Translationale Forschung – A year in review 2019

Christian Schem

Mammazentrum Hamburg
Projekthighlights aus dem Jahr 2019

- Wichtige klinische Erkenntnisse für den Alltag
- Datenbank und seltene klinische Ereignisse
- Marker für das Ansprechen der NACT
- Immunmarker – auf der Suche nach dem richtigen Kollektiv
- Tumor mutational burden
Impact in delay of start of chemotherapy and surgery on pCR and survival in breast cancer – a pooled analysis of individual patient data from six prospectively neoadjuvant trials

Sibylle Loibl1, Gustavo Werutsky1, Valentine Nekludova2, Sabine Seiler1, Jens-Uwe Blomher2, Carsten Denkert3, Claus Hanusch4, Jens Huober4, Christian Jackisch5, Sherko Kümmel5, Andreas Schneeweiss6, Michael Umtch7, Kerstin Rhiem7, Peter A. Fasching8, Gunter von Minckwitz9, Jenny Furlanetto10

1German Breast Group, Heidelberg; 2Chair, Berlin; 3Helmholtz Zentrum München, Munich; 4University Hospital, Essen; 5Accentova, Essen; 6University Hospital Mainz, Mainz; 7University Hospital Heidelberg.

*Notas Klinikum Berlin-Buch, Charite-Universitätsmedizin Berlin, Berlin; Charité Universitätsmedizin Berlin, Berlin; Department of Gynecology and Obstetrics, University Hospital Erlangen, Comprehensive Cancer Center Erlangen-SUPR, Erlangen, Germany

Objectives

1. Describe the median (range) time to NACT (TBC) and time to surgery after NACT (TCS)
2. Analyze the frequency of TBC and TCS length ≤28d vs >28d (predefined cut-off)
3. Analyze the impact of TBC length on pCR and of TBC and TCS length on DFS and OS

Materials and Methods

Overall 9127 patients with early breast cancer (BC) from 6 German neoadjuvant trials receiving an anthracycline-taxane based NACT were included. pCR (ypT0-N0), disease free survival (DFS) and overall survival (OS) were compared according to the time interval from diagnostic biopsy to NACT (TBC) start and from last chemotherapy application to surgery (TCS) length (cut-off of ≤28 vs >28 days (d)). The analysis has been conducted in the overall cohort and in subgroups (BC subtypes [luminal, HER2+, triple-negative breast cancer (TNBC)] and pCR [yes vs no]) for survival endpoints, adjusted by study.

Table 1: Baseline characteristics

<table>
<thead>
<tr>
<th>CHARACTERISTICS</th>
<th>N (%)*</th>
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<tr>
<td>TARGETED THERAPY</td>
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<td>Anti-HR2</td>
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<td>Anti-HER2</td>
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Table 2: Interval from diagnostic biopsy to NACT (TBC) and to surgery after NACT (TCS)

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<td>28d</td>
<td>76.7%</td>
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<td>&gt;28d</td>
<td>55.4%</td>
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Materials

Overall 9127 patients with early breast cancer (BC) from 6 German neoadjuvant trials receiving an anthracycline-taxane based NACT were included. pCR (ypT0/N0), disease free survival (DFS) and overall survival (OS) were compared according to the time interval from diagnostic biopsy to NACT (TBC) start and from last chemotherapy application to surgery (TCS) length (cut-off of ≤28 vs >28 days (d)). The analysis has been conducted in the overall cohort and in subgroups (BC subtypes [luminal, HER2+, triple-negative breast cancer (TNBC)]) and pCR [yes vs no] for survival endpoints, adjusted by study.

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<td>&gt;28d</td>
<td>55.4%</td>
<td>44.6%</td>
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Conclusions

A delay in starting NACT does neither impact the pCR rate nor DFS or OS. Delay in surgery after NACT in patients without pCR seems to influence outcome. Our analysis is explorative, however, indicates for the first time, that time interval of starting NACT might be uncritical, but timing of surgery seems to be relevant especially for those patients not achieving a pCR.

