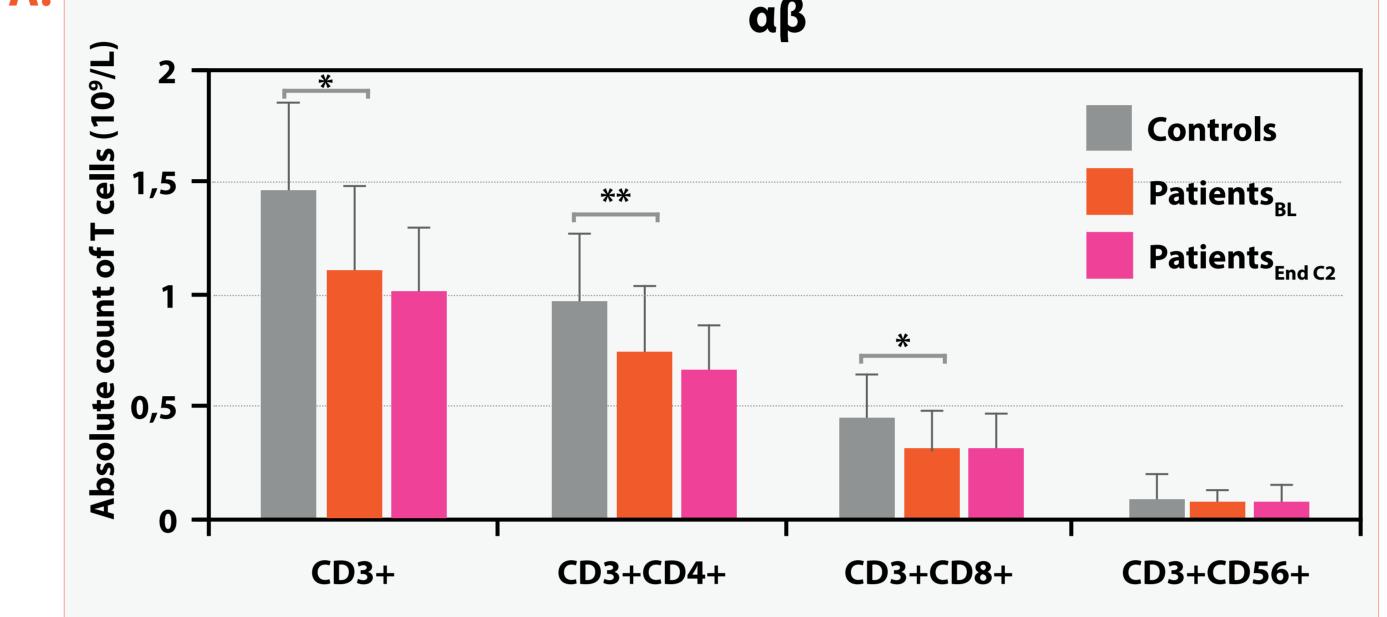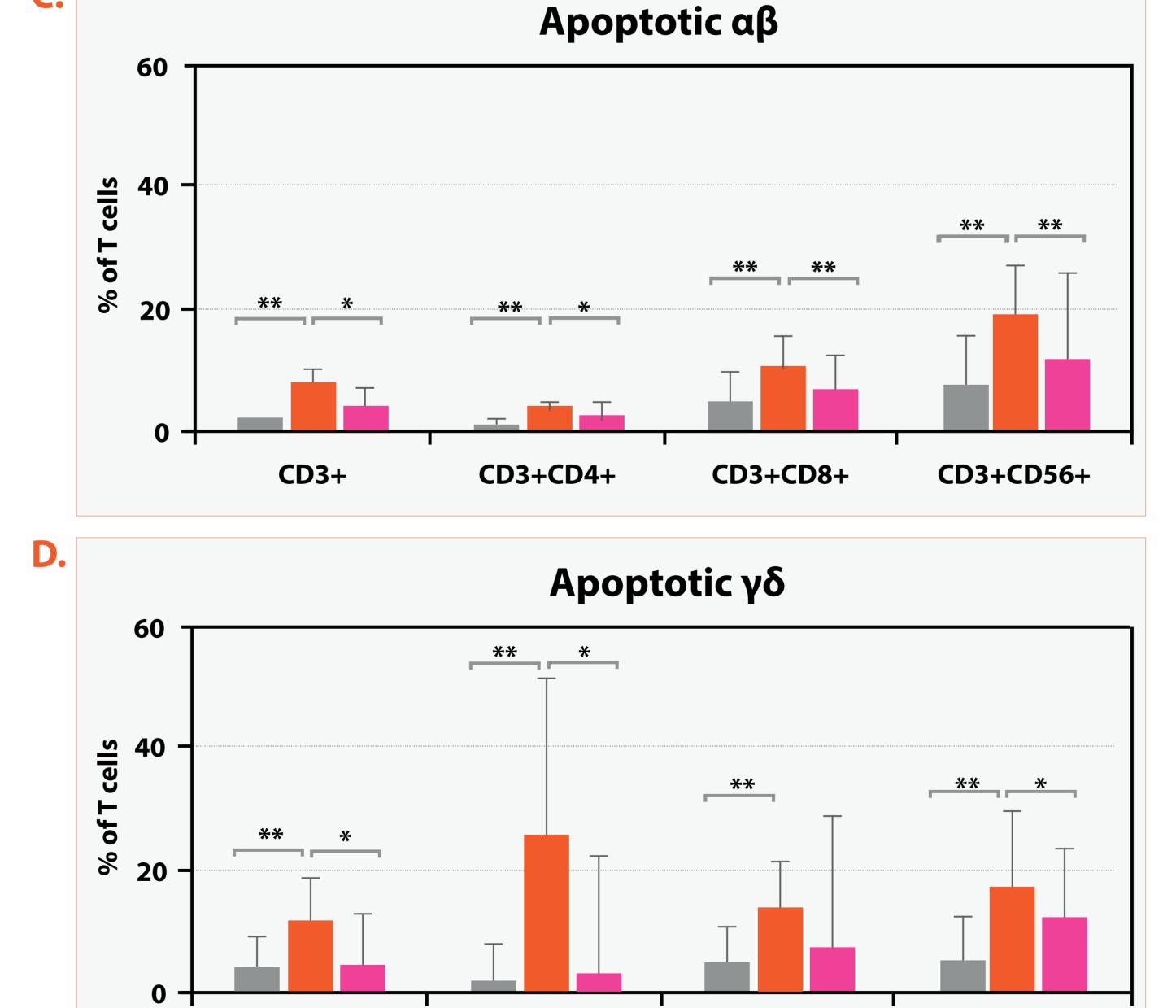| Characteristic	Patients (N= 24)	Controls (n=48)
Age, years	50 (45–59)	52 (41–58.3)
≤50	12 (50)	23 (47.9)
>50	12 (50)	25 (52.1)
Female	24 (100)	48 (100)
ECOG Performance Status		
0	23 (95.8)	-
1	1 (4.2)	-
Postmenopausal		
No	11 (45.8)	-
Yes	13 (54.2)	-
Stage		
I	3 (12.5)	-
II	17 (70.8)	-
IIIA	4 (16.7)	-
