Translationale Projekte zur Immunoonkologie

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Conflict of Interest

- Keine Interessenskonflikte

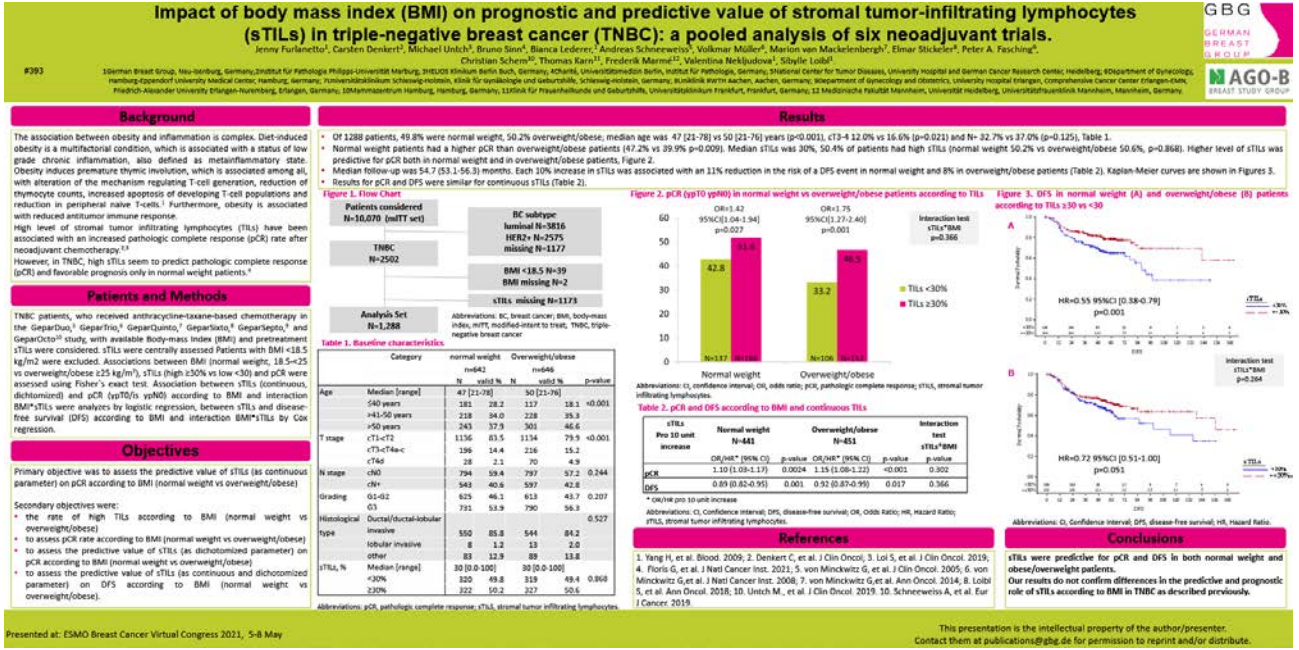
Translationale Projekte zur Immunoonkologie

- **Einfluss des BMI auf prädiktiven Wert von TILs**
- **Immun-Marker bei Brustkrebs in der Schwangerschaft**
- **GeparNuevo: Immun-Checkpoint-Blockade**
  - Validierung von RNA-Signaturen
  - Immunzell-Profile im peripheren Blut
  - Spatial Profiling
  - Survival-Daten

Einfluss des BMI auf prädiktiven Wert von TILs

- Adipositas mit reduzierter Anti-Tumor-Immunantwort assoziiert
- Prädiktiver Wert von TILs für pCR/Prognose auf normalen BMI beschränkt?

Gepoolte Untersuchung von TNBC aus G2, G3, G5, G6, G7



Impact of body mass index (BMI) on prognostic and predictive value of stromal tumor-infiltrating lymphocytes (sTILs) in triple-negative breast cancer (TNBC): a pooled analysis of six neoadjuvant trials.

Jenny Furlanetto[1], Carsten Denkert[2], Michael Untch[3], Bruno Sinn[4], Blanca Lederer,[5] Andreas Schneeweiss[5], Volkmar Müller[6], Marion van Mackelenbergh[7], Elmar Stickeler[8], Peter A. Fasching[9], Christian Schem[10], Thomas Karn[11], Frederik Marmé[12], Valentina Nekljudova[1], Sibylle Loibl[1].

#393

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Background

The association between obesity and inflammation is complex. Diet-induced obesity is a multifactorial condition, which is associated with a status of low grade chronic inflammation, also defined as metainflammatory state. Obesity induces premature thymic involution, which is associated among all, with alteration of the mechanism regulating T-cell generation, reduction of thymocyte counts, increased apoptosis of developing T-cell populations and reduction in peripheral naive T-cells.[1] Furthermore, obesity is associated with reduced antitumor immune response.

