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Immune-related Gene Expression Predicts Response to Neoadjuvant Chemotherapy but not Additional Benefit from PD-L1 Inhibition in Women with Early Triple-negative Breast Cancer

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ABSTRACT

Purpose: We evaluated mRNA signatures to predict response to neoadjuvant PD-L1 inhibition in combination with chemotherapy in early triple-negative breast cancer.

Experimental Design: Targeted mRNA sequencing of 2,559 transcripts was performed in formalin-fixed, paraffin-embedded samples from 162 patients of the GeparNuevo trial. We focused on validation of four predefined gene signatures and differential gene expression analyses for new predictive markers.

Results: Two signatures [GeparSixto signature (G6-Sig) and IFN signature (IFN-Sig)] were predictive for treatment response in a multivariate model including treatment arm [G6-Sig: OR, 1.558; 95% confidence interval (CI), 1.130–2.182; $P = 0.008$ and IFN-Sig: OR, 1.695; 95% CI, 1.234–2.376; $P = 0.002$), while the CYT metric predicted pathologic complete response (pCR) in the durvalumab arm, and the proliferation-associated gene signature in the placebo arm. Expression of PD-L1 mRNA was associated with better response in both arms, indicating that increased levels of PD-L1 are a general predictor of neoadjuvant therapy response. In an exploratory analysis, we identified seven genes that were higher expressed in responders in the durvalumab arm, but not the placebo arm: *HLA-A, HLA-B, TAP1, GBP1, CXCL10, STAT1*, and *CD38*. These genes were associated with cellular antigen processing and presentation and IFN signaling.

Conclusions: Immune-associated signatures are associated with pCR after chemotherapy, but might be of limited use for the prediction of response to additional immune checkpoint blockade. Gene expressions related to antigen presentation and IFN signaling might be interesting candidates for further evaluation.

Introduction

There is accumulating evidence that triple-negative breast cancer (TNBC) is an immunogenic disease and that the level of immune activation in tumor tissue indicates improved response to neoadjuvant chemotherapy and improved patient survival (1). The main clinical challenge is to translate this knowledge into therapeutic concepts, and to develop predictive biomarkers for combinations of immuno- and chemotherapy (2). Immune checkpoint inhibition is a promising approach for the treatment of solid neoplasms and has proven its value in the treatment of a number of tumors, among them metastatic TNBC (3, 4), with several current clinical trials (2).

The GeparNuevo trial was a prospective, randomized, multicenter, phase II study that evaluated the addition of the PD-L1 inhibitor, durvalumab, to neoadjuvant chemotherapy for patients with early TNBC (5). Addition of durvalumab numerically increased the rate of pathologic complete response (pCR; ref. 5). The effect was statistically significant in the subgroup of patients who received the first dose of durvalumab before the onset of chemotherapy within a window of opportunity and in higher-stage tumors. This suggests that the window treatment might result in an immune priming of the tumor facilitating immunologic effects triggered by cytotoxic treatment.

We have identified previously a set of immune genes that predicted response to neoadjuvant chemotherapy in triple-negative and HER2-positive breast cancer in the GeparSixto trial (GeparSixto signature; G6-Sig; ref. 6) and a proliferation-associated gene signature (Prolif-Sig) was shown to be predictive for response independent of the G6-Sig (7).

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Translational Relevance

There is a clinical need to develop predictive biomarkers for combination of immuno- and chemotherapy. We use targeted RNA sequencing of samples of the GeparNuevo clinical trial to investigate the use of gene signatures of tumor-infiltrating lymphocytes and proliferation to predict response to neoadjuvant therapy with and without immune checkpoint inhibition. The results validate the predictive value of immune-associated gene expression, including PD-L1, for response to chemotherapy, but not for additional benefit from PD-L1 inhibition. Prediction of response to PD-L1 inhibition might be challenging in the context of neoadjuvant chemotherapy in early breast cancer.

Higgs and colleagues described an IFN-associated gene signature that was predictive for response to durvalumab in lung and urothelial cancer (8). A simple metric of two key cytolytic effector transcripts (GZMA and PRF1) was described previously as a measure of immune cytolytic activity (CYT; ref. 9).

The aim of this study was to validate these signatures in the neoadjuvant GeparNuevo study for response to neoadjuvant chemotherapy in general, and in addition for the combination therapy with neoadjuvant durvalumab. We report the results of our gene expression analysis study based on the comprehensive biomaterial collection within the GeparNuevo trial.

