Pembrolizumab in combination with gemcitabine for patients with HER2-negative advanced breast cancer: GEICAM/2015-04 (PANGEA-Breast) study

Luis de la Cruz-Merino (luis.cruz.sspa@juntadeandalucia.es)
Virgen Macarena University Hospital, University of Seville

María Gion
Hospital Universitario Ramón y Cajal

Josefina Cruz
Hospital Universitario de Canarias, Santa Cruz de Tenerife

Jose Luis Alonso-Romero
Hospital Clínico Universitario Virgen de la Arrixaca-IMIB

Vanesa Quiroga
Badalona Applied Research Group in Oncology (B-ARGO Group). ICO Badalona.

Fernando Moreno
Hospital Clínico Universitario San Carlos

Raquel Andrés
Hospital Clínico Universitario Lozano Blesa

Marta Santisteban
Navarra Institute for Health Research

Manuel Ramos
Centro Oncológico de Galicia, A Coruña

Esther Holgado
Hospital La Luz

Javier Cortés
International Breast Cancer Center (IBCC), Quiron Group, Barcelona and Madrid

Elena López-Miranda
Hospital Universitario Ramón y Cajal

Alfonso Cortés
Hospital Universitario Ramón y Cajal

Fernando Henao
Virgen Macarena University Hospital, University of Seville

Natalia Palazón-Carrión
Virgen Macarena University Hospital, University of Seville

Luz Milva Rodriguez
Hospital Universitario de Canarias, Santa Cruz de Tenerife

Isaac Ceballos
Hospital Universitario de Canarias, Santa Cruz de Tenerife

Asunción Soto
Hospital Clínico Universitario Virgen de la Arrixaca-IMIB

Ana Puertes
Hospital Clínico Universitario Virgen de la Arrixaca-IMIB

Maribel Casas

GEICAM – Spanish Breast Cancer Group
Sara Benito
GEICAM – Spanish Breast Cancer Group
Massimo Chiesa
GEICAM – Spanish Breast Cancer Group
Susana Bezares
GEICAM – Spanish Breast Cancer Group
Rosalía Caballero
GEICAM – Spanish Breast Cancer Group
Carlos Jiménez-Cortegana
Virgen Macarena University Hospital, University of Seville
Víctor Sánchez-Margalet
Virgen Macarena University Hospital, University of Seville
Federico Rojo
IIS-Fundación Jiménez Díaz

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Abstract

Background: We evaluated a combination of two immunomodulatory agents considering a synergism that may induce long-term clinical benefit in advanced breast cancer (ABC).

Methods: HER2-negative ABC patients received 21-day cycles of pembrolizumab 200 mg (day 1) and gemcitabine (days 1 and 8). A run-in-phase (6+6 design) was planned with two dose levels (DL) of gemcitabine (1,250 mg/m^2 [DL0]; 1,000 mg/m^2 [DL1]) to determine the recommended phase II dose (RP2D). The primary objective was objective response rate (ORR). Tumor infiltrating lymphocytes (TILs) density and PD-L1 expression in tumors and myeloid-derived suppressor cells (MDSCs) levels in peripheral blood were analyzed.

Results: Fourteen patients were treated with DL0, resulting in RP2D. Thirty-six patients were evaluated during the first stage of Simon's design. Recruitment was stopped as statistical assumptions were not met. The median age was 52; 21 (58%) patients had triple-negative disease, 28 (78%) visceral involvement, and 27 (75%) ≥2 metastatic locations. Progression disease was observed in 29 patients. ORR was 15% (95% CI, 5–32). Eight patients were treated ≥6 months before progression. Fourteen patients reported grade ≥3 treatment-related adverse events. No association was found between TILs density and treatment efficacy. Patients with negative PD-L1 expression showed significantly prolonged progression-free survival. MDSCs levels were associated with treatment response.

Conclusion: Pembrolizumab 200 mg and gemcitabine 1,250 mg/m^2 were considered as RP2D. The objective of ORR was not met; however, 22% patients were on treatment for ≥6 months. Tumor PD-L1 expression and MDSCs levels suggested a potential predictive role in this population.

Trial registration number: ClinicalTrials.gov and EudraCT (NCT03025880 and 2016-001779-54, respectively).

Background

Immunotherapy based on immune checkpoint inhibitors (ICIs) has revolutionized treatment of several cancer types. An impact on overall survival (OS) is documented in advanced setting(1–3). The potential role of immunotherapy in breast cancer (BC) remains unclear. Efforts have been focused on the triple-negative (TN) BC subtype, as it has high levels of potential immune biomarkers in its microenvironment. Two phase III clinical trials(4–6) reported positive results in PD-L1 + metastatic TNBC patients. However, other trials(7) with similar designs have recently reported negative outcomes. Therefore, the real value of immunotherapy for TNBC needs still to be clarified.

In this trial, we aimed to test whether the combination of gemcitabine and pembrolizumab could be feasible and provide meaningful responses in pretreated advanced BC (ABC) patients with TNBC or luminal A/B subtype according to St. Gallen recommendations(8), irrespective of their PD-L1 statuses. Gemcitabine was combined with pembrolizumab as immunogenic properties through the elimination of myeloid-derived suppressor cells (MDSCs) and regulatory T lymphocytes (Tregs) were previously suggested in preclinical tumor models(9–12).

