



# RNA expression levels from peripheral immune cells, a minimal-invasive liquid biopsy source to predict response to therapy, survival and immune-related adverse events in patients with triple negative breast cancer enrolled in the GeparNuevo trial

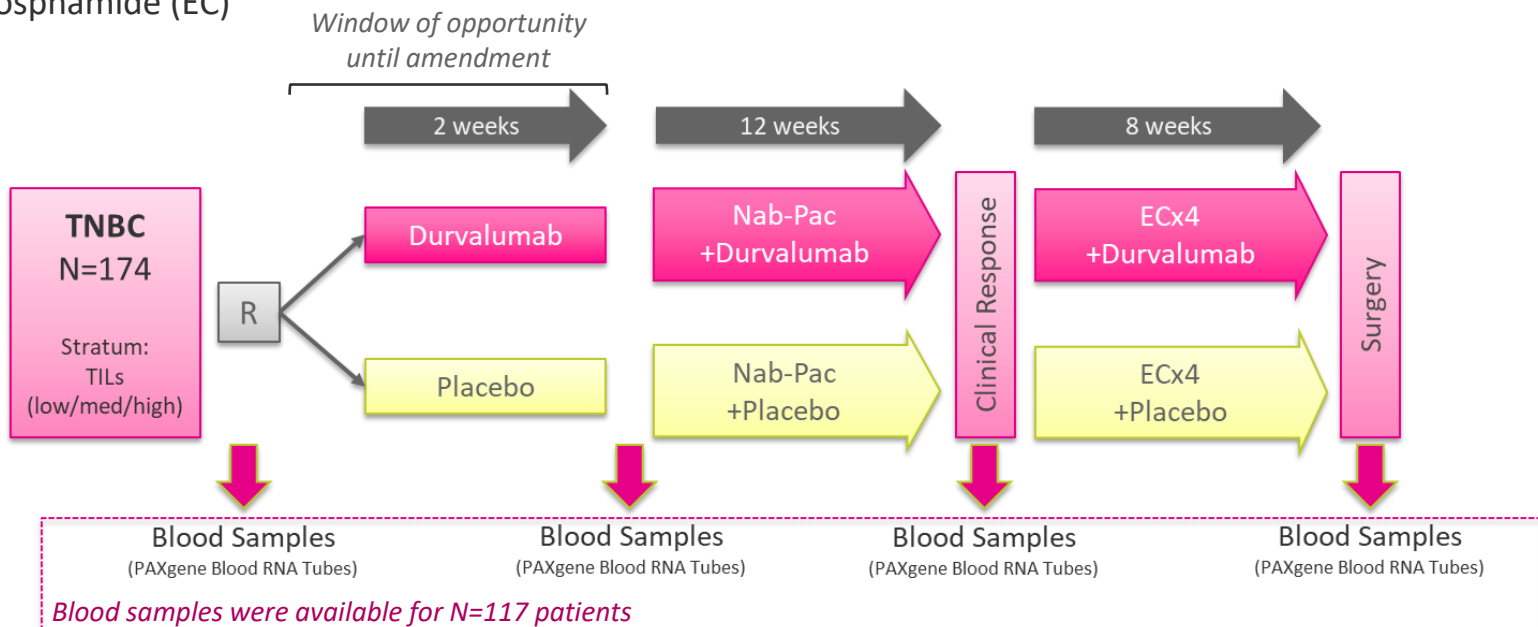
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# GeparNuevo: Study Design

- GeparNuevo:** A randomised phase II double-blind placebo-controlled study randomizing patients with TNBC to durvalumab or placebo given every 4 weeks in addition to nab-paclitaxel followed by standard Epirubicin/ Cyclophosphamide (EC)



