Exploring Circulating Leukocyte RNA Expression: Implications for Treatment Outcomes and Immune-Related Adverse Events in Patients with Triple Negative Breast Cancer Enrolled in the GeparNuevo Trial

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PS14-07

Background

Significant research has been conducted on the influence of immune checkpoint inhibitor therapy on tumor microenvironment, particularly with regard to tumor-infiltrating immune cells. Nevertheless, our understanding of the circulating immune repertoire and its association with treatment outcomes remains limited. While the tumor microenvironment provides detailed information about the interaction of tumor and immune cells, biomarkers from the blood could provide a more easily accessible source for monitoring treatment response and predicting outcome. Consequently, our presented project aimed to explore the RNA phenotype of circulating leukocytes – stabilized at blood draw – and its impact on overall survival (OS), and adverse events of patients enrolled in the GeparNuevo trial[1].

Patients and Methods

The GeparNuevo (GBG 89; NCT02685059) phase II trial focused on the effects of neoadjuvant nab-paclitaxel followed by epirubicin/cyclophosphamide (nabP-EC) chemotherapy combined with the anti-PD-L1 immune checkpoint inhibitor durvalumab versus placebo in patients with non-metastatic triple-negative breast cancer (**Figure 1A**). In order to conduct a comprehensive analysis of circulating leukocyte RNA levels, immediate stabilization of RNA at time of blood draw is of crucial importance[1,2]. Thus, RNA-stabilizing PAXgene tubes were used to collect blood samples prior to treatment initiation. These tubes enable immediate stabilization of RNA during collection and shipment of samples and thus, RNA expression patterns do not change after collection. RNA was extracted from circulating leukocytes of 117 patients and analyzed using a custom NanoString nCounter CodeSet, including 290 immune-related target genes (**Figure 1B**). The associations between 16 immune cell scores, 26 immune signaling scores, 31 individual gene expression patterns, OS, and immune-related adverse events (irAEs) were analyzed. irAEs were defined as toxicities reported as adverse events (AEs) irrespective of relatedness to study treatment based on NCI-CTC criteria v4.0 and being immune-related. irAES included pneumonitis, hepatitis, infusion-related reaction, thyroid dysfunction, hypothyroidism, hyperthyroidism, other thyroid hormone alterations, neuropathy, hepatotoxicity, dermatitis, hypophysitis, and AEs affecting cranial nerves. 174 patients were enrolled into the main study cohort (**Table 1**). From 117 of those blood samples before start of therapy were available. These patients were assigned to the subproject cohort. There were no significant differences regarding patient characteristics between the treatment arms of the subcohort (**Table 2**).

Figure 1. Study and Methods Flow Chart.

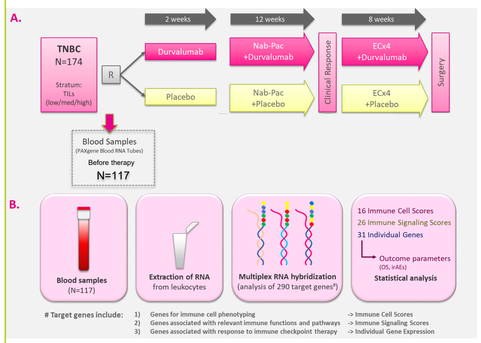


Table 1. Patient Characteristics – Main Study Cohort

Parameter		Durvalumab N=63, N(%)	Placebo N=54, N(%)
Age (yrs), median (range)		49.5 (25.0-74.0)	49.5 (23.0-76.0)
cT3/4		7 (8.0)	3 (3.5)
cN+		27 (30.7)	27 (31.4)
Grading	G3	74 (84.1)	71 (82.6)
Window		59 (67.0)	58 (67.4)
PDL1 status	neg.	9 (11.5)	11 (13.8)
	pos.	69 (88.5)	69 (86.2)
pCR	yes	47 (53.4)	38 (44.2)

Table 2. Patient Characteristics - Subcohort

Parameter		Durvalumab N=63, N(%)	Placebo N=54, N(%)
Age (yrs), median (range)		50.0 (25.0-68.0)	50.5 (23.0-76.0)
cT3/4		4 (6.4)	1 (1.9)
cN+		17 (27.4)	16 (29.6)
Grading	G3	52 (82.5)	44 (81.5)
Window		34 (54.0)	28 (51.9)
PDL1 status	neg.	6 (10.9)	8 (15.1)
	pos.	49 (89.1)	45 (84.9)
pCR	yes	33 (52.4)	29 (53.7)