Methods

Study design

The PANGEA-Breast was an open-label, single-arm, multicenter phase II trial conducted in Spain. Gemcitabine plus pembrolizumab was evaluated in human epidermal growth factor receptor 2 (HER2)-negative ABC patients, with balanced distribution between TN and hormone receptor-positive (HR+) cohorts. In an initial exploratory run-in-phase with a 6 + 6 design, toxicity was evaluated within the first cycle. Fixed doses of pembrolizumab on day 1 and gemcitabine on days 1 and 8 of each 21-day cycle were administered to determine the recommended phase II dose (RP2D) based on the occurrence of any dose-limiting toxicity (DLT). Dose level (DL) 0 comprised pembrolizumab 200 mg and gemcitabine 1,250 mg/m^2 as an intravenous (IV) infusion. De-escalation to DL-1 (gemcitabine 1,000 mg/m^2) was planned if DL0 was not tolerable.

Once the RP2D was defined, eligible patients were enrolled in phase II. The primary objective was the objective response rate (ORR), defined as the number of patients with ≥ 1 treatment dose and complete response (CR) plus partial response (PR) according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. The secondary objectives were clinical benefit rate (CBR) with stable disease (SD) of ≥ 24 weeks, duration of response (DoR), progression-free survival (PFS), OS, and safety and tolerability according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.0. The exploratory objectives were to assess the efficacy based on immune-related response criteria, search for tumor tissue and peripheral blood biomarkers for clinical activity, correlate a set of immune biomarkers with disease evolution and efficacy of the combination, and comparison of this set of biomarkers from cohorts of healthy volunteers and patients from this trial.

The study was conducted according to the International Conference on Harmonization Good Clinical Practice Guidelines and Declaration of Helsinki and was approved by the institutional ethical review boards of the participating sites and Spanish health authorities. It was registered at ClinicalTrials.gov and EudraCT (NCT03025880 and 2016-001779-54, respectively). Written informed consent was obtained from all patients before performing any protocol-specific procedures.

Patients

The key inclusion criteria were women aged ≥ 18 years; HER2-negative ABC by immunohistochemistry (IHC) and/or *in situ* hybridization based on local testing of the most recent tumor biopsy; ≥10 mm measurable lesion as per the RECIST 1.1; an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1; prior anthracyclines and taxanes (unless contraindicated), and ≥ 2 endocrine therapy (ET) and ≤ 4 chemotherapy (CT) lines for ABC; patient agreement for a fresh metastatic tumor biopsy at inclusion and progressive disease (PD); adequate organ function; negative pregnancy test for women of child-bearing potential (WCBP) and adequate contraception. Patients with treated brain metastases and stable without steroids were allowed. Healthy controls from the University Hospital Virgen Macarena in Seville, Spain, provided written informed consent to participate in the analysis.

Treatment plan

Patients received pembrolizumab (200 mg IV) on day 1 and gemcitabine (1,250 mg/m^2 IV) on days 1 and 8 of each 21-day cycle until objective PD, clinical PD (under investigator's judgment), unacceptable toxicity, death, or consent withdrawal, whichever occurred first.

Evaluation procedures

Baseline assessments were performed ≤ 28 days from starting treatment. These included tumor assessment using radiological tests accepted by the RECIST 1.1, standard 12-lead electrocardiogram, hematology, biochemistry, coagulation test, thyroid hormones, urinalysis, physical examination, ECOG PS evaluation, and pregnancy test in WCBP. Tumor assessments were performed every 9 weeks until PD. AEs were graded using the NCI-CTCAE v4.0. Other safety endpoints included regular monitoring of vital signs and laboratory tests.

Biomarker analyses

TILs and PD-L1 assessments

Thirty (83%) available pre-treatment metastatic tumor samples were assessed for TILs density, according to the suggested international guidelines(13). Cut-offs explored for TILs evaluation were ≥ 5%, ≥ 10%, and ≥ 20%. PD-L1 expression measured using IHC was assessed in 29 pre-treatment metastatic tumor samples using the mAb anti-PD-L1 clone 22C3 (Merck, Kenilworth, NJ). Immune cell (IC) scores and combined positive score (CPS) were obtained. PD-L1 IC score and CPS ≥ 1 were considered positive. Cut-offs (≥ 5, ≥ 10, and ≥ 20) were also explored for CPS.

The logistic and Cox regression models were used to evaluate the association of TILs density and PD-L1 expression with treatment efficacy in terms of ORR, CBR (with SD ≥ 24 weeks) and PFS according to the RECIST 1.1.

Immunophenotyping of whole peripheral blood using flow cytometry analysis

Blood samples were collected in EDTA-K3 tubes at baseline, before cycles 3 (C3) and 6 (C6), or at the end of treatment (EOT), whichever occurred first, to determine MDSCs counts. Cell populations were determined using flow cytometry of the whole blood using the BD FACSCanto™ system (BD Biosciences, San Jose, CA, USA). Monocytic-MDSCs (M-MDSCs) were defined as CD45 + CD11b + CD33 + HLA-DR − CD14 + CD15 − and granulocytic-MDSCs (G-MDSCs) as CD45 + CD11b + CD33 + HLA-DR − CD14 − CD15+.

Monoclonal antibodies

Antibodies were obtained from Becton Dickinson Immunocytometry Systems (BD Biosciences, CA, USA) and were used at the manufacturer's recommended concentrations.