Loibl S, et al. Annals of Oncology, 2019; 30, 1279–1288

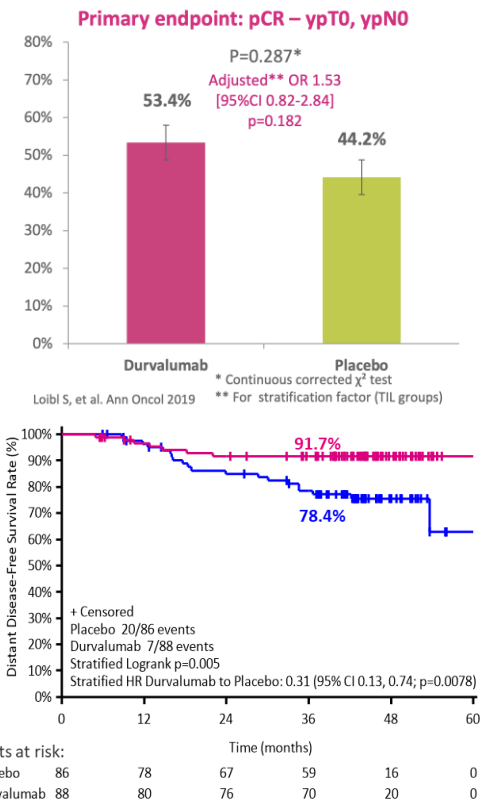
# GeparNuevo: Study results

- **Population:** Primary non-metastatic TNBC
- **Recruitment:** June 2016 - October 2017
- **Primary endpoint:** pCR (ypT0 ypN0) after neoadjuvant therapy
- **Main secondary endpoints:** iDFS, DDFS, OS
- **pCR rate** with durvalumab was **53.4%** versus placebo **44.2%** (OR=1.53, 95% CI 0.82-2.84, p=0.182)
- **pCR rate of the “Window” cohort<sup>1</sup>** with durvalumab was **61.0%** versus placebo **41.0%** (OR=2.22, 95% CI 1.06–4.64, p=0.035; interaction p=0.048)
- **Significant differences were observed for 3-year DDFS [HR= 0.31 (95% CI 0.13, 0.74), p=0.0078] and OS [HR=0.24 (95% CI 0.08, 0.72), p=0.0108] independent of pCR effect**

<sup>1</sup> Patients who started with durvalumab or placebo monotherapy prior to chemotherapy

Loibl S, et al. Annals of Oncology, 2019; 30, 1279–1288

Loibl S, et al. Annals of Oncology, 2022, 33, 1149-1158





- **Objective 1:** Descriptive analysis of **changes of leukocyte RNA expression** per signature levels during therapy at all four measurement times
- **Objective 2:** Association of leukocyte RNA expression per signature levels **before therapy** with **pathologic complete response (pCR) rate (ypT0/ypN0)**
- **Objective 3:** Association of leukocyte RNA expression per signature levels **before therapy** with **distant disease-free survival (DDFS)**



# Baseline Characteristics

From 117 patients enrolled in the GeparNuevo Trial blood samples from before therapy were available for RNA testing (Subproject Cohort).

## Subproject Cohort

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)

## Main Study Cohort

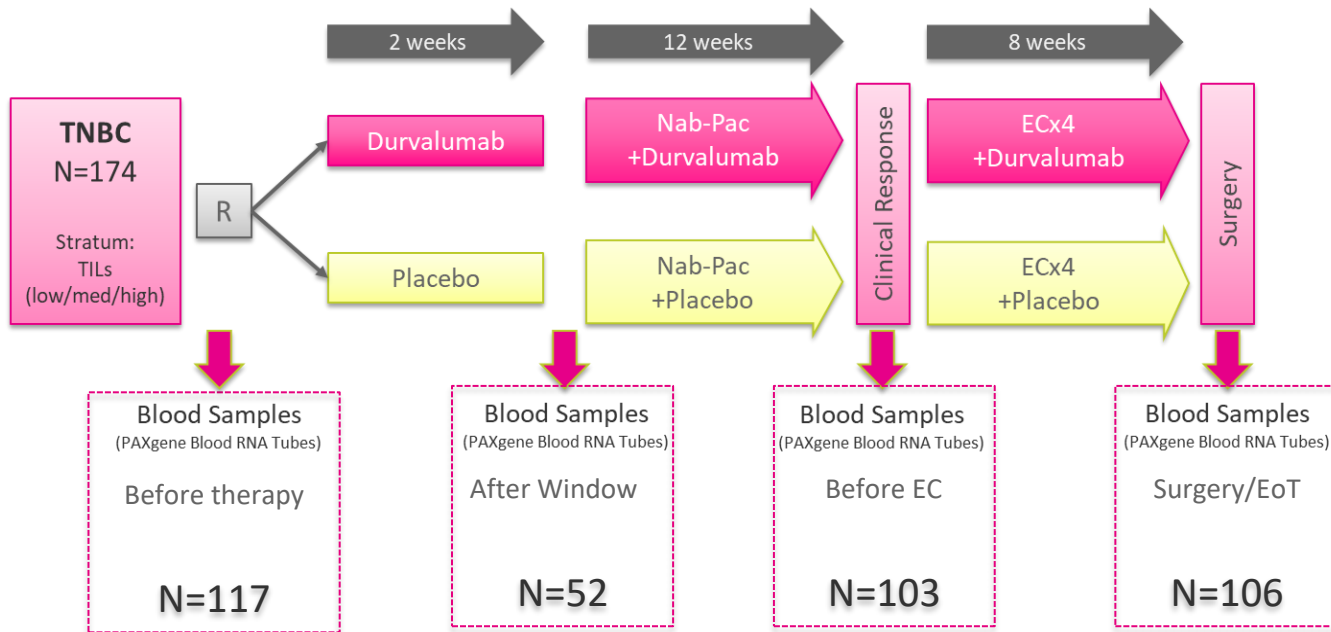
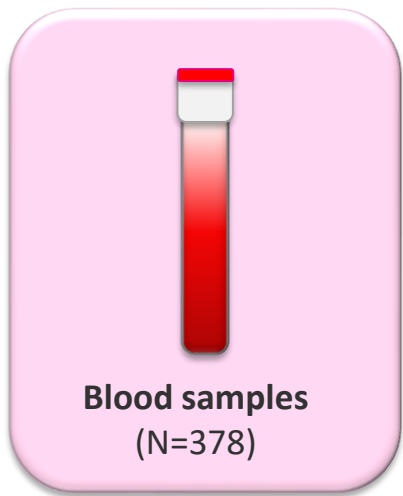
Parameter	Durvalumab N=88, N(%)	Placebo N=86, N(%)
Age (yrs), median (range)	49.5 (25.0-74.0)	49.5 (23.0-76.0)
cT3/4	7 (8.0)	3 (3.5)
cN+	27 (30.7)	27 (31.4)
Grading G3	74 (84.1)	71 (82.6)
Window	59 (67.0)	58 (67.4)
PDL1 status neg.	9 (11.5)	11 (13.8)
pos.	69 (88.5)	69 (86.2)
pCR yes	47 (53.4)	38 (44.2)