Nodal status		
Negative	13 (54.2)	-
Positive	11 (45.8)	-
Hormone receptor status		
ER[+] or PR[+] or both	19 (79.2)	-
ER[-] and PR[-]	5 (20.8)	-
HER2 status		
3+	19 (79.2)	-
2+ and ISH[+]	5 (20.8)	-
Ki67%		
≤20%	6 (25)	-
>20%	17 (70.8)	-
Not evaluated	1 (4.2)	-
Tumor grade		
I (well differentiated)	2 (8.3)	-
II (moderately differentiated)	10 (41.7)	-
III (poorly differentiated)	8 (33.3)	-
Unknown *	4 (16.7)	-
pCR		
Yes	13 (54.2)	-
No	11 (45.8)	-
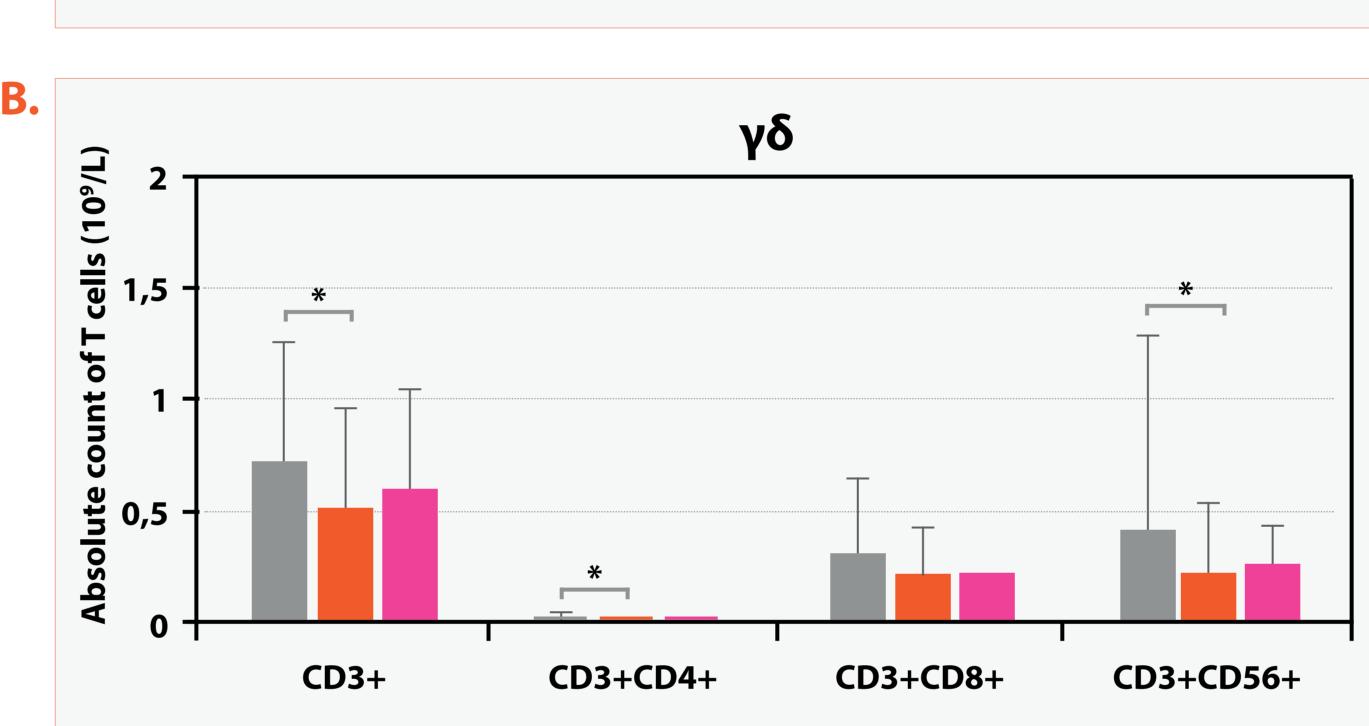
Data are expressed as median (IQR) or n (%).
* Tumor grade could not be assessed.
Abbreviations: ECOG, Eastern Cooperative Oncology Group; ER, estrogen receptor; IQR, interquartile range; ISH, in-situ hybridization; pCR, pathological complete response (ypT0/is ypN0); PR, progesterone receptor.