Statistical analyses

Simon's minimax two-stage design was employed for the phase II part of the study with the option of early stopping owing to lack of response. The sample size was calculated by testing the null hypothesis (H0) that gemcitabine resulted in an ORR of approximately 20%. With the study combination, the alternative hypothesis was 35% (an absolute increase of 15%); with an alpha error of 0.05 and a power of 80%, 53 evaluable patients were required. The first stage should include 31 evaluable patients, and if at least seven presented a response, recruitment would continue to include 53 evaluable patients. The H0 of 20% was rejected if ≥ 16 responses were observed in 53 patients. The SAS Enterprise Guide (version 7.15) was used for all analyses.

Results

Patients' characteristics

From June 2017 to May 2018, 36 patients were recruited for the first stage of Simon's minimax two-stage design (Fig. 1); however, only five patients achieved a response, and recruitment was stopped. Their characteristics are presented in Table 1. Approximately 58% of the tumors were TN. Twenty-three (64%) patients had up to two metastatic locations, and 28 (78%) had visceral involvement. Patients had a median of four prior therapy lines for ABC.

Table 1
Patients and disease characteristics

Characteristics	N = 36
Age, years (median [range])	51 (31–77)
<65, n (%)	33 (91.7)
≥65, n (%)	3 (8.3)
Menopausal status, n (%)	
Postmenopausal	26 (72.2)
Premenopausal	10 (27.8)
ECOG PS, n (%)	
0	23 (63.9)
1	12 (33.3)
2	1 (2.8)
Time since 1st BC diagnosis to study inclusion, years (median [range])	4 (1–37)
Time since M1 diagnosis to study inclusion, years (median [range])	2 (0.2–14)
Number of metastatic locations, n (%)	
1	9 (25.0)
2	14 (38.9)
≥ 3	13 (36.2)
Metastatic locations, n (%)	
Visceral	28 (77.8)
• Liver	25 (69.4)
• Lung	7 (19.4)
• Adrenal gland, pericardial effusion, or pleural involvement	6 (16.6)
Non-visceral only	8 (22.2)
• Bone	20 (55.6)
• Breast	2 (5.6)
• Lymph nodes	13 (36.1)
• Skin	4 (11.1)
• Soft tissue	5 (13.9)
Histological type, n (%)	
Invasive ductal carcinoma	33 (91.7)
Invasive lobular carcinoma	2 (5.6)
Invasive squamous carcinoma	1 (2.8)

Abbreviations: n, number of patients; ECOG PS, Eastern Cooperative Oncology Group performance status; BC, breast cancer; M1, metastases or metastatic disease; HR, hormone receptor; IHC, immunohistochemistry; TN, triple negative; CT, chemotherapy; ET, endocrine therapy; BT, biological therapy; ABC, advanced breast cancer.

Characteristics	N = 36
Histological grade, n (%)	
1	1 (2.8)
2	14 (38.9)
3	16 (44.4)
Unknown	5 (13.9)
HR status (local), n (%)	
Negative	21 (58.3)
Positive	15 (41.7)
Ki67 expression (local) (%)	
Median (range)	30 (15–95)
< 20%, n (%)	5 (13.9)
≥ 20%, n (%)	21 (58.3)
Unknown	10 (27.8)
BC subtype by IHC (local), n (%)	
TN	21 (58.3)
HR-positive disease	15 (41.7)
Prior therapy, n (%)	
CT	36 (100.0)
• ABC	34 (94.4)
ET	26 (72.2)
• ABC	19 (52.8)
BT	25 (69.4)
• ABC	24 (66.7)
Number of prior lines for ABC, n (%)	
None	1 (2.8)
1	9 (25.0)
2	2 (5.6)
3	3 (8.3)
4	5 (13.9)
5	7 (19.4)
≥ 6	9 (25.0)
Median (range)	4 (0–11)

Abbreviations: n, number of patients; ECOG PS, Eastern Cooperative Oncology Group performance status; BC, breast cancer; M1, metastases or metastatic disease; HR, hormone receptor; IHC, immunohistochemistry; TN, triple negative; CT, chemotherapy; ET, endocrine therapy; BT, biological therapy; ABC, advanced breast cancer.

Characteristics	N = 36
• TN	2 (1–8)
• HR-positive disease	5 (3–11)

Abbreviations: n, number of patients; ECOG PS, Eastern Cooperative Oncology Group performance status; BC, breast cancer; M1, metastases or metastatic disease; HR, hormone receptor; IHC, immunohistochemistry; TN, triple negative; CT, chemotherapy; ET, endocrine therapy; BT, biological therapy; ABC, advanced breast cancer.

Run-in phase and determination of RP2D

Fourteen patients were treated with DL0. Three patients were replaced since they experienced early PD (Supplementary Figure, SF1). Of the first six patients, one experienced DLT of grade 3 tumor pain and grade 2 anemia and thrombocytopenia; six additional patients were recruited at this DL without any reported DLT; therefore, DL0 was declared as the RP2D.

Treatment exposure

A median of 4 (range, 1–24) cycles for pembrolizumab and 4.5 (range, 1–24) cycles for gemcitabine were administered. The median relative dose intensity at the RP2D was 100% (100–100) and 80% (39–101), respectively.