High level of stromal tumor infiltrating lymphocytes (TILs) have been associated with an increased pathologic complete response (pCR) rate after neoadjuvant chemotherapy.[2,3]

However, in TNBC, high sTILs seem to predict pathologic complete response (pCR) and favorable prognosis only in normal weight patients.[4]

Patients and Methods

TNBC patients, who received anthracycline-taxane-based chemotherapy in the GeparDuo,[5] GeparTrio,[6] GeparQuinto,[7] GeparSixto,[8] GeparSepto,[9] and GeparOcto[10] study, with available Body-mass Index (BMI) and pretreatment sTILs were considered. sTILs were centrally assessed Patients with BMI <18.5 kg/m2 were excluded. Associations between BMI (normal weight, 18.5-<25 vs overweight/obese ≥25 kg/m^2), sTILs (high ≥30% vs low <30) and pCR were assessed using Fisher's exact test. Association between sTILs (continuous, dichtomized) and pCR (ypT0/is ypN0) according to BMI and interaction BMI*sTILs were analyzes by logistic regression, between sTILs and disease-free survival (DFS) according to BMI and interaction BMI*sTILs by Cox regression.

Objectives

Primary objective was to assess the predictive value of sTILs (as continuous parameter) on pCR according to BMI (normal weight vs overweight/obese)

Secondary objectives were:

- the rate of high TILs according to BMI (normal weight vs overweight/obese)
- to assess pCR rate according to BMI (normal weight vs overweight/obese)
- to assess the predictive value of sTILs (as dichotomized parameter) on pCR according to BMI (normal weight vs overweight/obese)
- to assess the predictive value of sTILs (as continuous and dichotomized parameter) on DFS according to BMI (normal weight vs overweight/obese).

Results

- Of 1288 patients, 49.8% were normal weight, 50.2% overweight/obese; median age was 47 [21-78] vs 50 [21-76] years (p<0.001), cT3-4 12.0% vs 16.6% (p=0.021) and N+ 32.7% vs 37.0% (p=0.125), Table 1.
- Normal weight patients had a higher pCR than overweight/obese patients (47.2% vs 39.9% p=0.009). Median sTILs was 30%, 50.4% of patients had high sTILs (normal weight 50.2% vs overweight/obese 50.6%, p=0.868). Higher level of sTILs was predictive for pCR both in normal weight and in overweight/obese patients, Figure 2.
- Median follow-up was 54.7 (53.1-56.3) months. Each 10% increase in sTILs was associated with an 11% reduction in the risk of a DFS event in normal weight and 8% in overweight/obese patients (Table 2). Kaplan-Meier curves are shown in Figures 3.
- Results for pCR and DFS were similar for continuous sTILs (Table 2).

Figure 1. Flow Chart

Table 1. Baseline characteristics

Category		normal weight n=642 N	valid %	Overweight/obese n=646 N	valid %	p-value
Age	Median [range]	47 [21-78]		50 [21-76]		
	≤40 years	181	28.2	117	18.1	<0.001
	>41-50 years	218	34.0	228	35.3	
	>50 years	243	37.9	301	46.6	
T stage	cT1-cT2	1136	83.5	1134	79.9	<0.001
	cT3-cT4a-c	196	14.4	216	15.2	
	cT4d	28	2.1	70	4.9	
N stage	cN0	794	59.4	797	57.2	0.244
	cN+	543	40.6	597	42.8	
Grading	G1-G2	625	46.1	613	43.7	0.207
	G3	731	53.9	790	56.3	
Histological type	Ductal/ductal-lobular invasive	550	85.8	544	84.2	0.527
	lobular invasive	8	1.2	13	2.0	
	other	83	12.9	89	13.8	
sTILs, %	Median [range]	30 [0.0-100]		30 [0.0-100]		
	<30%	320	49.8	319	49.4	0.868
	≥30%	322	50.2	327	50.6	

Abbreviations: pCR, pathologic complete response; sTILs, stromal tumor infiltrating lymphocytes

Figure 2. pCR (ypT0 ypN0) in normal weight vs overweight/obese patients according to TILs

Abbreviations: CI, confidence interval; OR, odds ratio; pCR, pathologic complete response; sTILs, stromal tumor infiltrating lymphocytes.

Table 2. pCR and DFS according to BMI and continuous TILs

sTILs Pro 10 unit increase	Normal weight N=441 OR/HR* (95% CI)	p-value	Overweight/obese N=451 OR/HR* (95% CI)	p-value	Interaction test sTILs*BMI p-value
pCR	1.10 (1.03-1.17)	0.0024	1.15 (1.08-1.22)	<0.001	0.302
DFS	0.89 (0.82-0.95)	0.001	0.92 (0.87-0.99)	0.017	0.366

* OR/HR pro 10 unit increase

Abbreviations: CI, Confidence interval; DFS, disease-free survival; OR, Odds Ratio; HR, Hazard Ratio; sTILs, stromal tumor infiltrating lymphocytes.

Figure 3. DFS in normal weight (A) and overweight/obese (B) patients according to TILs ≥30 vs <30

Abbreviations: CI, Confidence interval; DFS, disease-free survival; HR, Hazard Ratio.

References

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Conclusions

sTILs were predictive for pCR and DFS in both normal weight and obese/overweight patients.
Our results do not confirm differences in the predictive and prognostic role of sTILs according to BMI in TNBC as described previously.

