Spatial and temporal heterogeneity of predictive and prognostic signatures in triple-negative breast cancer treated with neoadjuvant combination immune-chemotherapy

PD4-02

Carsten Denkert[1], Andreas Schneeweiss[2], Julia Rey[3], Akira Hattesohl[1], Thomas Karn[4], Michael Braun[5], Paul Jank[1], Jens Huober[6], Hans-Peter Sinn[7], Dirk-Michael Zahm[8], Claus Hanusch[5], Frederik Marmé[9], Jenny Furlanetto[3], Jörg Thomalla[10], Jens-Uwe Blohmer[11], Marion van Mackelenbergh[12], Thorsten Stiewe[13], Peter Staib[14], Christian Jackisch[15], Julia Teply-Szymanski[1], Peter A. Fasching[16], Bruno V. Sinn[17], Michael Untch[18], Karsten Weber[3], Sibylle Loibl[3]

[1]Institut für Pathologie, Philipps Universität Marburg und Universitätsklinikum Marburg (UKGM), Germany, [2]Nationales Centrum für Tumorerkrankungen, Universitätsklinikum und Deutsches Krebsforschungszentrum, Heidelberg, Germany, [3]GBG, Nei-Isenburg, Germany, [4]Universitätsklinikum Frankfurt, Frankfurt am Main, Germany, [5]Rotkreuzklinikum München, Germany, [6]Kantonsspital St.Gallen, Brustzentrum, Departement Interdisziplinäre medizinische Dienste, St. Gallen, Switzerland, [7]Pathologie, Universitätsklinikum Heidelberg, Germany, [8]SRH Waldklinikum Gera GmbH, Germany, [9]Medizinische Fakultät Mannheim, Universität Heidelberg, Universitätsfrauenklinik Mannheim, Germany, [10]Institut für Versorgungsforschung in der Oncologie Koblenz am Rhein, Germany, [11]Gynäkologie mit Brustzentrum, Charité-Univesitätsmedizin Berlin, Germany, [12]Universitätsklinikum Schleswig-Holstein, Klinik für Gynäkologie und Geburtshilfe, Schleswig-Holstein, Germany, [13]Institute of Molecular Oncology, Genomics Core Facility, Philipps Universität Marburg, Germany, [14]St Antonius Hospital Eschweiler, Germany, [15]Sana Klinikum Offenbach, Germany, [16]Universitätsklinikum Erlangen, Germany, [17]Institut für Pathologie, Charité Berlin, Germany, [18]HELIOS Klinikum Berlin Buch, Germany

Background

Immunological pathways are relevant for response to classical neoadjuvant chemotherapy as well as combined chemo-immunotherapy. In addition, it has been shown that combined chemo-immunotherapy significantly improves survival, even in the context of only moderate effects on pCR. Due to the window therapy with durvalumab-alone, the administration of durvalumab only in the neo-adjuvant phase and the option to analyze multiple consecutive biopsies, the GeparNuevo trial[1,2] offers the opportunity to

- determine gene expression patterns for pCR and DDFS endpoints in two different neoadjuvant therapy arms
- identify pathways most relevant for pCR and DDFS in the different therapy arms
- analyze the temporal heterogeneity at different time points, with a focus on genes regulated by durvalumab vs placebo
- evaluate the tumor microenvironment by spatial sequencing of tumor cell and stroma areas.

Patients and Methods

264 tumor samples were evaluated by gene expression analysis: 148 pretherapeutic core biopsies, 72 post-window biopsies, 27 biopsies during chemotherapy and 17 biopsies of the residual tumor after therapy. These samples were analyzed by HTG OBP panel targeting 2549 genes which are assigned to 25 different biological mechanisms or cellular pathways. In addition, spatial profiling was compared in a subset of pre-and post-window samples using the Nanostring GeoMx spatial RNA profiling system guided by cytokeratine immunofluorescence. We compared areas with high tumor cell content with stromal areas with or without TILs. Endpoints were pCR and DDFS.