2. Functional and Apoptotic T Cells in the Peripheral Circulation of HER2^{+} EBC Patients and Normal Controls
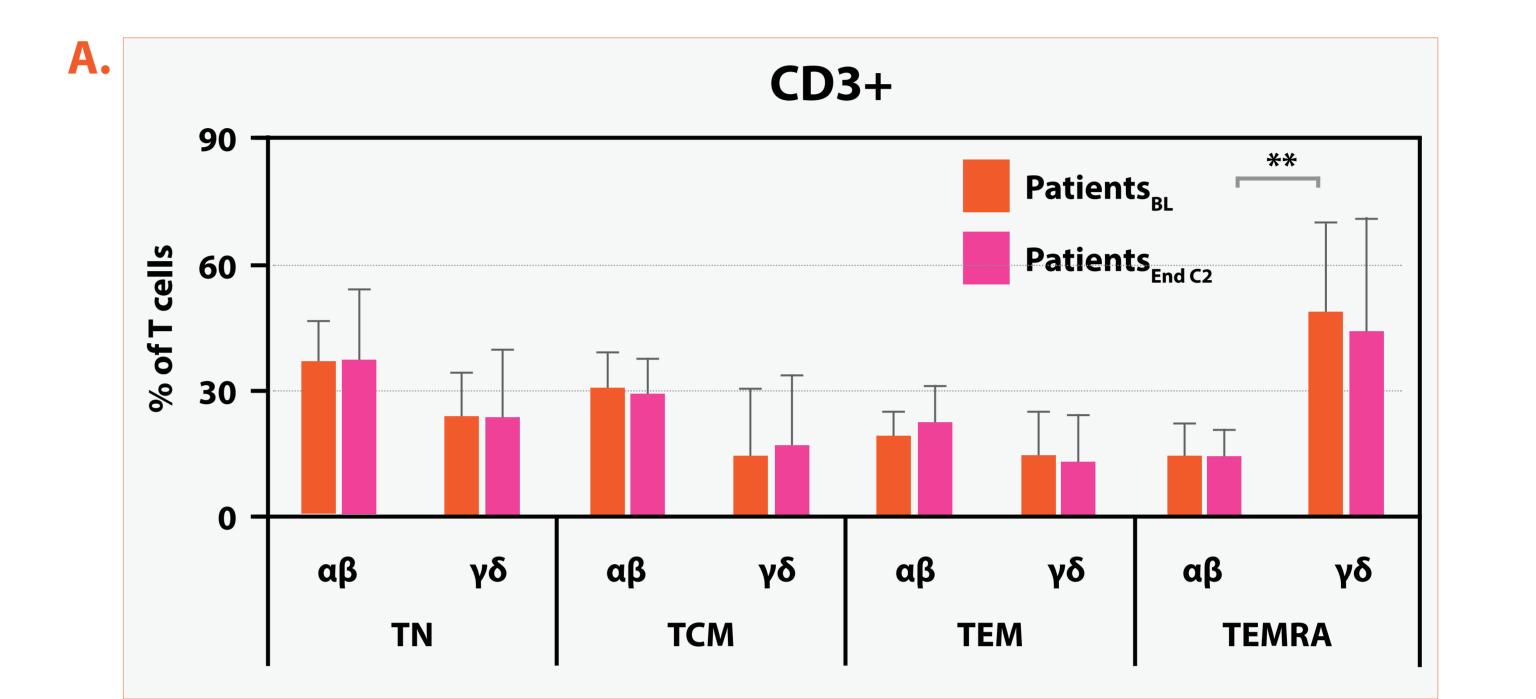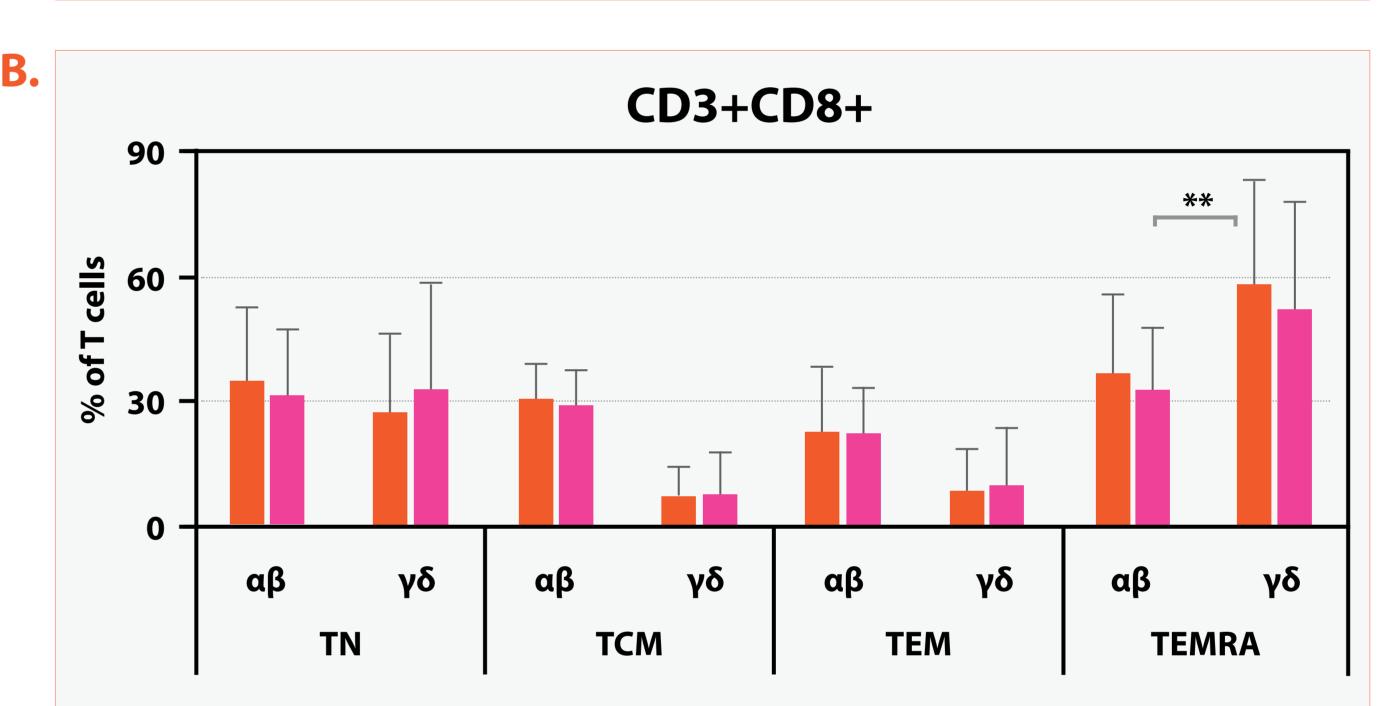