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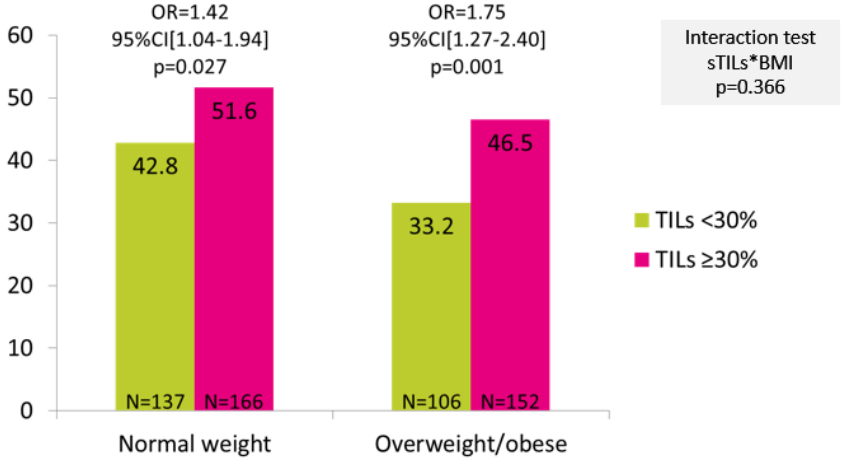
Furlanetto et al. ESMO-Breast 2021

Einfluss des BMI auf prädiktiven Wert von TILs

- Prädiktiver Wert von TILs für pCR/Prognose auf normalen BMI beschränkt?
- sTILs sowohl bei normalgewichtigen wie auch Patientinnen mit erhöhtem BMI sowohl mit pCR als auch besserem Survival assoziiert.
- Ergebnisse konnten beschriebenen Unterschied in Prädiktion und Prognose entsprechend des BMI nicht bestätigen.

Figure 2. pCR (ypT0 ypN0) in normal weight vs overweight/obese patients according to TILs

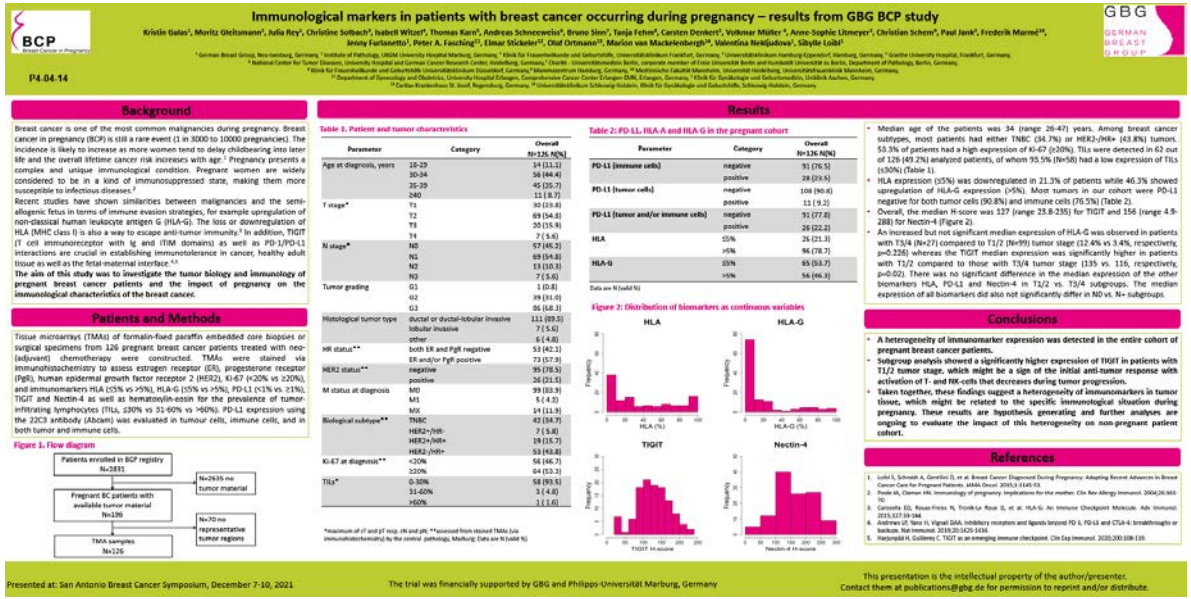


Furlanetto et al. ESMO-Breast 2021

Immun-Marker bei Brustkrebs in der Schwangerschaft

- Schwangerschaft als immunsupprimierter Status
- Untersuchungen zum Einfluss der Schwangerschaft auf die immunologischen Eigenschaften von Tumoren

TMA-Kollektiv von schwangeren Patientinnen aus der BCP-Studie



Immunological markers in patients with breast cancer occurring during pregnancy – results from GBG BCP study

Kristin Galas[1], Moritz Gleitsmann[2], Julia Rey[3], Christine Solbach[4], Isabell Witzel[5], Thomas Karn[6], Andreas Schneeweiss[6], Bruno Sinn[7], Tanja Fehm[8], Carsten Denkert[9], Volkmar Müller [4], Anne-Sophie Litmeyer[1], Christian Schem[8], Paul Jank[9], Frederik Marmé[10], Jenny Furlanetto[1], Peter A. Fasching[11], Elmar Stickeler[12], Olaf Ortmann[13], Marion van Mackelenbergh[14], Valentina Nekljudova[1], Sibylle Loibl[1]