Materials and Methods

Patients and samples

GeparNuevo (NCT02685059) was a multicenter, prospective, randomized, double-blind, placebo-controlled phase II trial investigating the pCR rate of neoadjuvant chemotherapy, including nab-paclitaxel, followed by dose-dense epirubicin/cyclophosphamide with durvalumab versus placebo in TNBC (5). Patients with untreated uni- or bilateral primary, nonmetastatic invasive TNBC (cT2–cT4a–d) were enrolled. Primary objective was the comparison of pCR rates (ypT0 ypN0) following neoadjuvant chemotherapy in combination with durvalumab versus placebo. As a secondary endpoint, correlative research was planned, including predefined and additional exploratory analyses to identify possible relationships between biomarkers and drug activity (5).

Supplementary Fig. S1 illustrates the study design: patients received one injection of durvalumab (0.75 g, i.v.) or placebo 2 weeks prior to the start of chemotherapy (window trial) followed by durvalumab (1.5 g, i.v.) or placebo every 4 weeks plus nab-paclitaxel (125 mg/m^2) weekly for 12 weeks, followed by durvalumab (1.5 g, i.v.) or placebo every 4 weeks plus epirubicin/cyclophosphamide every 2 weeks for four cycles. On the basis of the recommendation of the Independent Data Monitoring Committee, the window phase was stopped as part of an amendment. Thereafter, all patients started with durvalumab or placebo plus chemotherapy on day 1.

Patients gave written informed consent for study participation and the use of biomaterial for translational research. The study protocol was approved by the respective ethics committee, institutional review board, and national competent authority and adheres to the ethical principles of the Declaration of Helsinki. Characteristics of the study cohort are detailed in **Table 1**.

IHC

Central histopathologic confirmation of negative hormone receptors (<1% estrogen receptor and <10% progesterone receptor expression by IHC), negative HER2 status (IHC 0/1 or IHC2+ with a ratio of HER2/CEP17 < 2 and <6 copies of HER2/cell), and Ki-67 was mandatory prior to randomization. Tissue from 158 patients was

Table 1. Clinical and pathologic patient characteristics.

		Durvalumab (subset)	%	Placebo (subset)	%	Durvalumab (all)	%	Placebo (all)	%	*P*
		83		79		88		86		
Age	<50	40	48	39	49	44	50	43	50	ns
	≥50	43	52	40	51	44	50	43	50	
cT stage	cT1–2	76	92	76	96	81	92	83	97	ns
	cT3–4	7	8	3	4	7	8	3	3	
cN stage	cN0	56	67	56	71	61	69	59	69	ns
	cN1	20	24	18	23	20	23	22	26	
	cN2–3	7	8	5	6	7	8	5	6	
Grading	G2	14	17	14	18	14	16	15	17	ns
	G3	69	83	65	82	74	84	71	83	
sTILs	0%–10%	32	39	28	35	27	31	27	31	ns
	11%–59%	40	48	38	48	49	56	46	53	
	≥60%	11	13	13	16	12	14	13	15	
Ki-67	<30	11	13	11	14	11	13	13	15	ns
	≥30	72	87	68	86	77	88	73	85	
Window trial	Yes	55	66	51	65	59	67	58	67	ns
	No	28	34	28	35	29	33	28	33	
Response	pCR	45	54	37	47	47	53	38	44	ns
	No pCR	38	46	42	53	41	47	48	56	
PD-L1 tumor	≥1%	32	39	31	39	33	38	32	37	ns
	0	43	52	44	56	45	51	48	56	
	NA	8	10	4	5	10	11	6	7	

Note: Clinical and pathologic patient characteristics of the subset of patients and samples with available pretreatment samples used in this study ($N = 162$) in comparison with all patients in the complete study cohort ($N = 174$).

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evaluable for PD-L1 IHC using the Ventana SP263 assay. We recorded the percentage of tumor cells with positive membranous staining and defined ≥1% as a threshold value for positive cases.

Evaluation of tumor-infiltrating lymphocytes

We evaluated tumor-infiltrating lymphocytes (TILs) in the stroma (stromal TILs, sTIL) and within the epithelial tumor cell nests (intratumoral TILs) as the percentage of area of the respective compartment that contains lymphocytes (10). This was based on hematoxylin and eosin (H&E) morphology using a standardized software-assisted method for sTILs (11).

Targeted RNA sequencing

Formalin-fixed, paraffin-embedded (FFPE) tissue was processed using a HTG EdgeSeq Instrument (HTG Molecular Inc) with the oncology biomarker panel according to the manufacturer's instructions. In brief, the tumor area was marked on an H&E-stained slide and the area of invasive breast cancer was recorded. Tissue (15 mm^2) was scraped off one or several unstained slides and used for library preparation. The method is based on an RNA extraction–free chemistry and a nuclease protection assay. Libraries were quantified, pooled, and sequenced on an Ion Torrent S5 Instrument (Thermo Fisher Scientific). Count tables were generated using the HTG parsing tool. For quality control, we transformed the reads to counts-per-million and calculated the mean of five negative and four positive internal controls for each sample. We excluded samples if the mean of its positive controls was below two SDs of the total mean across all samples or if the mean of its negative controls was above two SDs from the total mean.