Box plots showing distribution of total number of CD3+, CD3+CD4+, CD3+CD8+, and CD3+CD56+ T cells in HER2^{+} EBC patients. **A,B.** Patients at baseline had significantly lower absolute αβ and γδ T cell subsets than controls. **C,D.** Patients at baseline showed higher apoptotic rates of αβ and γδ T cell subsets than controls. After 2 cycles of HP, apoptosis in both these groups was significantly reduced.
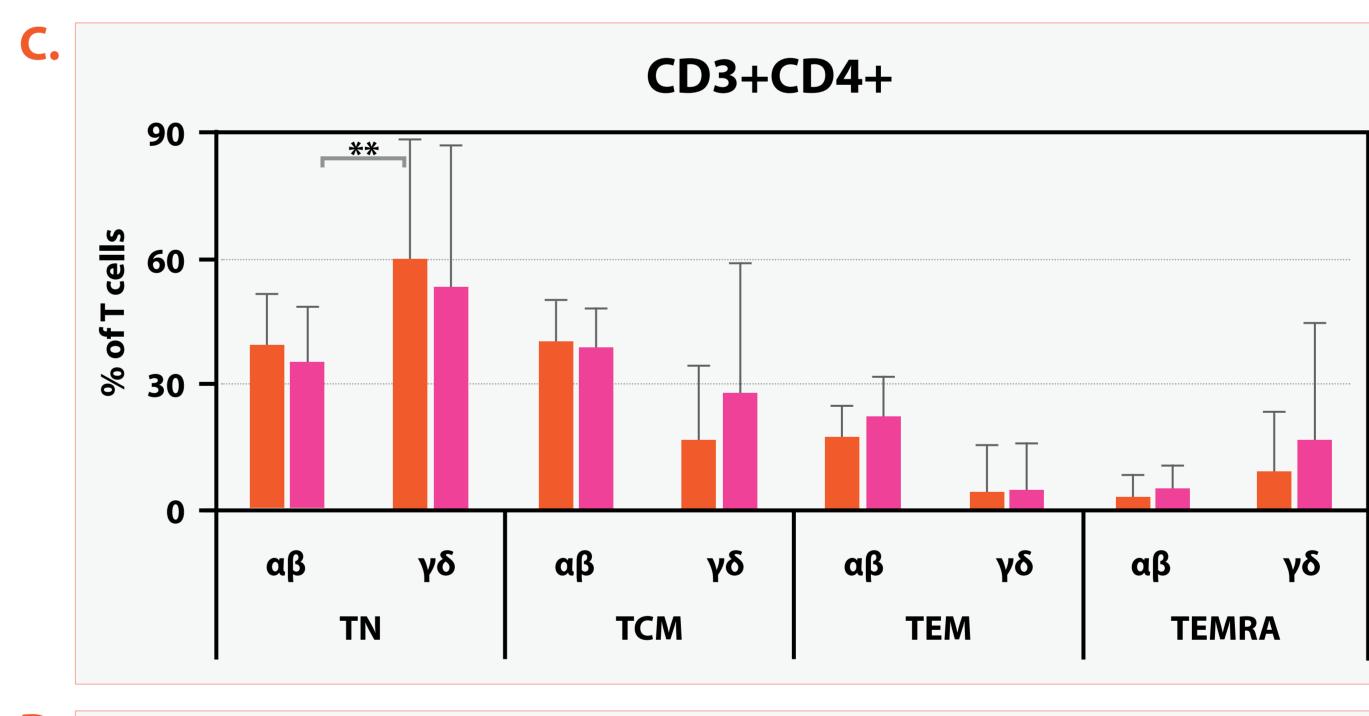Results are expressed as mean ± standard deviation.
* $P \leq 0.05$; ** $P \leq 0.001$.