P4.04.14

Background

Breast cancer is one of the most common malignancies during pregnancy. Breast cancer in pregnancy (BCP) is still a rare event (1 in 3000 to 10000 pregnancies). The incidence is likely to increase as more women tend to delay childbearing into later life and the overall lifetime cancer risk increases with age.[1] Pregnancy presents a complex and unique immunological condition. Pregnant women are widely considered to be in a kind of immunosuppressed state, making them more susceptible to infectious diseases.[2] Recent studies have shown similarities between malignancies and the semi-allogenic fetus in terms of immune evasion strategies, for example upregulation of non-classical human leukocyte antigen G (HLA-G). The loss or downregulation of HLA (MHC class I) is also a way to escape anti-tumor immunity.[3] In addition, TIGIT (T cell immunoreceptor with Ig and ITIM domains) as well as PD-1/PD-L1 interactions are crucial in establishing immunotolerance in cancer, healthy adult tissue as well as the fetal-maternal interface.[4,5]

The aim of this study was to investigate the tumor biology and immunology of pregnant breast cancer patients and the impact of pregnancy on the immunological characteristics of the breast cancer.

Patients and Methods

Tissue microarrays (TMAs) of formalin-fixed paraffin embedded core biopsies or surgical specimens from 126 pregnant breast cancer patients treated with neo-(adjuvant) chemotherapy were constructed. TMAs were stained via immunohistochemistry to assess estrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2), KI-67 (<20% vs ≥20%), and immunomarkers HLA (≤5% vs >5%), HLA-G (≤5% vs >5%), PD-L1 (<1% vs. ≥1%), TIGIT and Nectin-4 as well as hematoxylin-eosin for the prevalence of tumor-infiltrating lymphocytes (TILs, ≤30% vs 31-60% vs >60%). PD-L1 expression using the 22C3 antibody (Abcam) was evaluated in tumour cells, immune cells, and in both tumor and immune cells.

Figure 1. Flow diagram

Results

Table 1. Patient and tumor characteristics

Parameter	Category	Overall N=126 N(%)
Age at diagnosis, years	18-29	14 (11.1)
	30-34	56 (44.4)
	35-39	45 (35.7)
	≥40	11 (8.7)
T stage*	T1	30 (23.8)
	T2	69 (54.8)
	T3	20 (15.9)
	T4	7 (5.6)
N stage*	N0	57 (45.2)
	N1	69 (54.8)
	N2	13 (10.3)
	N3	7 (5.6)
Tumor grading	G1	1 (0.8)
	G2	39 (31.0)
	G3	86 (68.3)
Histological tumor type	ductal or ductal-lobular invasive	111 (89.5)
	lobular invasive	7 (5.6)
	other	6 (4.8)
HR status**	both ER and PgR negative	53 (42.1)
	ER and/or PgR positive	73 (57.9)
HER2 status**	negative	95 (78.5)
	positive	26 (21.5)
M status at diagnosis	M0	99 (82.9)
	M1	5 (4.2)
	MX	14 (11.9)
Biological subtype**	TNBC	43 (34.7)
	HER2+/HR-	7 (5.6)
	HER2+/HR+	19 (15.7)
	HER2-/HR+	53 (43.8)
Ki-67 at diagnosis**	<20%	56 (46.7)
	≥20%	64 (53.3)
TILs*	0-30%	58 (93.5)
	31-60%	3 (4.8)
	>60%	1 (1.6)

*maximum of cT and pT resp. cN and pN; **assessed from stained TMAs (via immunohistochemistry) by the central pathology, Marburg; Data are N (valid %)

Table 2: PD-L1, HLA-A and HLA-G in the pregnant cohort

Parameter	Category	Overall N=126 N(%)
PD-L1 (immune cells)	negative	91 (76.5)
	positive	28 (23.5)
PD-L1 (tumor cells)	negative	108 (90.8)
	positive	11 (9.2)
PD-L1 (tumor and/or immune cells)	negative	91 (77.8)
	positive	26 (22.2)
HLA	≤5%	26 (21.3)
	>5%	96 (78.7)
HLA-G	≤5%	65 (53.7)
	>5%	56 (46.3)

Data are N (valid %)

Figure 2: Distribution of biomarkers as continuous variables

- Median age of the patients was 34 (range 26-47) years. Among breast cancer subtypes, most patients had either TNBC (34.7%) or HER2-/HR+ (43.8%) tumors. 53.3% of patients had a high expression of KI-67 (≥20%). TILs were detected in 62 out of 126 (49.2%) analyzed patients, of whom 93.5% (N=58) had a low expression of TILs (≤30%) (Table 1).
- HLA expression (≤5%) was downregulated in 21.3% of patients while 46.3% showed upregulation of HLA-G expression (>5%). Most tumors in our cohort were PD-L1 negative for both tumor cells (90.8%) and immune cells (76.5%) (Table 2).
- Overall, the median H-score was 127 (range 23.8-235) for TIGIT and 156 (range 4.9-288) for Nectin-4 (Figure 2).
- An increased but not significant median expression of HLA-G was observed in patients with T3/4 (N=27) compared to T1/2 (N=99) tumor stage (12.4% vs 3.4%, respectively, p=0.226) whereas the TIGIT median expression was significantly higher in patients with T1/2 compared to those with T3/4 tumor stage (135 vs. 116, respectively, p=0.02). There was no significant difference in the median expression of the other biomarkers HLA, PD-L1 and Nectin-4 in T1/2 vs. T3/4 subgroups. The median expression of all biomarkers did also not significantly differ in N0 vs. N+ subgroups.