Table 1: Consort table

Patient set	N
G9 mITT analysis set	174
… QC cleaned HTG data available	148
- durvalumab arm	77
- non pCR	35
- pCR	42
- placebo arm	71
- non pCR	35
- pCR	36

Table 2. Baseline characteristics

Parameter	*Category*	*Durvalumab N=77 N (%)*	*Placebo N=71 N (%)*	*Overall N=148 N (%)*	*p-value*
Age, years	<40	24 (31.2)	16 (22.5)	40 (27.0)	0.237
	>=40	53 (68.8)	55 (77.5)	108 (73.0)	
Breast Cancer Stage	Stage 0 or I	27 (35.1)	26 (36.6)	53 (35.8)	0.865
	Stage IIA and higher	50 (64.9)	45 (63.4)	95 (64.2)	
Grading	G2	13 (16.9)	13 (18.3)	26 (17.6)	0.820
	G3	64 (83.1)	58 (81.7)	122 (82.4)	
Tumor type	Duct. or duct.lob.	60 (77.9)	58 (81.7)	118 (79.7)	0.437
	lobular	0 (0.0)	1 (1.4)	1 (0.7)	
	other	17 (22.1)	12 (16.9)	29 (19.6)	
sTILs	low (0-10%)	27 (35.1)	26 (36.6)	53 (35.8)	0.852
	Interm. (11-59%)	39 (50.6)	33 (46.5)	72 (48.6)	
	high (60-100%)	11 (14.3)	12 (16.9)	23 (15.5)	
PD-L1	negative	8 (11.3)	10 (14.9)	18 (13.0)	0.616
	positive	63 (88.7)	57 (85.1)	120 (87.0)	
	missing	6	4	10	
pCR(ypT0 ypN0)	no	35 (45.5)	35 (49.3)	70 (47.3)	0.742
	yes	42 (54.5)	36 (50.7)	78 (52.7)	

Results

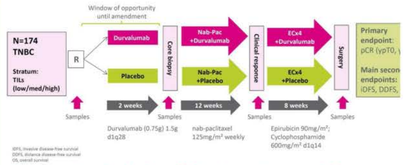

Figure 1: Study design of the GeparNuevo trial

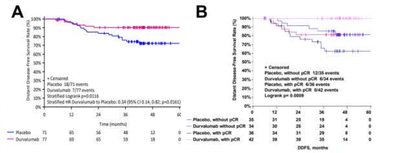

Figure 2: DDFS by treatment arm (A) and pCR (B)
(ypT0 ypN0, landmark analysis, biomarker cohort, n=148)

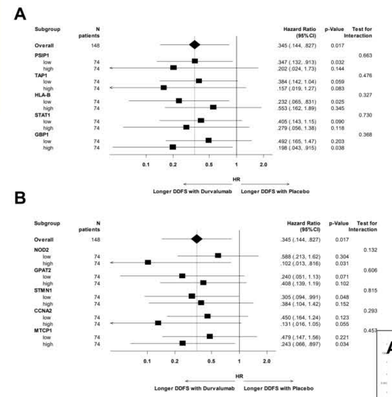

Figure 5. Survival analysis for selected genes: **A** Cox-regression (DDFS) for the most significant pCR genes in the durvalumab arm; **B** Cox-regression (DDFS) for the most significant pCR genes in the placebo arm

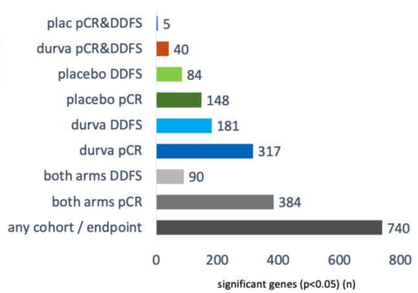

Figure 3. Number of significant genes for different endpoints and cohorts

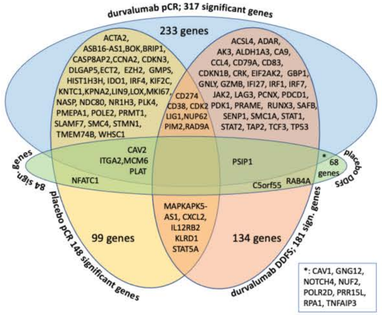

Figure 4. Overlap between significant genes for different endpoints and study arms