Nine (25%) patients experienced delays in pembrolizumab administration because of AEs in five (13.9%) patients; these AEs included hematological and liver function test alterations, nausea, arthralgia, and infections. Thirty (83.3%) patients experienced dose modifications for gemcitabine, with omissions being the most frequent modification followed by reductions and delays. AEs were the most common reasons for all types of modifications. The main reasons for treatment discontinuation included PD in 29 (80.6%) patients; death in two (5.6%); and AE (grade 3 respiratory failure), patient`s decision, and physician’s decision in one (2.8%) patient each. At the time of analysis, two patients were still on treatment.

Efficacy

Considering the efficacy population (n = 33), the ORR was 15.2% (n = 5/33; 95% confidence interval [CI] 5.1–31.9), with only PR reported, and the ORR in patients with TN or HR + BC was similar to the ORR in the whole population. Twelve (36.4%) patients achieved SD (one case lasted for > 6 months), and 12 (36.4%) experienced PD (Supplementary SF2). The CBR, including SD of any duration, was 51.5% (n = 17/33; 95% CI 33.5–69.2). The median DoR was 4.3 months (95% CI 2.3–7.4), median PFS was 3.1 months (95% CI 2.0–4.3), and median OS was 7.9 months (95% CI 6.5–10.3). Eight patients were on treatment for ≥ 6 months before PD (11.4 and 16.1 months in two cases). Efficacy based on immune-related response criteria showed similar results.

Safety

Almost all patients (35 [97.2%]) experienced AE, which was related to the study treatment in 25 (69.4%) patients. AEs led to treatment discontinuation in five (13.9%) patients. Grade 3 AEs were reported in 15 (41.7%) patients and grade 4 AEs in 7 (19.4%), and of these AEs, their relationship with treatment was established by the investigators in 14 (38.9%) patients. Serious AEs were reported in 12 (33.3%) patients and were related to treatment in three cases. No grade 5 AEs related to treatment were reported. Treatment-emergent AEs, irrespective of their causal relationship with the study treatment, are summarized in Table 2.

Table 2
Treatment-emergent adverse events by grade according to the NCI-CTCAE (version 4.03) and with a frequency of at least 5% in any grade

Safety Population (n = 36)				
Adverse Event Term	Grade 1, n (%)	Grade 2, n (%)	Grade 3, n (%)	Grade 4, n (%)
Patients with any TEAE	27 (75.0)	27 (75.0)	18 (50.0)	7 (19.4)
Pyrexia	**10 (27.8)**	1 (2.8)	0	0
Fatigue	**11 (30.6)**	10 (27.8)	2 (5.6)	0
Anemia	**8 (22.2)**	2 (5.6)	3 (8.3)	0
Nausea	**6 (16.7)**	2 (5.6)	0	0
Decreased appetite	**4 (11.1)**	1 (2.8)	0	0
Musculoskeletal chest pain	**3 (8.3)**	0	0	0
Pruritus	**3 (8.3)**	0	0	0
Upper respiratory tract infection	**3 (8.3)**	0	0	0
Albuminuria	**2 (5.6)**	0	0	0
Back pain	**2 (5.6)**	0	1 (2.8)	0
Hot flush	**2 (5.6)**	0	0	0
Hypothyroidism	**2 (5.6)**	0	0	0
Abdominal pain upper	1 (2.8)	**2 (5.6)**	0	0
Elevated AST level	1 (2.8)	0	**2 (5.6)**	0
Constipation	1 (2.8)	**2 (5.6)**	1 (2.8)	0
Diarrhea	1 (2.8)	**3 (8.3)**	1 (2.8)	0
Dyspnea	1 (2.8)	**2 (5.6)**	0	0
Painful skin	1 (2.8)	**2 (5.6)**	0	0
Rash	1 (2.8)	**2 (5.6)**	0	0
Tachycardia	1 (2.8)	**2 (5.6)**	0	0
Hypertension	0	**4 (11.1)**	0	0
Neutrophil count decreased	0	6 (16.7)	**10 (27.8)**	3 (8.3)
Weight decreased	0	**2 (5.6)**	0	0
Pembrolizumab-related TEAEs included rash, anemia, decreased neutrophil count, diarrhea, and increased AST and ALT levels.				
Abbreviations: TEAE, treatment-emergent adverse events; NCI-CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events; n, number of patients; AST, aspartate aminotransferase				

Association of TILs density and PD-L1 expression with treatment efficacy

TILs density values are listed in Table 3. No significant associations were observed between TILs density and either ORR/CBR or PFS (Table 3). Analysis of the median TILs density distribution according to tumor subtype and ORR showed that TNBC patients who achieved ORR tended to present higher levels of lymphocyte infiltration (Supplementary SF3).

Table 3
Logistic and Cox regression models to analyze the association between TILs density and PD-L1 expression (IC and CPS scores) with ORR (CR + PR), CBR (CR + PR + SD of > 6 months), and PFS