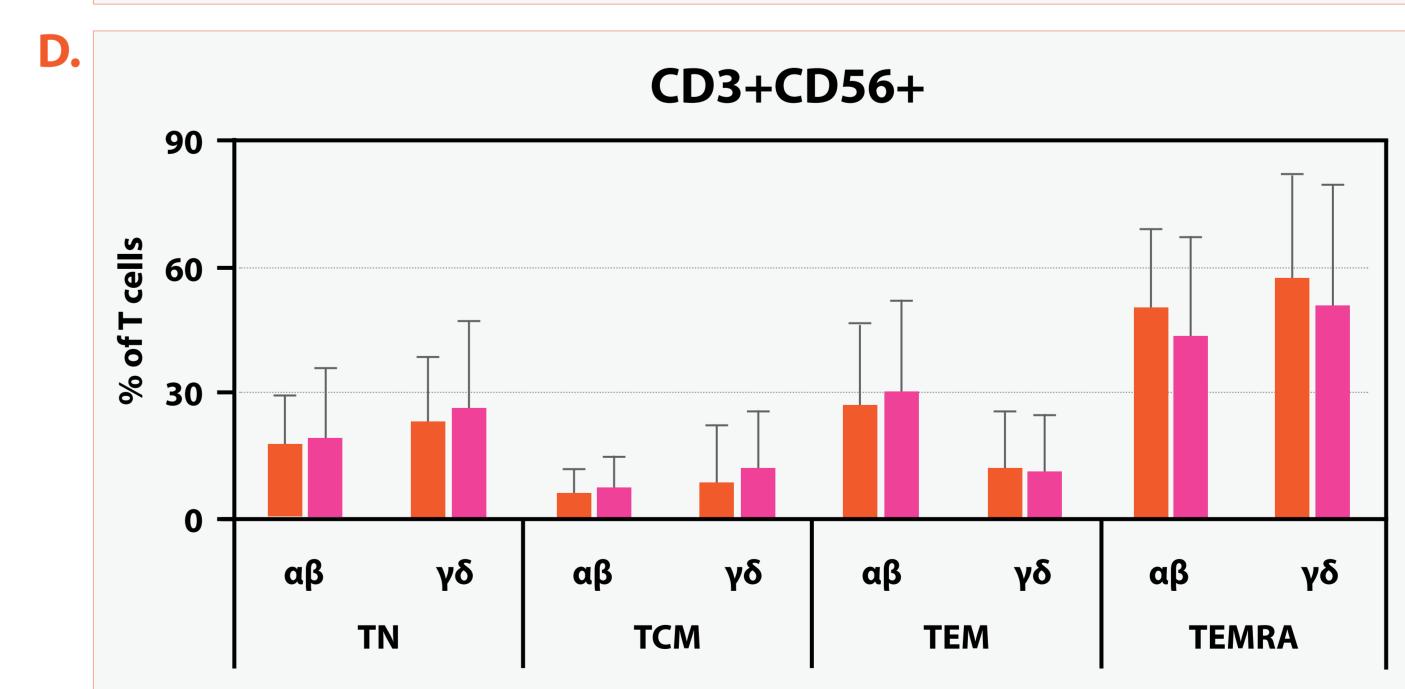### 3. Differentiation Subsets of T Cells in the Peripheral Circulation of HER2^{+} EBC Patients Before and After Neoadjuvant Treatment