The data were then normalized to counts-per-million reads according to:

$$\widetilde{\xi}_i := \max\{3, \xi_i\} = \max\left\{3, log2\left(10^6 \times \frac{x_i+0.5}{X+1}\right)\right\},\ i=1,\ldots,2559.$$

Where x_i is the count of gene *i*, *X* is the sum of counts of all 2,559 genes, and $\widetilde{\xi}_i$ is the bounded transformed expression value of gene *i*.

Raw count data and normalized gene expression data are available at https://my.idgard.de/#/guest-access?b=6aeq1yhjibvsa68mmh92si7k62jkqn5s5xq95afilp3n0rpkgu. For access to clinical data please refer to https://gbg.de/en/research/trafo.php.

Predefined gene expression signatures

We calculated four predefined gene signatures as the mean expression of its members. We evaluated a gene signature predictive for neoadjuvant response that we have defined previously in the GeparSixto study [G6-Sig: *CXCL9, CCL5, CD8A, CD80, CXCL13, IDO1, PDCD1, CD274* (*PD-L1*), *CTLA4, FOXP3, CD21*, and *IGKC*; ref. 6]. The genes *CD21* and *IGKC* had to be omitted because they were not covered by the sequencing assay. We also evaluated a proliferation-associated signature (PAM50 proliferation signature; Prolif-Sig: *BIRC5, CCNB1, CDC20, NUF2, CEP55, NDC80, MKI67, PTTG1, RRM2, TYMS*, and *UBE2C*; ref. 7) and a previously described four-gene IFN signature that was associated with durvalumab response in urothelial and non–small cell lung cancer (IFN-Sig: *IFNG, CD274* (*PD-L1*), *LAG3*, and *CXCL9*; ref. 8). We also evaluated the CYT metric of cytolytic activity based on the two genes *GZMA* and *PRF1* (9).

Statistical analysis

pCR was defined as no residual cancer (invasive and noninvasive) in the breast (ypT0) and axillary lymph nodes (ypN0). We used logistic regression analyses to evaluate the association of gene signatures or single genes with pCR. All statistical analyses were computed in R 3.5.2 (R Project for Statistical Computing, RRID:SCR_001905) and Bioconductor (Bioconductor, RRID:SCR_006442). *P* values were computed two-sided and considered as statistically significant if <0.05. Adjustment for multiple testing was applied where indicated using the method of Benjamini and Hochberg. For the differential gene expression analysis according to treatment response, we fit linear models with empirical Bayes moderation (LIMMA, RRID:SCR_010943). We preselected genes by unspecific filtering based on minimal expression and variability across samples (mean, >4 and interquartile-range, >1). We used a 2×2 factorial design to account for the two different treatment arms and the response variable (pCR vs. residual disease), and report the differentially expressed genes in the durvalumab arm.

Results

From the 174 FFPE samples available in the GeparNuevo biobank, 164 had a successful histologic quality control, and 162 of these samples passed the sequencing control. An overview is given in the consort statement (Supplementary Fig. S2). The baseline characteristics of the sequenced samples did not significantly differ from the overall GeparNuevo patients (**Table 1**).

Evaluation of predefined molecular signatures

We tested the four previously defined gene signatures (G6-Sig, IFN-Sig, CYT, and Prolif-Sig) for prediction of response to treatment. G6-Sig and the CYT were significantly associated with increased pCR rate in the complete cohort and the durvalumab arm. IFN-Sig was associated with better response in the complete cohort and both therapy arms (**Fig. 1A**). Prolif-Sig was associated with a higher probability of pCR in all patients and the placebo arm.

G6-Sig and the IFN-Sig were predictive for treatment response in a multivariate analysis adjusted for clinical and pathologic risk factors and treatment arm (**Table 2**).

Hierarchical clustering using the 12 genes of the G6-signature resulted in three clusters of samples with high, intermediate, or low immune activation with significantly different pCR rates (P = 0.002; **Fig. 1B**). Of the individual genes of the G6-signature, *CXCL9, CCL5, CD8A, CD80, CXCL13, IDO1, PDCD1, CTLA4*, and *CD274* (*PD-L1*) were associated with a better response in all patients and (except *CD80*) within the durvalumab arm (**Fig. 1C–E**). Expression of CD274 (PD-L1) mRNA was positively correlated with all three immune signatures and showed a high concordance with PD-L1 IHC (Supplementary Fig. S3). It was associated with better response in all patients and in the placebo and durvalumab arm (**Fig. 1C–E**).