Results

Univariate Cox regression analysis using continuous scores revealed a significant correlation between *PIP3 activates AKT signaling*, T cells, *CDK2*, and *TIMP1* expression with OS in the **placebo arm** (**Table 3**). Higher expression of *PIP3 activates AKT signaling*, T cells, and *CDK2*, as well as lower expression of *TIMP1,* were associated with prolonged survival (**Table 3**). Notably, T cell scores and *CDK2* expression exhibited a significant **interaction** with the treatment arm in the Cox-PH-Model with continuous scores (p=0.0489 and 0.0210, respectively). For those, Kaplan-Meier-Plots were presented for dichotomized expression levels (cut-off: median, **Figure 2**). Patients with low T cell scores had a significant better OS when treated with durvalumab (**Figure 2A,** p=0.0497). Multivariate Cox regression analysis demonstrated a significant association of *DPP4*, *ICOS* and *MYC* expression with OS (**Table 3**). Additionally, continuous scores of *CDK2*, *CDKN2A*, *F5* and *HLA-DRA* expression were linked to the presence of irAEs (**Table 4**). In the durvalumab arm, *TNFR2 non canonical NFkB pathway* signalling, *CDK2* and *CDKN2A* expression showed an inverse association with the presence of irAEs (**Table 4).** Analysis dichotomized leukocyte RNA expression levels (cut-off: median) of these five genes revealed a significant difference between high versus low expression and the presence of irAEs for *CDKN2A* and the *TNFR2 non canonical NFkB pathway* signalling signature (**Figure 3B and E**, p=0.0193 and p=0.0468, respectively). Significant interaction with the treatment arm was observed for dichotomized RNA expression of *CDKN2A* (**Figure 3B,** p=0.0193).

Overall Survival

Figure 2. Kaplan-Meier-Plots for dichotomized leukocyte RNA expression levels per treatment arm with overall survival (OS)

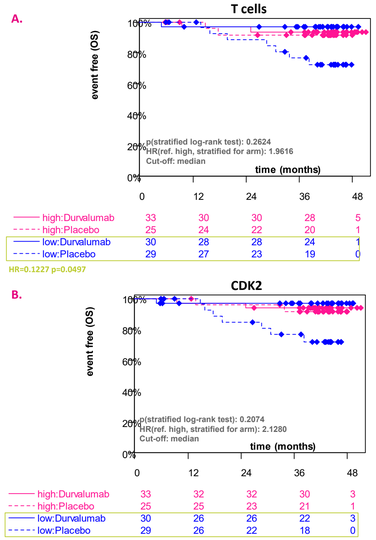


Table 3. Association of leukocyte RNA expression levels with overall survival (OS)

Signature		N	Events	HR (95% CI)	P-value
CDK2	Durvalumab	63	3	51.874 (0.284-9.46e+03)	0.1371
	Placebo	54	9	0.074 (0.009-0.606)	**0.0152**
	Multivariate#	116	12	0.687 (0.056-8.371)	0.7686
	Univariate	117	12	0.158 (0.016-1.544)	0.1126
DPP4	Durvalumab	63	3	0.201 (0.004-10.729)	0.4294
	Placebo	54	9	0.187 (0.031-1.141)	0.0693
	Multivariate#	116	12	0.120 (0.016-0.914)	**0.0407**
	Univariate	117	12	0.147 (0.026-0.823)	**0.0292**
ICOS	Durvalumab	63	3	0.069 (0.002-1.991)	0.1193
	Placebo	54	9	0.244 (0.045-1.334)	0.1038
	Multivariate#	116	12	0.094 (0.009-0.940)	**0.0442**
	Univariate	117	12	0.174 (0.036-0.845)	**0.0301**
MYC	Durvalumab	63	3	0.142 (0.003-6.895)	0.3241
	Placebo	54	9	0.469 (0.069-3.209)	0.4403
	Multivariate#	116	12	0.123 (0.018-0.851)	**0.0338**
	Univariate	117	12	0.320 (0.055-1.867)	0.2055
TIMP1	Durvalumab	63	3	1.677 (0.101-27.828)	0.7182
	Placebo	54	9	7.028 (1.403-35.197)	**0.0177**
	Multivariate#	116	12	3.687 (0.741-18.345)	0.1110
	Univariate	117	12	4.560 (1.121-18.546)	**0.0340**
T cells	Durvalumab	63	3	12.671 (0.057-2.83e+03)	0.3576
	Placebo	54	9	0.018 (0.001-0.443)	**0.0140**
	Multivariate#	116	12	0.175 (0.013-2.424)	0.1937
	Univariate	117	12	0.157 (0.011-2.180)	0.1677
PIP3 activates AKT signaling	Durvalumab	63	3	0.817 (0.172-3.882)	0.7990
	Placebo	54	9	0.287 (0.097-0.844)	**0.0234**
	Multivariate#	116	12	0.596 (0.258-1.378)	0.2260
	Univariate	117	12	0.502 (0.233-1.079)	0.0775