Box plots showing the effect of neoadjuvant treatment with HP on the number of TN, TCM, TEM, and TEMRA T cells in HER2^{+} EBC patients. **A,B.** A significantly higher fraction of γδ TEMRA compared with αβ TEMRA was observed in CD3+ and CD3+CD8+ T cell subsets. **C.** CD3+CD4+ T cell subset had a significantly higher fraction of γδ TN compared with αβ TN. **D.** No statistically significant differences were observed.
Results are expressed as mean ± standard deviation.
** $P \leq 0.001$.

4. Association of Immunological Parameters with Baseline Characteristics and Clinical Outcome

- Among 24 patients, 54.2% achieved a pathological complete response (ypT0/is ypN0) after 8 cycles of HP with or without endocrine therapy.

- No evidence of T cell association was found with pathological complete response nor clinical characteristics in EBC patients.

CONCLUSIONS

- To our knowledge, LINGain is the first study to assess the evolution of peripheral αβ and γδ T cells in the context of neoadjuvant chemotherapy-free regimen with HP for HER2^{+} EBC patients.

- Our data suggest that proportions of αβ and γδ T cells were significantly lower in patients compared with T cells obtained from the peripheral circulation of normal blood donors.

- Increased rate of spontaneous apoptosis was observed in all subsets of peripheral αβ and γδ T cells obtained from patients compared with normal blood donors.

- Intriguingly, treatment with HP significantly decreased the proportion of apoptotic T cells in with HER2^{+} EBC patients.

- Further investigation is required in larger cohorts to validate these findings and provide more complete evidence on the functional impairment of immune system and the anti-apoptotic effect mediated by dual HER2 blockade with HP in HER2^{+} EBC.

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