The PAM50 Prolif-Sig and G6-Sig were previously shown to be independently predictive for response to neoadjuvant chemotherapy for TNBC (7). In GeparNuevo, the G6-Sig was predictive for therapy response in a bivariate logistic regression model (Supplementary Table S1), with increasing response rates with increasing values of both signatures (**Fig. 2**).

Exploratory identification of novel markers for response to immunotherapy

To identify genes that might be associated with response to immune checkpoint inhibition, we performed an exploratory differential gene expression analysis according to outcome (pCR vs. no pCR) using

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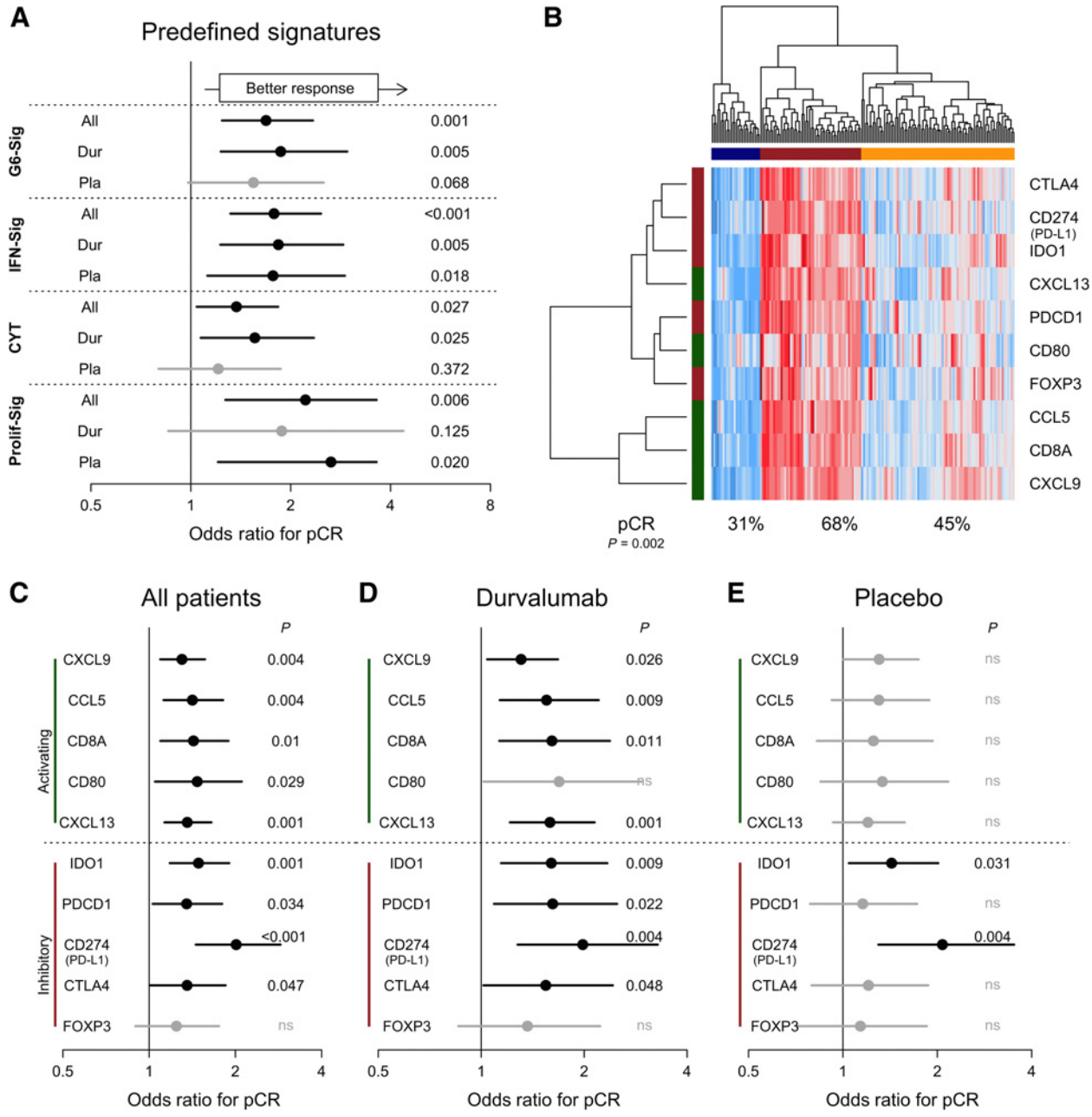

Figure 1.