		ORR p-value Odds ratio (95% CI)	CBR p-value Odds ratio (95% CI)	PFS p-value Hazard ratio (95% CI)
TILs	**≥ 5%** (24, 80%)	NA	0.556	0.534
(n = 30)	**< 5%** (6, 20%)		0.47	1.37
Cut-Off (n, %)			(0.04, 5.90)	(0.51, 3.64)
	≥ 10% (13, 43%)	0.773	0.554	0.311
	< 10% (17, 57%)	1.36	0.50	1.51
		(0.17, 11.23)	(0.05, 4.98)	(0.68, 3.36)
	≥ 20% (6, 20%)	0.789	0.699	0.073
	< 20% (24, 80%)	1.40	0.56	2.50
		(0.12, 16.46)	(0.03, 10.93)	(0.92, 6.82)
PD-L1 Score (n = 29)	**IC ≥ 1%** (11, 38%)	0.595	0.635	0.321
Cut-Off (n, %)	**IC < 1%** (18, 62%)	1.78	0.57	1.51
		(0.21, 14.86)	(0.06, 5.77)	(0.67, 3.40)
	CPS ≥ 1 (14, 48%)	0.941	0.668	**0.038**
	CPS < 1 (15, 52%)	1.08	1.60	2.56
		(0.13, 8.95)	(0.19, 13.69)	(1.05, 6.23)
	CPS ≥ 5 (11, 38%)	0.571	0.482	**0.053**
	CPS < 5 (18, 62%)	0.50	2.50	2.34
		(0.05, 5.51)	(0.19, 32.19)	(0.99, 5.53)
	CPS ≥ 10 (8, 28%)	0.901	0.793	0.173
	CPS < 10 (21, 72%)	0.86	1.43	1.84
		(0.08, 9.69)	(0.10, 20.44)	(0.77, 4.42)
	CPS ≥ 20 (7, 24%)	0.965	0.793	0.072
	CPS < 20 (22, 76%)	1.06	1.43	2.36
		(0.09, 12.14)	(0.10, 20.44)	(0.93, 6.01)

Abbreviations: TILs, tumor-infiltrating lymphocytes; ORR, objective response rate; CBR, clinical benefit rate; PFS, progression-free survival; IC, immune cell; CPS, combined positive score; CR, complete response; PR; partial response; SD, stable disease; CI, confidence interval

Eleven (38%) and 14 (48%) patients showed PD-L1 + IC score and CPS at a cut-off of 1; 10 (28%) of them were positive for both scores. No associations were observed between PD-L1 IC score and ORR, CBR, or PFS. For CPS, we observed that patients with a CPS of ≥ 1 showed significantly worse PFS (hazard ratio [HR] 2.56; 95% CI 1.05–6.23; $p = 0.038$), and the same tendency was observed with a CPS of ≥ 5 (HR 2.34; 95% CI 0.99–5.53; $p = 0.053$); this association was not maintained with CPS cut-offs of ≥ 10 and ≥ 20 (HR 1.84; 95% CI 0.77–4.42; $p = 0.173$, and HR 2.36; 95% CI 0.93–6.01; $p = 0.072$, respectively) (Table 3). No association was observed between CPS, ORR, or CBR. Nine (53%) TN patients were PD-L1 + for IC scores ≥ 1% and nine (53%) for CPS ≥ 1 (seven were positive for both the scores). In the HR + subpopulation, five (38%) patients were positive for CPS, and two

(15%) were positive for IC score. PD-L1 + scores were not associated with ORR in either TN or HR+ (data not shown). PD-L1 expression by tumor subtype and ORR is shown in Supplementary SF4. PD-L1 + IC scores seemed to be more frequent in TNBC patients with no ORR, while the majority of HR + patients not achieving ORR showed PD-L1 – IC scores (Supplementary SF4B).

Finally, a significant positive correlation between TILs levels and PD-L1 expression was observed (correlation coefficient, 0.567; $p = 0.00134$) (Supplementary SF5).

Association between MDSCs levels and efficacy

Patients showed significantly higher concentrations of total MDSCs at baseline than did the healthy control cohort (median values of 44.5 vs. 17.5 cells/μL, respectively, $p = 0.0018$). This difference was especially marked for M-MDSCs (median values of 33.5 vs. 10.3 cells/μL, $p = 0.0010$) rather than G-MDSCs (median values of 8.6, vs. 5.0 cells/μl, $p = 0.1880$) (Fig. 2).

Analysis of variation in MDSCs levels along the treatment revealed significant differences in M-MDSCs levels associated with CBR between baseline-C3 and baseline-C6/EOT ($p = 0.0313$ and 0.0469, respectively) (Table 4). A similar trend, although not significant ($p < 0.1$), was also observed for differences between baseline and C6/EOT levels for total MDSCs ($p = 0.0781$).

Table 4
MDSCs levels (cells/μL) according to tumor response

MDSCs												
	Total				M-MDSCs				G-MDSCs			
	CB (SD ≥ 24 weeks) (n = 7)		PD (n = 12)		CB (SD ≥ 24 weeks) (n = 7)		PD (n = 12)		CB (SD ≥ 24 weeks) (n = 7)		PD (n = 12)	
	Median	IR	Median	IR	Median	IR	Median	IR	Median	IR	Median	IR
Baseline	38.6 (n = 7)	53.0	47.6 (n = 11)	42.8	30.7 (n = 7)	32.8	39.8 (n = 11)	33.2	7.9 (n = 7)	20.9	5.2 (n = 11)	27.9
C3	17.4 (n = 7)	27.0	60.7 (n = 11)	247.4	4.3 (n = 7)	13.9	47.4 (n = 11)	223.1	5.2 (n = 7)	10.3	7.7 (n = 11)	24.3
C6/EOT	12.4 (n = 7)	8.5	23.2 (n = 5)	37.2	6.3 (n = 7)	8.5	8.0 (n = 5)	31.2	4.3 (n = 7)	10.2	12.4 (n = 5)	8.6
Baseline vs. C6/EOT p-value	0.0781		0.8750		**0.0469**		0.8750		0.6875		0.6250	
Baseline vs. C3 p-value	0.2969		0.6953		**0.0313**		0.7695		0.9375		0.6250	
CB vs. PD (C6/EOT) p-value	0.0513				0.7453				**0.0348**			
CB vs. PD (C3) p-value	0.1743				0.0572				0.5261			
CB vs. PD (baseline) p-value	0.3651				0.1743				0.9278			