No significant differences were observed between the treatment arms

PDL1 status: Percentage of tumor cells with membranous staining and percentage of PDL1 positive TILs with membranous or cytoplasmic staining (assessed by using the SP263 antibody and a cut-off of  $\geq 1\%$ )

# Methods

## Analysis of RNA expression levels of peripheral immune cells from patients enrolled into GeparNuevo trial.





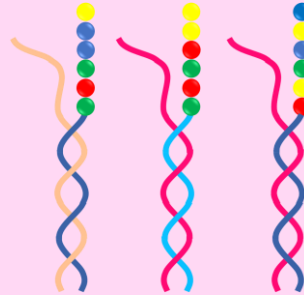
## Analysis of RNA expression levels of peripheral immune cells from patients enrolled into GeparNuevo trial.



**Blood samples**  
(N=378)



**Extraction of RNA**  
from leukocytes



**Multiplex RNA hybridization**  
(analysis of 290 target genes#)

16 Immune Cell Scores  
26 Immune Signaling Scores  
31 Individual Genes

↳ Outcome parameters  
(pCR, DDFS)

**Statistical analysis**

# Target genes include:

- 1) Genes for immune cell phenotyping
- 2) Genes associated with relevant immune functions and pathways
- 3) Genes associated with response to immune checkpoint therapy

-> Immune Cell Scores  
-> Immune Signaling Scores  
-> Individual Gene Expression