Conclusions

- **A heterogeneity of immunomarker expression was detected in the entire cohort of pregnant breast cancer patients.**
- **Subgroup analysis showed a significantly higher expression of TIGIT in patients with T1/2 tumor stage, which might be a sign of the initial anti-tumor response with activation of T- and NK-cells that decreases during tumor progression.**
- **Taken together, these findings suggest a heterogeneity of immunomarkers in tumor tissue, which might be related to the specific immunological situation during pregnancy. These results are hypothesis generating and further analyses are ongoing to evaluate the impact of this heterogeneity on non-pregnant patient cohort.**

References

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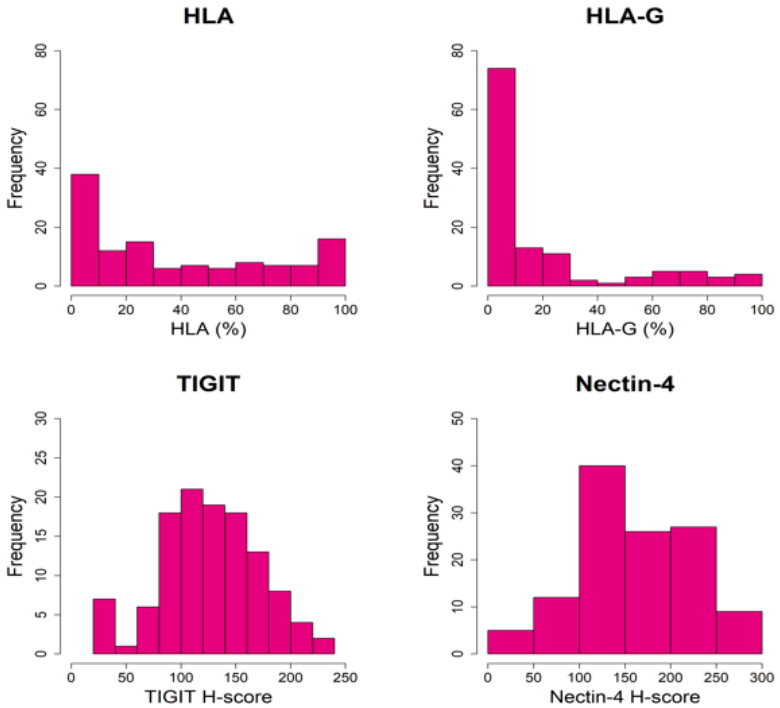
Galas et al. SABCS 2021

Immun-Marker bei Brustkrebs in der Schwangerschaft

- Heterogenität verschiedener Immunmarker im Tumorgewebe
- Könnte mit der spezifischen immunologischen Situation während der Schwangerschaft in Beziehung stehen.
- Weitere Analysen in Kontrollkohorten von nicht schwangeren Patientinnen laufen.



Galas et al. SABCS 2021

GeparNuevo: Immun-Checkpoint-Blockade

- Validierung von RNA-Signaturen
- Immunzell-Profile im peripheren Blut
- Spatial Profiling
- Survival Daten

Validierung von RNA-Signaturen in GeparNuevo

- Yale-Trial mit identischem Therapie-Schema wie GeparNuevo-Durvalumab-Arm
- Bioinformatische Auswertung von RNA-Seq und TMB-Daten