Predefined signatures and response to treatment. Predefined gene signatures in pretreatment biopsies and response to therapy. **A,** The G6-Sig and the CYT metric were associated with better response in all patients and in the durvalumab (Dur) arm. The Prolif-Sig predicted response in the placebo (Pla) arm. **B,** Hierarchical clustering using the genes from the G6-Sig results in three distinct groups with different response to therapy ("cold," blue; "hot," red; and "intermediate," orange column annotation). The row annotation indicates genes with predominantly activating (green) or inhibitory (red) function. Response to treatment according to the individual genes in the complete cohort (**C**), in the durvalumab arm (**D**), and in the placebo arm (**E**).

pretreatment samples of patients treated with durvalumab (**Fig. 3**; Supplementary Table S2).

Eight genes were significantly associated with response after adjustment for multiple testing (*TAP1, GBP1, HLA-A, HLA-B, CXCL10, STAT1, CD38*, and *ITGA2*). These genes predicted pCR in the durvalumab arm, but not (except *ITGA2*) in the placebo arm.

Discussion

In this study, we performed a comprehensive analysis of predictive gene expression profiles in the GeparNuevo neoadjuvant trial. Immune-associated gene expression signatures were associated with better response to neoadjuvant chemotherapy with high significance, irrespective of the treatment arm. We could validate our previously

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Table 2. Multivariate logistic regression analyses for predefined gene signatures.

Covariate	OR (95% CI)	*P*	Covariate	OR (95% CI)	*P*
G6-Sig	1.558 (1.13–2.182)	0.008	Prolif-Sig	1.871 (1.009–3.574)	0.051
Age ≥50 (vs. <50)	0.829 (0.423–1.623)	0.582	Age ≥50 (vs. <50)	0.935 (0.471–1.869)	0.849
cT3–4 (vs. cT1–2)	0.196 (0.028–0.866)	0.051	cT3–4 (vs. cT1–2)	0.164 (0.024–0.715)	0.029
cN+ (vs. cN−)	0.842 (0.404–1.757)	0.646	cN+ (vs. cN−)	0.832 (0.401–1.727)	0.620
G3 (vs. G2)	3.473 (1.376–9.664)	0.011	G3 (vs. G2)	3.393 (1.332–9.476)	0.013
Durvalumab (vs. pla)	1.630 (0.833–3.243)	0.157	Durvalumab (vs. pla)	1.539 (0.793–3.021)	0.205
Covariate	**OR (95% CI)**	***P***	**Covariate**	**OR (95% CI)**	***P***
CYT metric	1.323 (0.987–1.793)	0.064	IFN-Sig	1.695 (1.234–2.376)	0.002
Age ≥50 (vs. <50)	0.802 (0.413–1.556)	0.514	Age ≥50 (vs. <50)	0.806 (0.409–1.588)	0.532
cT3–4 (vs. cT1–2)	0.162 (0.023–0.703)	0.028	cT3–4 (vs. cT1–2)	0.188 (0.026–0.851)	0.048
cN+ (vs. cN−)	0.835 (0.403–1.729)	0.626	cN+ (vs. cN−)	0.808 (0.384–1.699)	0.573
G3 (vs. G2)	3.751 (1.506–10.331)	0.006	G3 (vs. G2)	3.485 (1.376–9.734)	0.011
Durvalumab (vs. pla)	1.608 (0.828–3.170)	0.164	Durvalumab (vs. pla)	1.659 (0.841–3.332)	0.148

Note: Multivariate logistic regression analyses for prediction of treatment response according to the GeparSixto TIL-associated signature (G6-Sig), the Prolif-Sig, the CYT metric and the IFN-Sig, respectively.
Abbreviation: Pla, Placebo.

described gene signature (6) for prediction of response to chemotherapy and confirm its independency from proliferation-associated gene expression (7). Our data indicate that there might be a relatively greater impact of immune-associated gene expression in patients receiving immunotherapy. However, further studies are necessary to address the hypothesis that the impact of immunotherapy might be pronounced in TNBC with lower proliferation, as tumors with high proliferation typically respond well to chemotherapy alone.

PD-L1 expression of ≥1% of tumor cells was associated with better response to durvalumab when evaluated by IHC (5). Expression of CD274 (PD-L1) mRNA was predictive for treatment response in both treatment arms, underscoring the observation that the prediction of response to immunotherapy is complicated by the predictive value of immune-associated gene expression for chemotherapy alone.

While biomarkers for response to PD-L1 inhibition, like PD-L1 IHC (4) and immune/IFN-associated gene signatures (8, 12–14), have been described to predict response to PD-L1 therapy, effects of these biomarkers might be masked by chemotherapy response in GeparNuevo.