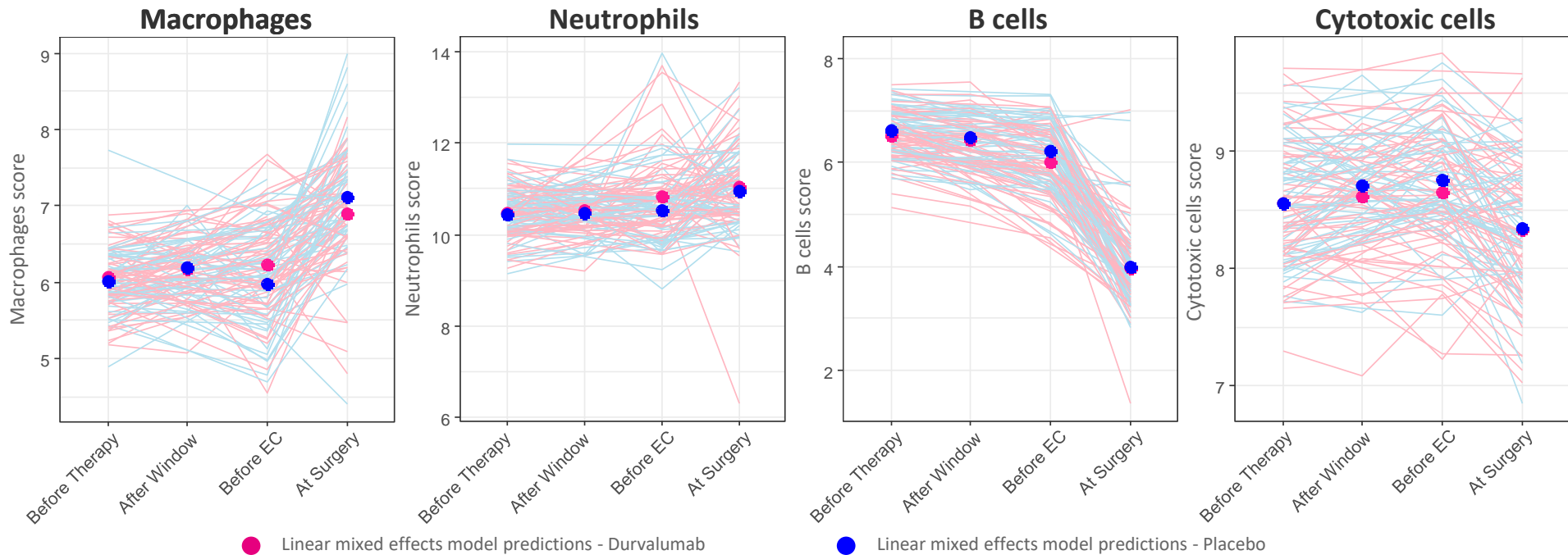
- **Objective 1:** Descriptive analysis of **changes of leukocyte RNA expression** per signature levels during therapy at all four measurement times





# Results: Change of immune cells

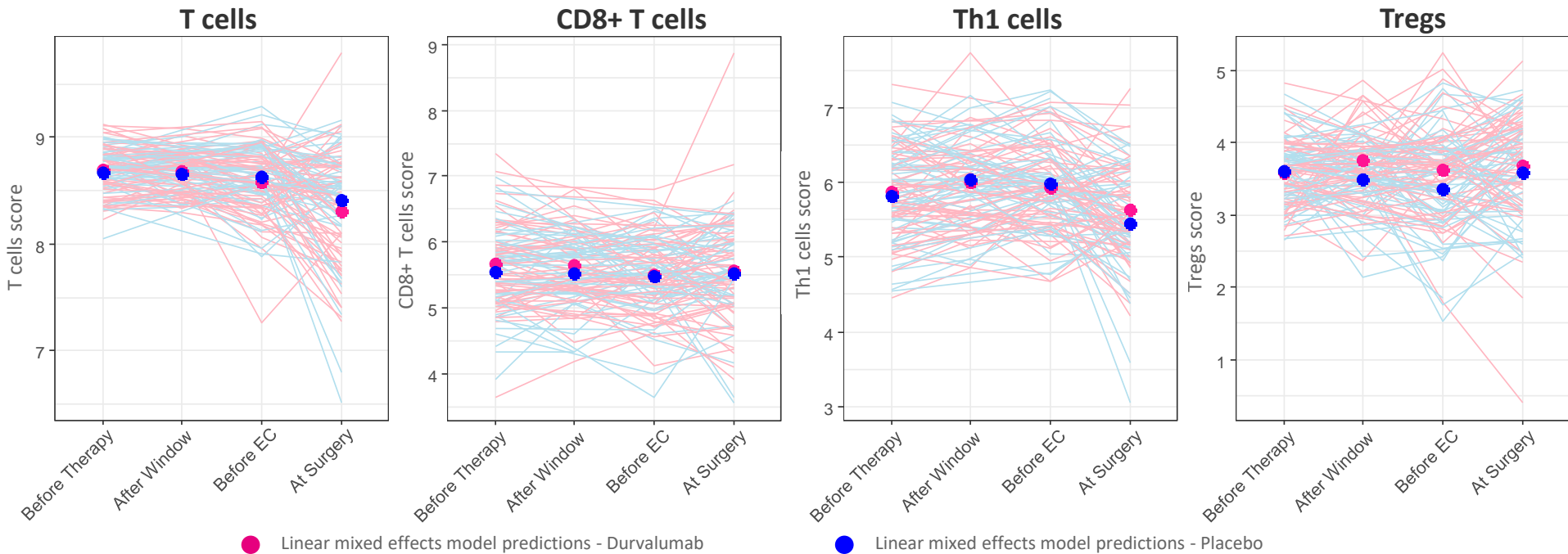
Immune cell type scores representing macrophages and neutrophils significantly increased during treatment, while B cell and Cytotoxic cell scores decreased ( $p < 0.0001$ , respectively) regardless of treatment arm.