1. Validierung des in GeparNuevo gefundenen unabhängigen prädiktiven Werts von Immunsignatur und TMB für pCR

2. Validierung von im Yale-Trial gefundenen komplexen Immunzell-Mustern in GeparNuevo mit prädiktiver Bedeutung

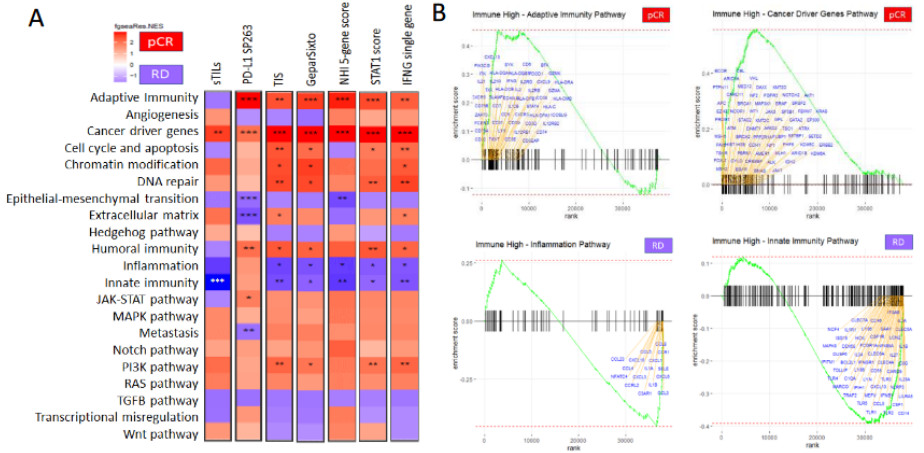
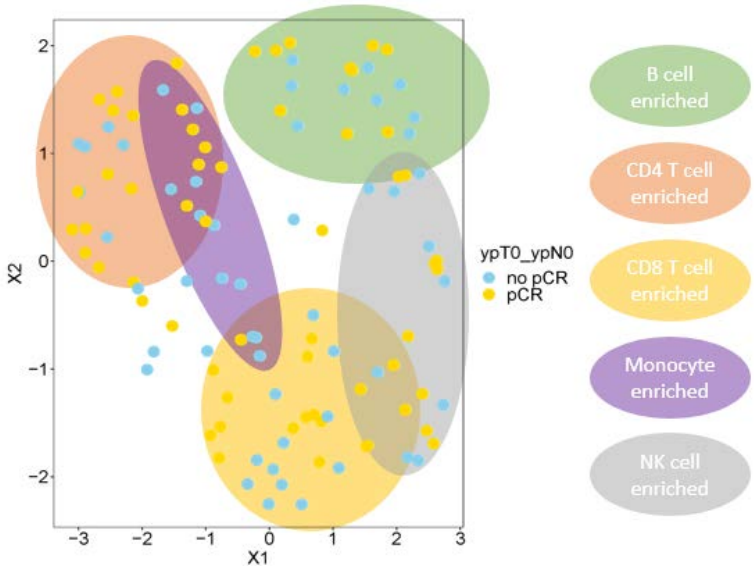


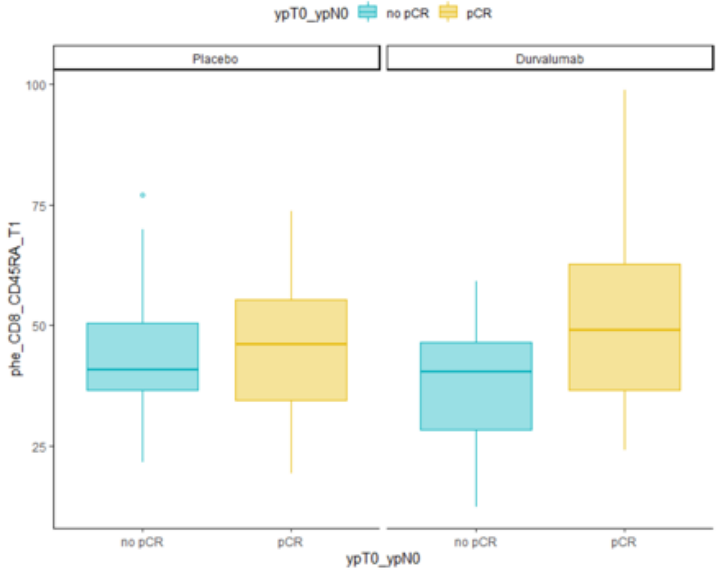
Figure 3. Pathway enrichment differences between pCR and RD in basal-like immune-rich TNBC.

Blenman et al. Clin Cancer Res 2022 in press

Immunzell-Profile im peripheren Blut

- Massa, Seliger: FACS-Profiling von peripherem Blut in GeparNuevo
- Cluster von Patienten zeigen Zusammenhänge mit klinischen Variablen wie Alter
- Hinweise auf Assoziation von pCR mit CD8 T_{naive} und T_{eff}





GeparNuevo: “Spatial Profiling”

Bulk

vs

Single cell

vs

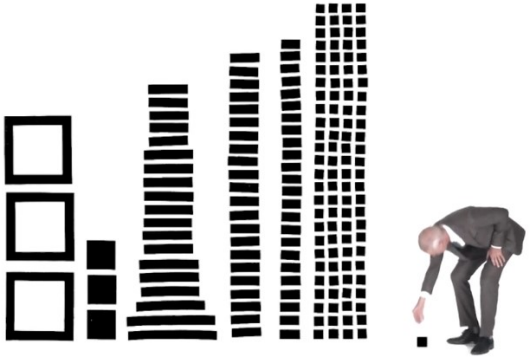
Single cell spatial analysis

GeparNuevo: “Spatial Profiling”

Spatial

Bulk

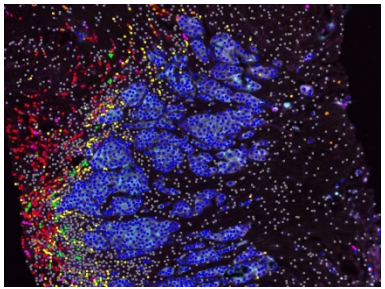
Single cell

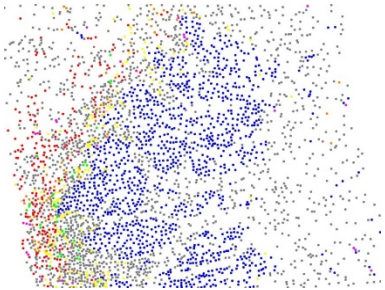


Kritische Information nur durch „Spatial Profiling“

GeparNuevo: “Spatial Profiling” durch MSI

Multi-Spectral-Imaging (MSI) mit Antikörper-Panel an >200 Biopsien (C. Massa):