Our study also confirms the observation that immune-associated gene expression is strongly correlated, and that this is even true when comparing genes with inhibitory function with those with stimulatory

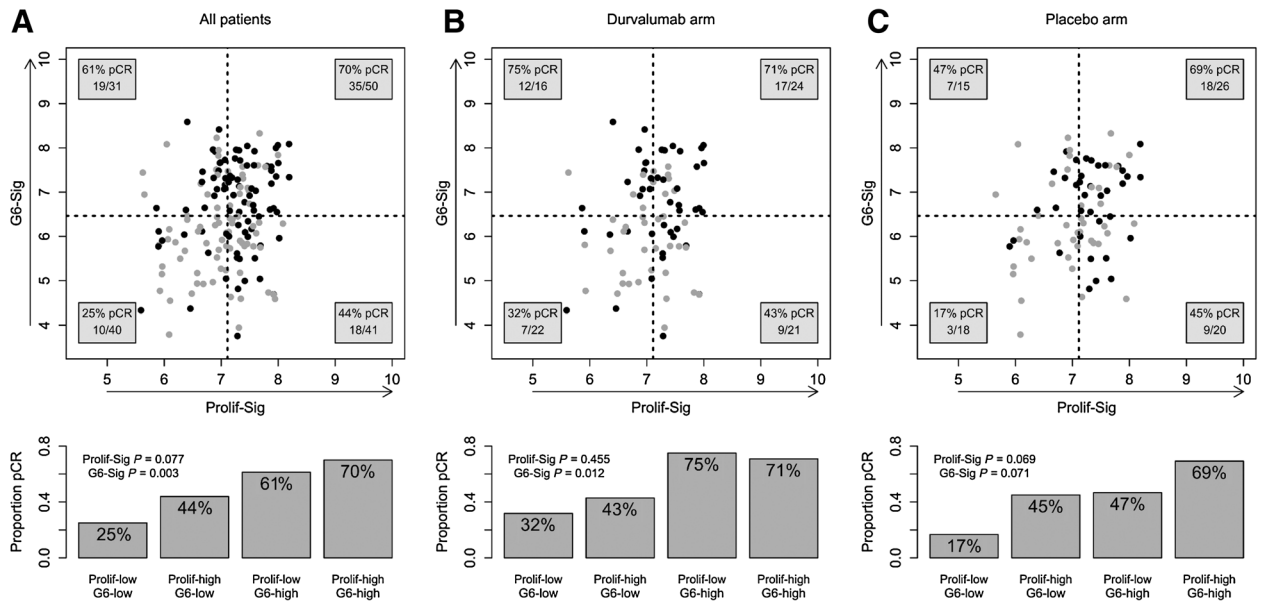

Figure 2.

Immune- and proliferation-associated gene signature. Relation of the G6-Sig and Prolif-Sig and response to therapy. **A,** Increasing levels of both signatures are associated with better response to therapy in all patients and **B,** within the durvalumab arm and **C,** placebo arm. The black dots highlight cases with pCR, the vertical and horizontal lines indicate the signature mean that was also used to classify the cases as low or high in the bar plots. The *P* values in the bar plots for the dichotomized signatures are derived from a bivariate logistic regression model (see Supplementary Table S1 for details).