# Results: Change of immune cells

Immune cell type scores representing Th1 cells significantly decreased ( $p < 0.0001$  and  $p < 0.0004$ , Durvalumab and Placebo) regardless of treatment arm.





- **Objective 2:** Association of leukocyte RNA expression per signature levels **before therapy** with pathologic complete response (**pCR**) rate (**ypT0/ypN0**)



# Results: Pathologic complete response

**Objective 2:** Association of leukocyte RNA expression per signature levels **before therapy** with pathologic complete response (pCR) rate (ypT0/ypN0)

## 16 Immune Cell Scores

B-cells

T-cells

Treg

Mast cells

NK cells

CD8

CD8/Treg

Th1

...

## 26 Immune Signaling Scores

PD1 signaling

NK Cell Activity

NFkB pathway

TCR signaling

AKT signaling

...

## 31 Individual Genes

CCL3

ERBB2

DPP4

ICOS

ITGA4

MYC

TIMP1

CDK2

...



# Results: Pathologic complete response

**Objective 2:** Association of leukocyte RNA expression per signature levels **before therapy** with pathologic complete response (**pCR**) rate (**ypT0/ypN0**)

## 16 Immune Cell Scores

B-cells

T-cells

Treg

Mast cells

NK cells

CD8

CD8/Treg

Th1

...



**1 score** was significantly associated with pCR

## 26 Immune Signaling Scores

PD1 signaling

NK Cell Activity

NFkB pathway

TCR signaling

AKT signaling

...



**No scores** were significantly associated with pCR

## 31 Individual Genes

CCL3

ERBB2

DPP4

ICOS

ITGA4

MYC

TIMP1

CDK2

...



**5 genes** were significantly associated with pCR

P-values < 0.05 were considered statistically significant



# Results: Pathologic complete response

Gene		N	OR (95% CI)	P-value	P-value Interaction arm
CCL3	Durvalumab	63	0.541 (0.294-0.996)	<b>0.0485</b>	<b>0.0175</b>
	Placebo	54	1.628 (0.830-3.195)	0.1564	
	Multivariate <sup>#</sup>	116	0.962 (0.611-1.516)	0.8685	
DPP4	Durvalumab	63	7.346 (1.130-47.733)	<b>0.0368</b>	0.2699
	Placebo	54	1.863 (0.391-8.882)	0.4352	
	Multivariate <sup>#</sup>	116	3.695 (1.057-12.918)	<b>0.0407</b>	
ITGA4	Durvalumab	63	10.825 (1.137-103.054)	<b>0.0383</b>	0.1889
	Placebo	54	1.412 (0.184-10.842)	0.7401	
	Multivariate <sup>#</sup>	116	5.631 (1.061-29.886)	<b>0.0424</b>	
TIMP1	Durvalumab	63	0.283 (0.074-1.084)	0.0655	0.7257
	Placebo	54	0.407 (0.089-1.874)	0.2488	
	Multivariate <sup>#</sup>	116	0.253 (0.084-0.760)	<b>0.0143</b>	
MYC	Durvalumab	63	3.987 (0.870-18.267)	0.0749	0.3283
	Placebo	54	1.408 (0.337-5.873)	0.6389	
	Multivariate <sup>#</sup>	116	3.357 (1.118-10.075)	<b>0.0308</b>	

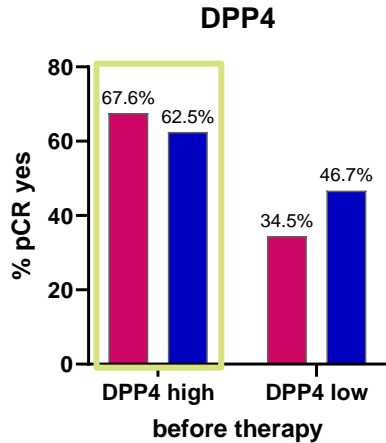
Logistic regression with continuous scores: ORs with 95%-CIs and Wald p-values

<sup>#</sup> 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%)