CD20 → B cells

CD163 → Macrophages (M2)

panCK → Tumor cells

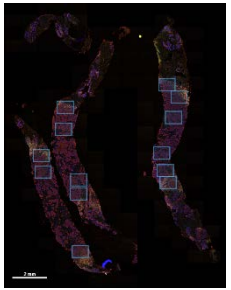
CD3
CD8
Foxp3

$CD3^+ CD8^+ (Foxp3^-)$ = CTL (CD8)

$CD3^+ Foxp3^+ (CD8^-)$ =„Treg“

$CD3^+ CD8^- Foxp3^-$ = T helper (CD4)

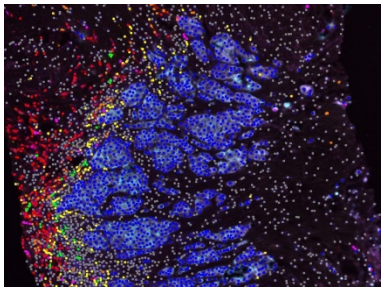
Only DAPI = „other“ (fibroblast, muscle…..

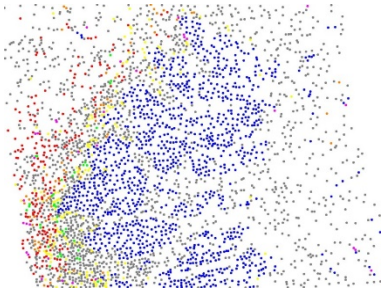


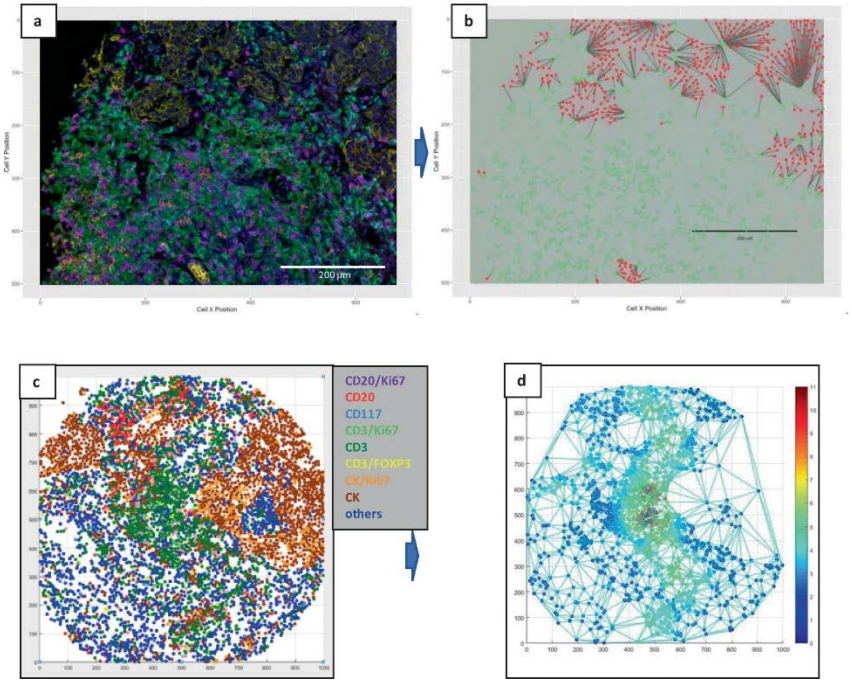
Massa et al.

GeparNuevo: “Spatial Profiling” durch MSI

Multi-Spectral-Imaging (MSI) mit Antikörper-Panel an >200 Biopsien (C. Massa):