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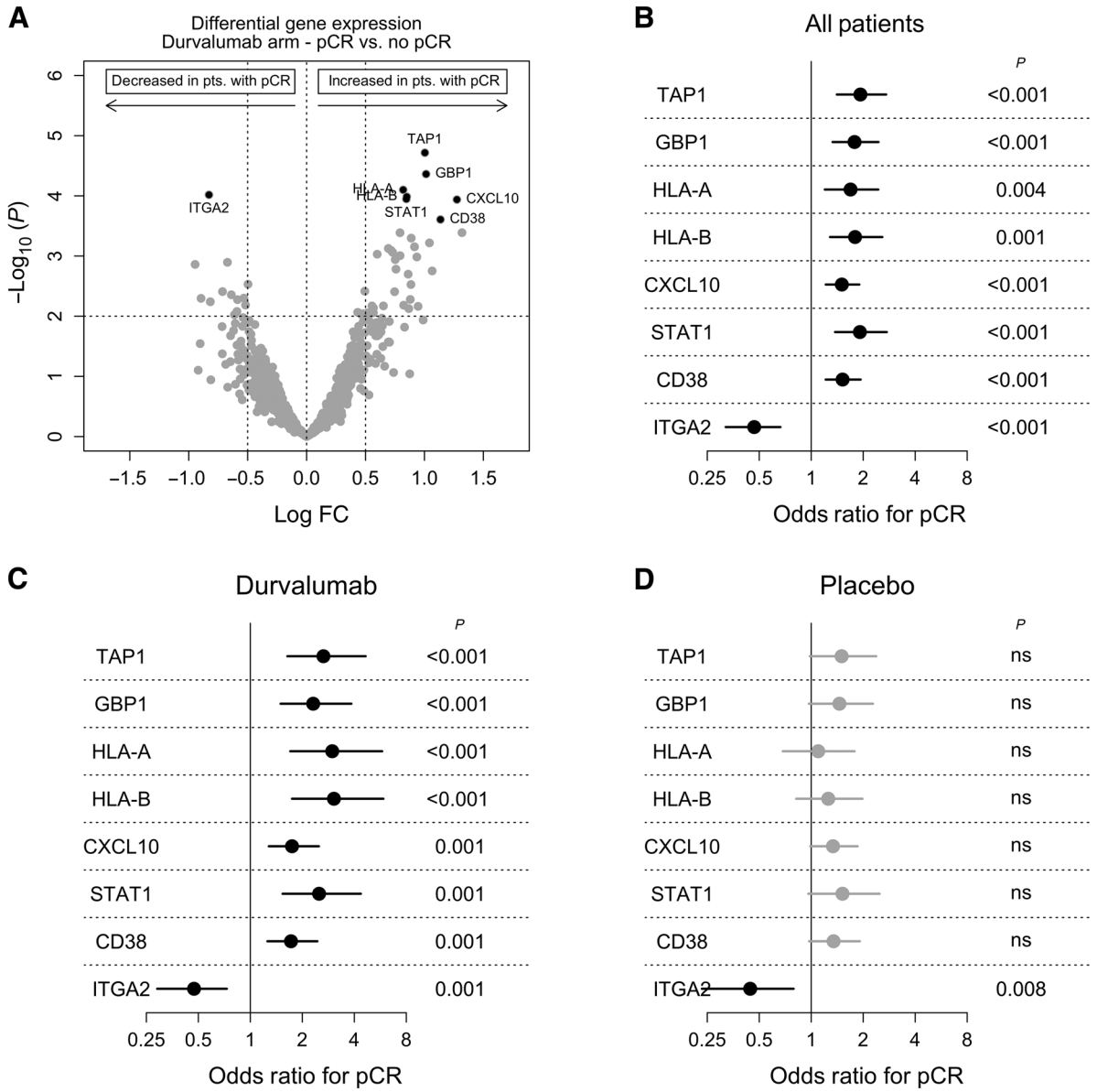

Figure 3.

Differential gene expression according to pCR. **A,** Differential gene expression analysis according to treatment response (pCR). For each gene, the log fold-change (log FC) and the −log10 *P* value are plotted. Black dots indicate significant *P* values after adjustment for multiple testing. Association of the potential candidate genes from **A** with patient (pt) outcome in the complete cohort (**B**) and in the durvalumab (**C**) and placebo arms (**D**).

functions (6). This strong coexpression makes it difficult to evaluate different functions or cellular components of the tumor-associated immune response independent from each other.

With these limitations in mind, we tried to explore whether our data can be used to identify genes that might specifically predict response to durvalumab. To this end, we performed a differential gene expression analysis according to response (pCR vs. residual disease) within the durvalumab arm. We found seven candidate genes for response to durvalumab that were associated with response in the durvalumab arm, but not the placebo arm. These genes represented constituents of the cellular antigen-presenting machinery and IFN-induced gene expression.

Among them were *HLA-A* and *-B* that encode for MHC class I molecules, which present antigens to cytotoxic T cells (15), and *TAP1* (transporter associated with antigen processing), which is part of cellular antigen processing. We have examined previously the expression of HLA in breast cancer (16), but not in the context of immunotherapy, where it might indicate that the presence of a functioning antigen-presenting machinery facilitates the effects of immunotherapy.

Other genes were *GBP1*, an IFN-induced gene that has been described previously as a marker for TILs in breast cancer (17), *CXCL10*, a cytokine involved in induction of a tumor-associated immune response (18), and *STAT1*, part of the IFN-induced JAK-

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STAT pathway that stimulates the expression of its target genes, like *HLA* and *PD-L1* (19).

We have reported previously that a higher quantity in sTILs on H&E-stained slides was associated with a higher probability of response in both treatment arms and observed a trend for increased response in PD-L1–positive tumors (5). We also demonstrated that the evaluation of tumor mutational burden adds independent information for response prediction. However, none of these markers seemed to be specific for PD-L1 inhibition (20). In this study, we evaluated immune- and proliferation-associated gene expression in more detail, but observed the same phenomenon that the biomarker under evaluation, immune-associated gene expression, is not specific for response prediction to PD-L1 inhibition in the context of chemotherapy.

The major caveat of the study is the relatively small sample size in this neoadjuvant phase II trial in the context of the high-dimensional gene expression analysis. While IFN-induced gene expression is a recurring observation in studies evaluating gene expression for prediction of response to PD-L1 inhibition (8, 12–14), the potential markers of durvalumab response identified in this study have to be considered exploratory until further validation in independent trials. With these limitations in mind, a strength of our study design is the availability of biopsy samples from a prospective, randomized trial with central evaluation of histology, receptor status, TILs, and PD-L1 expression. Also, we limited the analysis to a small number of predefined gene expression signatures.