Abbreviations: MDSCs, myeloid derived suppressor cells; M-MDSCs, monocytic MDSCs; G-MDSCs, granulocytic MDSCs; CB, clinical benefit; SD, stable disease; PD, progressive disease; IR, interquartile range; C3, cycle 3; C6, cycle 6; EOT, end of treatment

Analysis of the difference in MDSCs levels between patients with CBR or PD at different time points showed significant differences only in case of G-MDSCs at C6/EOT ($p = 0.0348$). No significant differences ($p < 0.1$) were observed at the same time point for total MDSCs levels and at C3 for M-MDSCs levels (Table 4).

Discussion

In the PANGEA-Breast trial, pembrolizumab plus gemcitabine achieved a modest ORR of 15.2%. No long-term responders were observed, although two patients were still alive at study closing data (22/07/2021). Some facts may explain these results. First, BC patients included in our study were heavily pretreated in most cases, with a median of four prior lines for ABC. Second, there

was no patient preselection with respect to PD-L1 expression or TILs density, which confirms that an unselected population is probably an adverse scenario for immunotherapy in ABC. Finally, gemcitabine probably does not harbor powerful immunogenic properties for treating ABC, as we expected. Therefore, other drugs that induce immunogenic cell death, such as anthracyclines, may be more appropriate. Some clinically outstanding results as those reported in the TONIC trial (35% ORR with doxorubicin [15 mg/m^2] followed by nivolumab in metastatic TNBC patients)(14) or KEYNOTE-522 study(15) in the neoadjuvant setting, support the hypothesis that the combination of immunotherapy and CT matters, and anthracyclines could trigger and boost the immune response better than other chemotherapeutic drugs. Discordant results between the IMpassion-130(15) and IMpassion-131(7) trials can also be partially explained with this theory. The difference among these two trials was the CT used (nab-paclitaxel or conventional paclitaxel, respectively) and premedication with corticosteroids in the IMpassion-131 trial.

Additionally, based on tissue immune biomarkers analysis of the PANGEA-Breast trial, we could not identify a subpopulation benefiting from the chemoimmunotherapy combination. Neither TILs density nor PD-L1 expression using CPS revealed a subgroup with a higher probability of response or better survival. The small sample size (n = 36) and highly heterogeneous population (e.g., TN and HR + BC, different previous lines of treatment) represent a major limitation at this point; therefore, our results should be considered cautiously, especially when the IMpassion-130 and KEYNOTE-522 trials have reported favorable results in PD-L1 enriched populations.

Regarding the peripheral blood immune biomarker analysis, MDSCs results were intriguing as their levels were clearly elevated in patients than in the healthy cohort ($p = 0.0018$), suggesting that an immunosuppressive status is induced by ABC. These results are concordant with previous findings that correlate higher levels of MDSCs with adverse prognostic factors and tumor burden in ABC(16). Interestingly, MDSCs decreased along treatment implementation in the CB group versus PD group with a clear trend at C3 and seem less obvious at C6. MDSCs may represent emerging and valuable biomarkers; however, the limited number of patients and samples in our study jeopardized any major interpretation of our data.

Conclusions

In summary, this trial reinforces the hypothesis that immunotherapy for ABC could eventually work only in highly selected and enriched populations, ideally for first-line therapy. Pretreated patients, especially those heavily pretreated in our work and in the KEYNOTE-119 trial(17) showed little benefit from ICIs, with a trend of improved efficacy with PD-L1 enrichment. For future clinical trials in this setting, better selection of patients with ABC would be advisable. Additionally, unless new results are available, different original strategies should be tested, as chemoimmunotherapy outcomes in ABC appear globally modest. Approaches aiming to induce the host immune system through effective immunogenic cell death modalities, depletion of immunosuppressive cells such as MDSCs or Tregs(18, 19), or favoring neoantigen presentation(20, 21) could widen the spectrum of immunotherapy for ABC.

Declarations

Ethics approval and consent to participate

The study was conducted according to the International Conference on Harmonization Good Clinical Practice Guidelines and Declaration of Helsinki and was approved by the institutional ethical review boards of the participating sites and Spanish health authorities. Written informed consent was obtained from all patients before performing any protocol-specific procedures.

Availability of data and material

The datasets used and/or analyzed in our study are available from the corresponding author upon reasonable request.