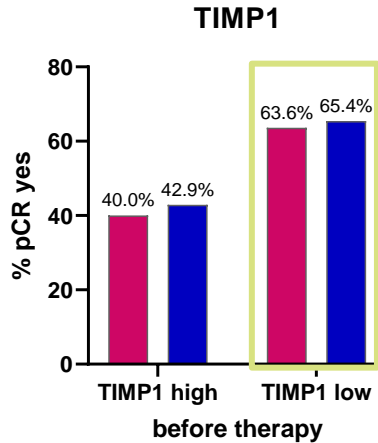


# Results: Pathologic complete response

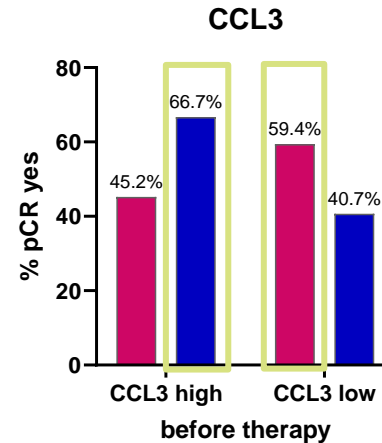
Cut-off: median



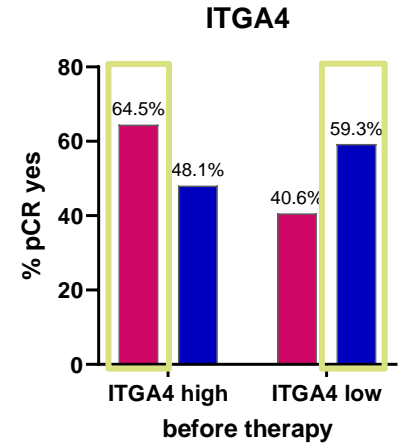
p(DPP4 high vs low)=0.0100  
p(interaction)=0.3421



p(TIMP1 high vs low)=0.0632  
p(interaction)=0.9568



p(CCL3 high vs low)=0.2604  
p(interaction)=0.0311



p(ITGA4 high vs low)=0.0602  
p(interaction)=0.0594

■ Durvalumab  
■ Placebo



- **Objective 3:** Association of leukocyte RNA expression per signature levels **before therapy** with **distant disease-free survival (DDFS)**





# Results: Distant disease-free survival

**Objective 3:** Association of leukocyte RNA expression per signature levels **before therapy** with distant disease-free survival (**DDFS**)

## 16 Immune Cell Scores

B-cells

T-cells

Treg

Mast cells

NK cells

CD8

CD8/Treg

Th1

...

## 26 Immune Signaling Scores

PD1 signaling

NK Cell Activity

NFkB pathway

TCR signaling

AKT signaling

...

## 31 Individual Genes

CCL3

ERBB2

DPP4

ICOS

ITGA4

MYC

TIMP1

CDK2

...



# Results: Distant disease-free survival

**Objective 3:** Association of leukocyte RNA expression per signature levels **before therapy** with distant disease-free survival (**DDFS**)

## 16 Immune Cell Scores

B-cells

T-cells

Treg

Mast cells

NK cells

CD8

CD8/Treg

Th1

...

## 26 Immune Signaling Scores

PD1 signaling

NK Cell Activity

NFkB pathway

TCR signaling

AKT signaling

...

## 31 Individual Genes

CCL3

ERBB2

DPP4

ICOS

ITGA4

MYC

TIMP1

CDK2

...

**2 scores** were significantly associated with DDFS

**1 scores** was significantly associated with DDFS

**5 genes** were significantly associated with DDFS

P-values < 0.05 were considered statistically significant

# Results: Distant disease-free survival

## Immune cell and signaling scores

Signature		N	Events	HR (95% CI)	P-value
<b>Mast cells</b>	Durvalumab	63	5	0.742 (0.245-2.244)	0.5974
	Placebo	54	12	0.632 (0.377-1.061)	0.0824
	Multivariate <sup>#</sup>	116	17	0.823 (0.436-1.552)	0.5476
	Univariate	117	17	0.604 (0.366-0.995)	<b>0.0479</b>
<b>Treg</b>	Durvalumab	63	5	0.053 (0.004-0.764)	<b>0.0309</b>
	Placebo	54	12	0.545 (0.155-1.917)	0.3439
	Multivariate <sup>#</sup>	116	17	0.420 (0.136-1.305)	0.1337
<b>PIP3 activ. AKT sign.</b>	Durvalumab	63	5	0.713 (0.219-2.323)	0.5749
	Placebo	54	12	0.373 (0.165-0.843)	<b>0.0178</b>
	Multivariate <sup>#</sup>	116	17	0.601 (0.301-1.197)	0.1472