To conclude, our results confirm that immune-associated gene expression is a robust marker of response to neoadjuvant chemotherapy for breast cancer. The definition of predictive markers specifically for response to additional immune checkpoint blockade is challenging, and it might be interesting to focus on the antigen processing and presentation machinery for further studies.

Authors' Disclosures

B.V. Sinn reports nonfinancial support from HTG Molecular Diagnostics Inc. during the conduct of the study, personal fees from Novartis outside the submitted work, as well as has a patent for EP18209672 pending. S. Loibl reports grants from AstraZeneca during the conduct of the study and Immunomedics; grants and other from Amgen, Roche, Celgene, Novartis, and Pfizer; grants, personal fees, and other from AbbVie and DSI; other from Seagen, BMS, Merck, and Puma; personal fees and other from Prime/Medscape and EirGenix; and personal fees from Chugai outside the submitted work; S. Loibl also has a patent for EP14153692.0 pending. C. Hanusch reports personal fees from Roche, Novartis, AstraZeneca, Pfizer, and Lilly outside the submitted work. M. Untch reports personal fees from AstraZeneca, Celgene, Daichi Sankyo, Roche Pharma, Pfizer, Mundipharma, MSD Oncology, Pierre Fabre, Seattle Genetics, Sanofi Aventis, Agendia, Amgen, AbbVie, Lilly, and Novartis outside the submitted work. K. Weber reports a patent for 18209672.7 - 1111 issued. T. Karn reports a patent for EP18209672 pending. F. Marmé reports personal fees from Pfizer, Clovis, Myriad, Seagen, Amgen, Celgene, Eisai, Janssen-Cilag, Roche, Novartis, MSD, and GenomicHealth and grants and personal fees from AstraZeneca outside the submitted work. W.D. Schmitt reports personal fees from AstraZeneca outside the submitted work. V. Müller reports personal fees from Amgen, AstraZeneca, Eisai, MSD, Hexal, Pierre Fabre, ClinSol, Lilly, Tesaro, and Nektar; grants and personal fees from Daiichi Sankyo, Pfizer, Novartis, Roche, and Seattle Genetics; and grants from Teva outside the submitted work. E. Stickeler reports personal fees from Roche outside the submitted work. M. von Mackelenbergh reports personal fees from AstraZeneca, Amgen, Novartis, Roche, Genomic Health, Pfizer, and Mylan outside the submitted work. P.A. Fasching reports personal fees from Novartis, Lilly, Pierre Fabre, Seattle Genetics, Roche, and Hexal, Pfizer, Daiichi Sankyo, AstraZeneca, Eisai, and Merck Sharp & Dohme, P.A. Fasching also reports grants from Biotech and Cepheid during the conduct of the study. C. Denkert reports grants from German Cancer Aid during the conduct of the study and Myriad Genetics, other from Sividon Diagnostics, personal fees from Novartis, Roche, MSD Oncology, Daiichi Sankyo, and AstraZeneca outside the submitted work, as well as has a patent for EP18209672 pending, EP20150702464 pending, and Software VMScope digital pathology pending. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

B.V. Sinn: Conceptualization, formal analysis, validation, visualization, writing–original draft. **S. Loibl:** Conceptualization, resources, data curation, supervision, writing–review and editing. **C.A. Hanusch:** Data curation, writing–review and editing. **D.-M. Zahm:** Data curation, writing–review and editing. **H.-P. Sinn:** Resources, writing–review and editing. **M. Untch:** Data curation, writing–review and editing. **K. Weber:** Conceptualization, resources, formal analysis, supervision, validation, writing–review and editing. **T. Karn:** Conceptualization, formal analysis, validation, writing–review and editing. **C. Becker:** Data curation, writing–review and editing. **F. Marmé:** Conceptualization, data curation, writing–review and editing. **W.D. Schmitt:** Data curation, writing–review and editing. **V. Müller:** Conceptualization, data curation, writing–review and editing. **C. Schem:** Conceptualization, data curation, writing–review and editing. **D. Treue:** Data curation, investigation, methodology, writing–review and editing. **E. Stickeler:** Conceptualization, data curation, writing–review and editing. **F. Klauschen:** Resources, Writing–review and editing. **N. Burchardi:** Data curation, writing–review and editing. **J. Furlanetto:** Data curation, writing–review and editing. **M. von Mackelenbergh:** Conceptualization, Data curation, writing–review and editing. **P.A. Fasching:** Conceptualization, data curation, writing–review and editing. **A. Schneeweiss:** Data curation, writing–review and editing. **C. Denkert:** Conceptualization, resources, supervision, funding acquisition, writing–review and editing.

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