Cox-PH-Model with continuous scores: HRs with 95%-CIs and Wald p-values
including Treatment arm (Durvalumab vs. Placebo), Breast cancer histopathologic grade (G2 vs. G3), Clinical lymph node status by sonography (cN0 vs. cN1-3) and sTILs (low: 0-10% vs. intermediate/high 11-100%)

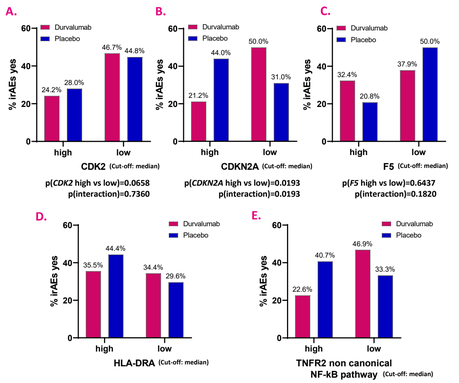
Immune-related adverse events

Table 4. Association of leukocyte RNA expression levels with presence of immune-related adverse events (irAEs)

Signature		N	Events	OR (95% CI)	P-value
CDK2	Durvalumab	63	22	0.065 (0.005-0.880)	**0.0398**
	Placebo	54	20	0.168 (0.013-2.254)	0.1782
	Multivariate#	116	42	0.070 (0.009-0.537)	**0.0104**
	Univariate	117	42	0.103 (0.016-0.645)	**0.0152**
CDKN2A	Durvalumab	63	22	0.424 (0.202-0.890)	**0.0234**
	Placebo	54	20	1.092 (0.454-2.630)	0.8440
	Multivariate#	116	42	0.650 (0.373-1.135)	0.1297
	Univariate	117	42	0.612 (0.359-1.044)	0.0717
F5	Durvalumab	63	22	0.601 (0.251-1.439)	0.2531
	Placebo	54	20	0.338 (0.115-0.998)	**0.0497**
	Multivariate#	116	42	0.384 (0.186-0.794)	**0.0098**
	Univariate	117	42	0.472 (0.242-0.921)	**0.0278**
HLA-DRA	Durvalumab	63	22	4.627 (0.680-31.465)	0.1173
	Placebo	54	20	3.580 (0.584-21.937)	0.1680
	Multivariate#	116	42	4.376 (1.069-17.916)	**0.0401**
	Univariate	117	42	4.060 (1.095-15.055)	**0.0361**
TNFR2 non canonical NF kB pathway	Durvalumab	63	22	0.454 (0.231-0.892)	**0.0220**
	Placebo	54	20	1.105 (0.555-2.201)	0.7760
	Multivariate#	116	42	0.618 (0.368-1.036)	0.0679
	Univariate	117	42	0.679 (0.427-1.079)	0.1015

Logistic regression with continous scores: ORs with 95%-CIs and Wald p-values
including Treatment arm (Durvalumab vs. Placebo), Breast cancer histopathologic grade (G2 vs. G3), Clinical lymph node status by sonography (cN0 vs. cN1-3) and sTILs (low: 0-10% vs. intermediate/high 11-100%)

Figure 3. Association of dichotomized leukocyte RNA expression levels per treatment arm with the presence of immune-related adverse events (irAEs)



Conclusions

Our study provides preliminary evidence that RNA derived from circulating leukocytes may serve as a potential biomarker for predicting treatment outcomes and identifying patients prone to develop side effects during standard-of-care chemotherapy or immune checkpoint therapy.

- Patients with low expression of T cell signature levels who have received durvalumab have a better overall survival compared to patients who received placebo
- For patients of the durvalumab arm lower levels of *CDK2* and *CDKN2A* expression as well as *TNFR2 non canonical NF kB pathway* signature scores were associated with the presence of irAE events
- Patients with high *CDKN2A* levels experience fewer irAE events when treated with durvalumab compared to placebo, while the converse is true for patients with *CDKN2A* expression below the median.

These findings highlight the potential utility of peripheral immune cell RNA profiling in improving treatment strategies and patient management. Further research and validation are necessary to fully comprehend the clinical significance and broader implications of these findings.

References

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Presented at: San Antonio Breast Cancer Symposium® - December 5-9, 2023

This study was funded by Walter Schulz Stiftung and the clinical trial was funded by AstraZeneca and Celgene.