## Individual Genes \*

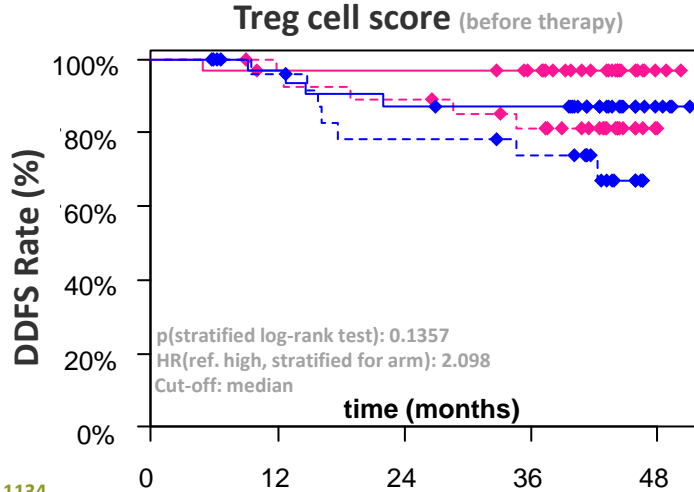
Gene		N	Events	HR (95% CI)	P-value
<b>CDK2</b>	Placebo	54	12	0.123 (0.017-0.896)	<b>0.0386</b>
	Univariate	117	17	0.191 (0.046-0.797)	<b>0.0231</b>
<b>DPP4</b>	Multivariate <sup>#</sup>	116	17	0.111 (0.017-0.715)	<b>0.0207</b>
	Univariate	117	17	0.232 (0.060-0.895)	<b>0.0339</b>
<b>MYC</b>	Multivariate <sup>#</sup>	116	17	0.208 (0.043-0.995)	<b>0.0493</b>
	Placebo	54	12	4.149 (1.058-16.278)	<b>0.0413</b>

Cox-PH-Model with continuous scores: HRs with 95%-CIs and Wald p-values

<sup>#</sup> 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%)

\* Only values with a p-value < 0.05 are presented

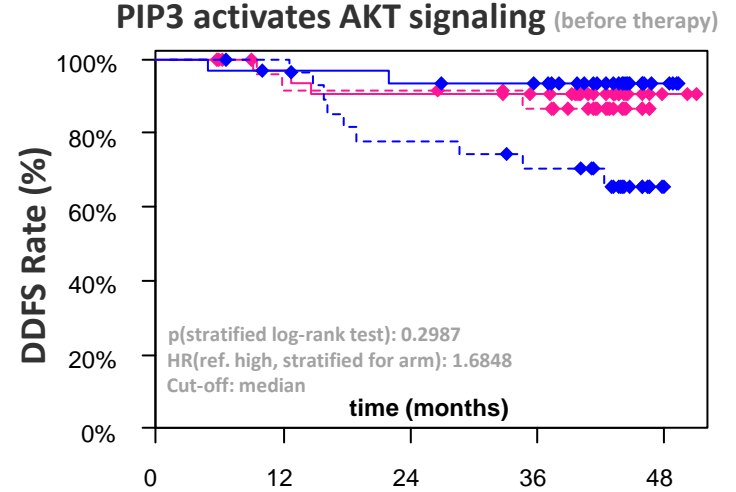
# Results: Distant disease-free survival



HR=5.6681, p=0.1134

— high:Durvalumab	1/30	28	28	25	2
- - - high:Placebo	5/28	26	24	20	1
— low:Durvalumab	4/33	30	27	26	4
- - - low:Placebo	7/26	23	18	16	0

Regulatory T cells (Treg) inhibit effector B and T cells and play a central role in suppression of anti-tumor immune responses.



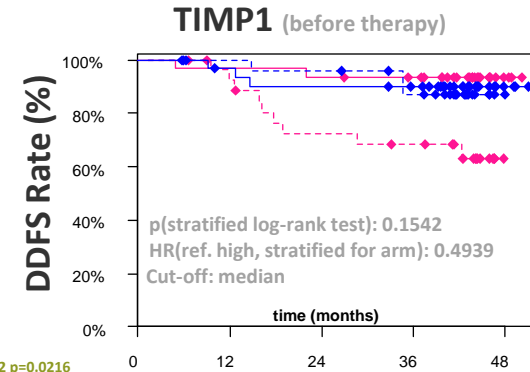
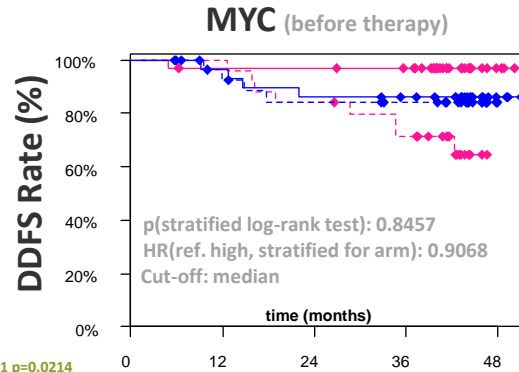
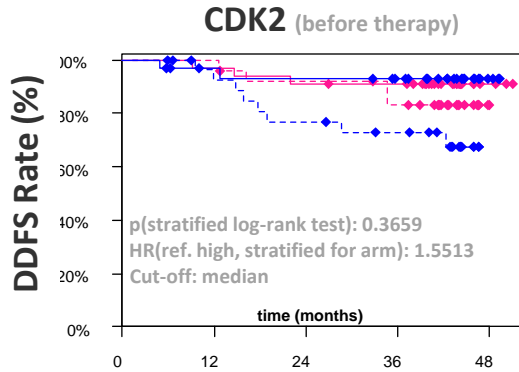
— high:Durvalumab	3/33	30	28	26	2
- - - high:Placebo	3/25	21	21	18	0
— low:Durvalumab	2/30	28	27	25	4
- - - low:Placebo	9/29	28	21	18	1