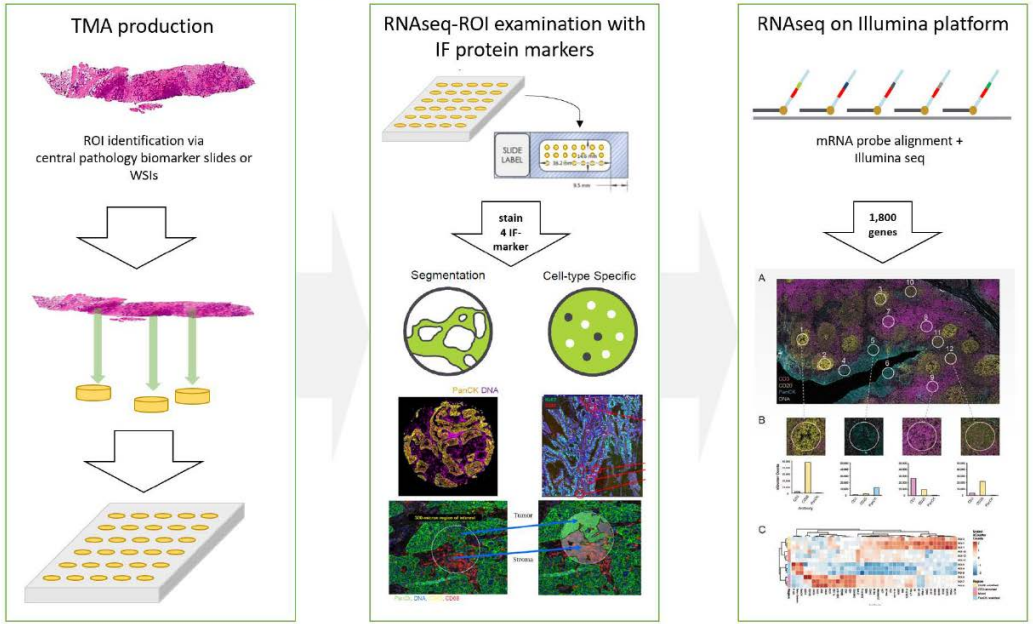


Nearest neighbor Analysen für Interaktion verschiedener Zelltypen

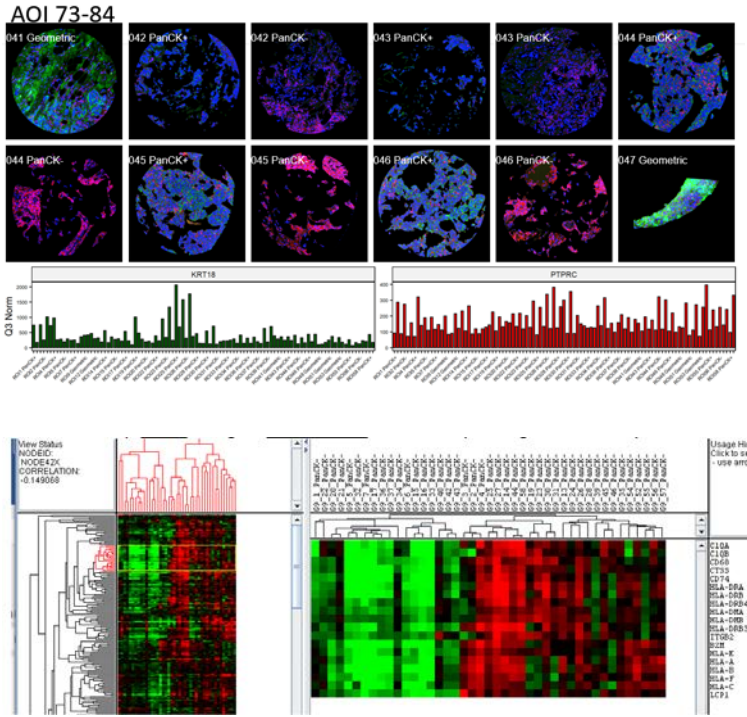
Massa et al. in preparation, Mori et al. 2021

GeparNuevo: “Spatial Profiling” durch GeoMX

Nanostring GeoMX DSP workflow

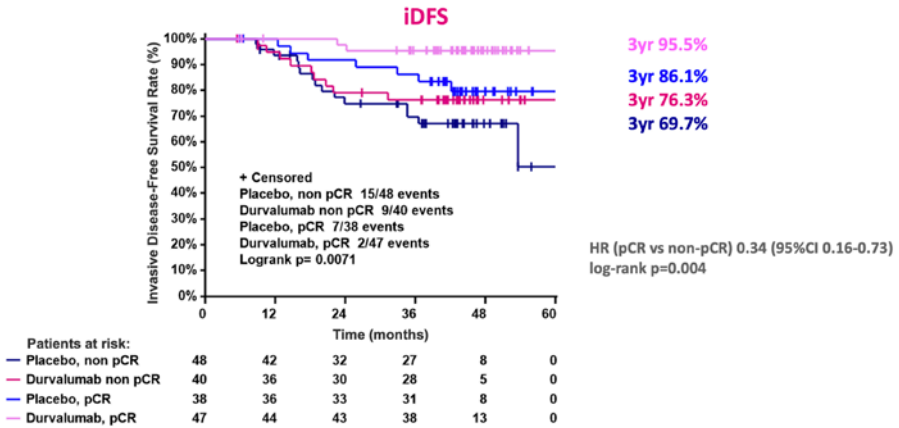


*DSP Digital Spatial Profiler



GeparNuevo: Immun-Checkpoint-Blockade

- **Survival-Daten aus GeparNuevo**



- Positiver Effekt von Durvalumab sowohl bei Patientinnen mit RD als auch pCR
- Innerhalb Durvalumab-Arm schlechterer Verlauf bei Patientinnen mit RD
- Können Biomarker Patientinnen mit späterem Event prognostizieren?

Loibl et al. ASCO 2021

Translationale Projekte zur Immunoonkologie 2021:

- **Diskutierter Einfluss des BMI auf prädiktiven Wert von TILs wurde nicht bestätigt**
- **Heterogene Immun-Marker bei Brustkrebs in der Schwangerschaft**
- **GeparNuevo: Immun-Checkpoint-Blockade**
  - Erfolgreiche Validierung von RNA-Signaturen
  - Hinweise auf möglichen Wert von Immunzell-Profilen im peripheren Blut
  - Spatial Profiling: Große Bedeutung und hohe Komplexität der Analysen
  - Survival-Daten: Wichtige neue Fragestellungen für Biomarker

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