Competing Interests

L. de la Cruz-Merino received consulting or advisory role fees from Merck Sharp & Dohme (MSD)-Merck, Roche, Bristol-Myers-Squibb (BMS), Pierre-Fabre, Amgen, and Novartis; research funding from MSD-Merck, Roche, and Celgene; speaker's honoraria from MSD-Merck, Roche, BMS, and Amgen; and grant support from Roche and BMS. M Gion received honoraria from Roche and

traveled accommodation from Pfizer. J. Cruz received consulting or advisory role fees from Roche, PharmaMar, Lilly, Novartis, Eisai, Pfizer Amgen, and Celgene; travel accommodation from Roche, Novartis, and PharmaMar. V. Quiroga received speakers' bureau honoraria from Pfizer, Novartis, and Roche; research funding from Celgene; and travel accommodation from Novartis, Roche, and Pfizer. F. Moreno received an advisory role honoraria from Pfizer, Roche, Novartis MSD, and AstraZeneca; speaker's bureau honoraria and research funding from Pfizer; and travel accommodation from Pfizer, Roche, and Novartis. R. Andrés received travel accommodation from Roche. M. Santisteban received honoraria from Roche, Novartis, and Pfizer; and consulting or advisory role honoraria from Gilead, MSD, Novartis, Pfizer, and Biomerieux. M. Ramos received speakers' bureau honoraria from Novartis, Roche, and Pfizer. J. Cortés has stock and other ownership interests in MedSIR and has received honoraria from Novartis, Eisai, Celgene, Pfizer, Roche, SAMSUNG, Lilly, MSD, and Daiichi Sankyo; consulting or advisory role honoraria from Celgene, Cellestia Biotech, AstraZeneca, Biothera, Merus, Roche, Seattle Genetics, Daiichi Sankyo, ERYTECH Pharma, Polyphor, Athenex, Lilly, Servier, MSD , GlaxoSmithKline (GSK), Leuko, Clovis Oncology, Bioasis, Boehringer Ingelheim, and Kyowa Kyrin; research funding from ARIAD, AstraZeneca, Baxalta GMBH/Servier Affaires, Bayer, Eisai Farmaceutica, Guardanth health, MSD, Pfizer, Puma CO, Queen Mary University of London, Roche, and Piqur; and travel accommodation from Roche, Pfizer, Eisai, Novartis, and Daiichi Sankyo. E. López received consulting or advisory role honoraria from AstraZeneca, Pfizer, Roche, and Novartis; and the speaker's bureau honoraria from Roche, Eisai, Pfizer, and Novartis. A. Cortés received consulting or advisory role honoraria from Lilly, Roche, Clovis, Ferrer, and Pfizer; speakers' bureau honoraria from MSD, GSK, AstraZeneca, Roche and Pfizer, and research funding and travel accommodation from Pfizer. L. M. Rodríguez received honoraria from Pfizer and Pierre Fabre. I. Ceballos received consulting or advisory role honoraria from Roche and Merk KGaA; speakers' bureau honoraria from Roche, Pfizer, BMS, and Celgene; and travel accommodation from Roche, Merck, Pfizer, and Novartis. F. Rojo received consulting or advisory role fees from Roche, AstraZeneca, Novartis, BMS, MSD, Lilly, Pfizer, Genomic Health, Guardant Health, Archer, and Pierre-Fabre; speaker bureau/expert testimony fees from Roche, AstraZeneca, Novartis, BMS, MSD, Lilly, Pfizer, and Pierre-Fabre; and travel accommodation from Roche and Novartis His institution received research grant/funding from Roche and Pfizer. The rest of authors declare no conflict of interest.

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Authors´ contribution

Conceptualization, L.dC., F.R. and R.C.; Methodology, LdC, F.R., SB, SBe, R.C. and M.Ch.; Software and Validation SB and S.Be.; Formal Analysis, M.C.; Investigation and Data Curation, L.dC., F.R., C.J-C., V.S-M., M.G., J.C., V.Q., R.A., F.M., J.L.A-R., M.R., E.H., J.C., E. L-M., F.H., N.P-C., L.M.R., I.C., M.S., A.C., A.S. and A.P. Resources, L.dC., F.R., C.J-C., V.S-M., M.G., J.C., V.Q., R.A., F.M., J.L.A-R., M.R., E.H., J.C., E. L-M., F.H., N.P-C., L.M.R., I.C., M.S., A.C., A.S., A.P., R.C., M.Ch., S.B and S.Be.; Writing – Original Draft Preparation, L.dC., F.R., S.B., M.C., R.C., M.Ch. and S.Be.; Writing – Review & Editing and Visualization, all authors.; Supervision, L.dC. and S.B.; Project Administration, L.dC., S.B. and S.Be.; Funding Acquisition, L.dC., R.C., and S.B.