HR=5.3208, p=0.0326

AKT is activated by the messenger PIP3, a phospholipid that is generated by PI3K. Active AKT plays important roles in cell survival and metabolism.



# Results: Distant disease-free survival



— high:Durvalumab	3/33	32	30	29	3
- - - high:Placebo	4/25	25	22	19	1
— low:Durvalumab	2/30	26	25	22	3
- - - low:Placebo	8/29	24	20	17	0

HR=11.5021 p=0.0214

— high:Durvalumab	1/33	31	31	29	3
- - - high:Placebo	8/25	25	21	17	0
— low:Durvalumab	4/30	27	24	22	3
- - - low:Placebo	4/29	24	21	19	1

HR=6.0372 p=0.0216

— high:Durvalumab	2/30	29	28	26	3
- - - high:Placebo	9/28	24	18	16	0
— low:Durvalumab	3/33	29	27	25	3
- - - low:Placebo	3/26	25	24	20	1

HR=4.4670 p=0.0585



- **Changes of peripheral immune cells** under therapy seemed to be dependent on **chemotherapy** but not immune checkpoint therapy
- It appears that patients in the **durvalumab** arm who had **high levels of CCL3** expression before therapy had a **lower pCR rate** compared to patients in the placebo arm (significant interaction)
- Patients of the **durvalumab** arm who had **high levels of regulatory T cell** scores before therapy had **lower risk of distant disease-free events** compared to those with low levels
- **DPP4, MYC and ICOS** expression was associated with **distant disease-free survival** regardless of treatment arm
- While these findings offer promising insights, **further research** is necessary to **validate** and **expand** upon these initial results

RNA expression levels from peripheral immune cells could enable differentiation between patients who might benefit from neoadjuvant immune checkpoint therapy compared to standard therapies



# Acknowledgement

- All patients who agreed to participate in the GeparNuevo trial
- All GeparNuevo study centers, investigators and study nurses
- All members of the GBG and AGO-B
- All cooperating partners:
  - Department of Gynecology and Obstetrics, University Clinic Erlangen
  - Institute for Pathology, University Clinic Erlangen
  - Financial and drug support: AstraZeneca, Bristol Myers Squibb
  - Funding Source: Walter Schulz Stiftung





## Complete list of analyzed scores and individual genes

### 16 Immune Cell Scores

B-cells score, T-cells score, Th1 cells score, Treg score, CD45 score, CD8 T cell score, Cytotoxic cells score, DC score, Macrophages score, Mast cells score, Neutrophils Score, NK cells score, NK CD56dim cells score, CD8 vs. Exhausted CD8, CD8 vs Treg, CD8 vs T cells

### 26 Immune Signaling Scores

PD1 signaling, Immunecheckpoint Function, T-cell Immunecheckpoint, CD4 T cell differentiation, lymphocyte activation, TH1 and TH2 response, TH17 response, T cell priming and activation, Killing of cancer cells, Immune Inhibition, NK Cell Activity, T-Cell Functions , NK Cell Functions, Cell Functions , B-Cell Functions, Myeloid Cell Activity, Immunometabolism, Costimulatory Signaling, Cytotoxicity, Lymphoid Compartment, Myeloid Compartment, Myeloid Inflammation, Immune Response to tumor, MHC Class II antigen presentation, TCR signaling, Costimulation by the CD28 family

### 31 Individual Genes

ADAM17, CCL3, CCR3, CDK2, CDKN2A, CTSD, CXCL1, DPP4, ERBB2, F5, GADD45A, HLA\_DRA, ICOS, IL18BP, IL2RA, IL5, IL8, IRAK3, ITGA4, MMP9, MYC, NLRC4, NRAS, PTGS2, RHOC, SOCS3, TGFB1, TIMP1, TLR9, TXNRD1, UBE2C