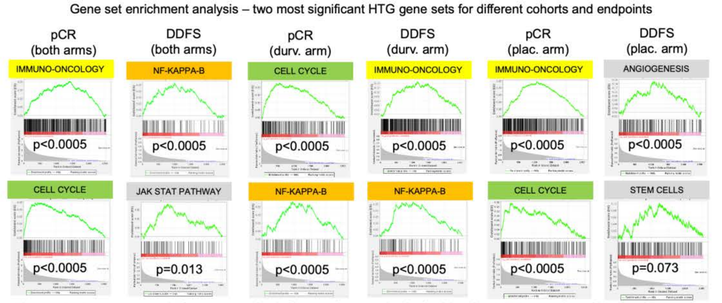

Figure 6. Gene set enrichment analysis for pathways included in the HTG oncology biomarker panel (2549 genes, 24 pathways)

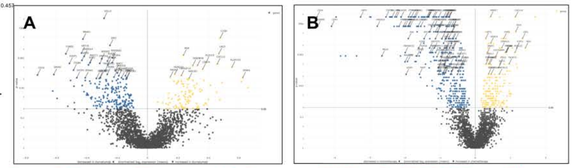

Figure 7. Temporal heterogeneity in sequential biopsies (HTG OBP panel):
A: durvalumab-induced changes (comparison of core biopsy before therapy and after one dose of durvalumab) B: chemotherapy-induced changes: comparions of core biopsy before therapy and after 4 cycles of chemotherapy)

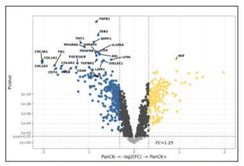

Figure 8. Spatial heterogeneity in different tumor regions: Tumor cell areas vs. stromal areas (GeoMx platform)

Results and Conclusions

1. The survival results of the biomarker cohort were similar to the complete GeparNuevo trial cohort, with an improved survival in the durvalumab arm (**Figure 2**). The pCR rate was not significantly different between both arms (not shown).
2. A total of 740 genes were significantly associated with either pCR or DDFS in either the complete GeparNuevo cohort or one of the two therapy arms.
3. There was a large number of predictive or prognostic genes (n=317 for pCR and n=181 for DDFS) in the durvalumab arm, while the number of genes in the placebo arm was considerably lower (n=148 for pCR and n=84 for DDFS) (**Figure 3**).
4. Overall, the overlap between the significant genes for pCR and DDFS in the two trial arms was limited. There was no single gene significant for all endpoints/cohorts, and only a limited set of genes for three of four scenarios (**Figure 4**). A considerable overlap of genes was observed for pCR and DDFS in the durvalumab arm (40 genes) and for pCR in the placebo and durvalumab arms (43 genes). Only 5 genes were significant in the placebo arm for both, pCR and DDFS.
5. Genes with high significance for pCR did typically not have a significant test for interaction with therapy in the survival analysis (**Figure 5**).
6. Gene set enrichment analyses identified immuno-oncology, NF-kappa B as well as cell cycle as the most common pathways for DDFS and pCR, with a considerable heterogeneity between the different trial arms and endpoints (**Figure 6**). Interestingly, immuno-oncology genes were relevant for pCR in the placebo arm, but not in the durvalumab arm. This is in line with the observation that a pre-activated immune system is relevant for response to conventional chemotherapy. In our cohort, this immune pre-activation is less relevant for pCR to durvalumab, but might play a role for DDFS after durvalumab.
7. Temporal as well as spatial heterogeneity of gene expression was observed, this might contribute to the differences observed in the different trial arms (**Figure 7-8**).

In our analysis, we show that immune gene signatures, NF-kappa B signaling as well as proliferation contribute to therapy response and prognosis after neoadjuvant durvalumab combined with chemotherapy, with considerable heterogeneity of the different genes and gene sets in the different clinical situations. The parallel targeting of immune- and proliferation pathways as well as the additional effect on survival might explain why a combined immunotherapy-chemotherapy approach is more successful than each single therapy strategy alone.

References

1. Loibl S, Schneeweiss A, Huober J, et al., Neoadjuvant durvalumab improves survival in early triple-negative breast cancer independent of pathological complete response (2022) Ann Oncol. GBG and AGO-B.
2. Loibl S, Untch M, Burchardi N, et al., A randomised phase II study investigating durvalumab in addition to an anthracycline taxane-based neoadjuvant therapy in early triple-negative breast cancer: clinical results and biomarker analysis of GeparNuevo study (2019) Ann Oncol.

Presented at: San Antonio Breast Cancer Symposium, December 6-10, 2022

This study was funded by INTEGRATE-TN Deutsche Krebshilfe, ONCOBIOME EUH2020.
The trial was financially supported by AstraZeneca and BMS.