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Figures

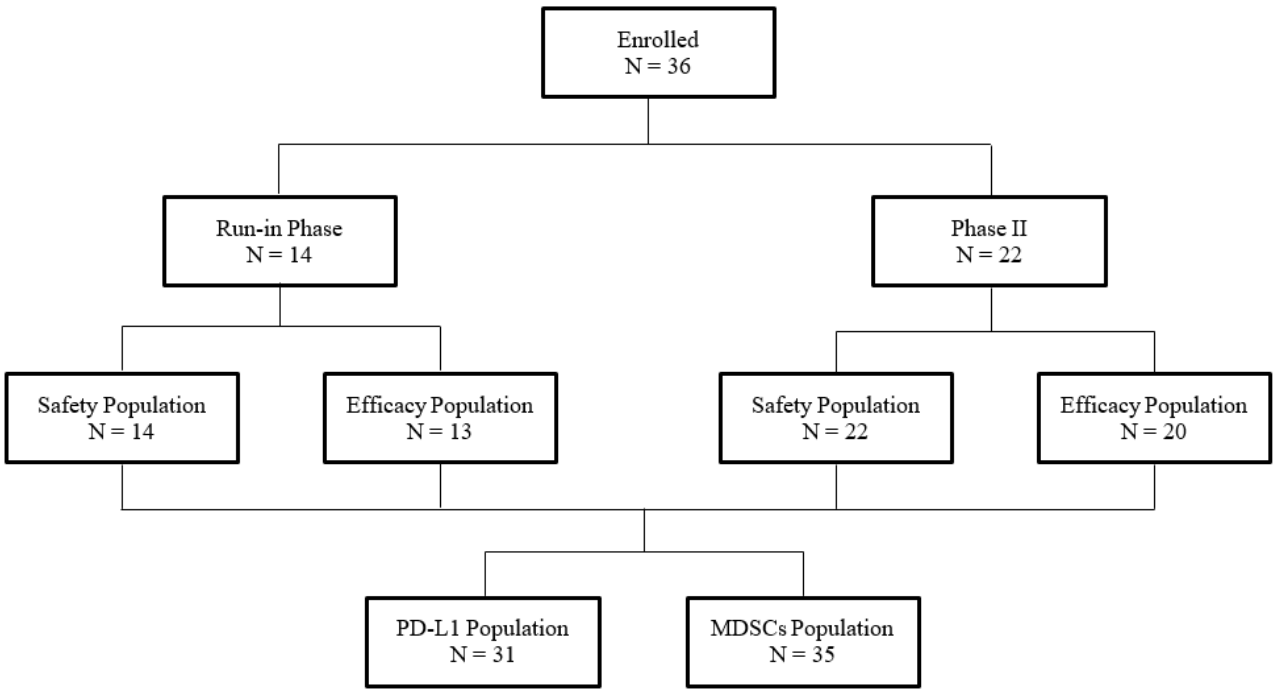

Figure 1

Study flowchart. PD-L1, programmed death-ligand 1; MDSCs, myeloid derived suppressor cell

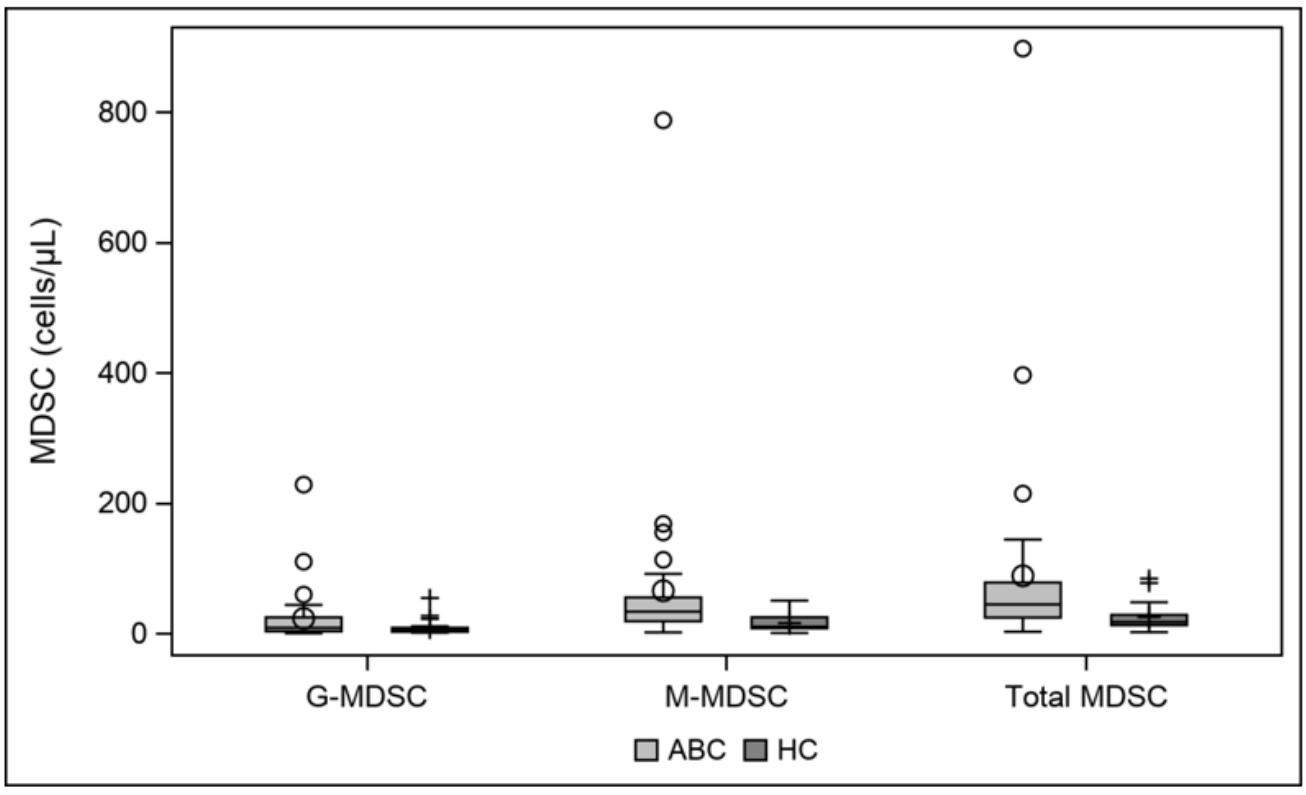

Figure 2

Baseline median values of MDSCs (G-, M-, Total; cells/μL) in ABC patients and healthy cohort (HC). MDSCs, myeloid derived suppressor cells; M-MDSCs, monocytic MDSCs; G-MDSCs, granulocytic MDSCs; ABC, advanced breast cancer.

Supplementary Files

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