References


Presented at: ASCO Annual Meeting 2017, Chicago, Illinois, USA, 2nd May-6th June 2017

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German Breast Group, Neu Isenburg, Germany; email: publications@germanbreastgroup.de

#171

Results

Median follow-up was 65 months (range 0-201). TBC did not influence the pCR rate, neither overall nor in subgroups. In the multivariable logistic regression analysis TBC length did also not independently predict pCR. TBC did not influence DFS or OS. There were 28b/d was associated with worse DFS overall (≥28d vs ≤28b HR adjusted for study=1,280; p=0.02) and in patients not achieving a pCR (≥28b vs ≤28b HR adjusted for study=1,120; [95%CI:1.02-1.23]) was not observed within BC subtypes. OS was not impacted by TCS length. In the multivariable Cox TBC or TCS ≥28d vs <28d did independently influence DFS or OS.

A the time of surgery after NACT (TCS) ≥28d vs ≤28d in the overall cohort (A) and in patients without...
Histological and epigenetic analyses of placenta tissue
Karolin Fröhlich1, Torsten Pilsch2, Fenja Selther2, Volkmar Müller3, Thomas Karr2, Elmar Stickeler4, Chris Marion van Mackelenbergh5, Bruno Sinn1, Valentina Nekloudo6
1Universitätsklinik Köln, Germany; 2University Medical Center Groningen, Netherlands; 3German Breast Group, Heidelberg, Germany; 4Universitätsklinikum Freiburg, Germany; 5Universitätsklinikum Schleswig-Holstein, Germany

Background
Placenta tissues from breast cancer patients and healthy participants were investigated semiquantitatively by immunohistochemistry for placental and breast cancer markers, as well as by epigenetic analyses to assess DNA methylation of specific gene promoters. The results demonstrated altered methylation patterns and decreased expression of epigenetic regulators in breast cancer compared to healthy controls, suggesting potential mechanisms for tumor development and progression.

Table 1. Demographic and delivery characteristics

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<th>Controls</th>
<th>P-value</th>
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<td>Age at delivery, years</td>
<td>mean = 39.22, SD = 5.78</td>
<td>mean = 38.36, SD = 5.27</td>
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<tr>
<td>Gestation period, weeks</td>
<td>mean = 38.7, SD = 1.5</td>
<td>mean = 39.0, SD = 1.4</td>
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<tr>
<td>Birth weight, kg</td>
<td>mean = 3.45, SD = 0.52</td>
<td>mean = 3.50, SD = 0.49</td>
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<td>Birth length, cm</td>
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<td>mean = 51.0, SD = 2.1</td>
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<td>Maternal stress</td>
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<td>mean = 2.8, SD = 2.0</td>
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Figure 1. Epigenetic analyses

Mean proliferation index was reduced (Fig.3, 36.3 vs 58.0, p < 0.001). Nuclear and cytoplasmic expression of negative cell cycle regulator p27Kip1 was reduced (mean IRS score 1.0/0.8 vs 4.3/4.6, p < 0.001). No evidence of enhanced apoptosis was found. Epigenetic analyses showed significant differences in mean cytokine methylation of EPO (68.4% vs 71.1%, p = 0.05) and CYP3A4 (87.8% vs 90.0%, p < 0.01) genes (Fig.4).

Mean expression of the G protein-coupled receptor 1F2-H9, HSD11B2, ER and P-gp genes were found.

Placentas from breast cancer patients seem to be harbored in contrast to placentas from normal pregnancies, shown by morphologic abnormalities and a decreased proliferation index. Nevertheless, no increase of apoptotic cells could be demonstrated. Alterations of expression of efflux pumps or drug-metabolizing enzymes might be a reason for good fetal tolerability of chemotherapy during pregnancy as methylation patterns were changed in P-gp and CYP3A4 genes.
CAV Gene Expression Predicts for Response and Clinical Outcomes of Patients Treated with Preoperative Paclitaxel-based Chemotherapy Regimens in Early Stage Breast Cancer

We hypothesized that Caveolin (CAV) genes are correlated with the clinical response of patients treated with paclitaxel.

CAV was associated with the clinical response of patients treated with paclitaxel. Higher CAV expression was associated with worse DFS and OS in all patients. For patients who received paclitaxel-based treatment, higher CAV expression was associated with worse DFS and OS.

Conclusions

- CAV1 and CAV2 RNA expression are correlated in breast cancer.
- The odds of obtaining pCR with nab-paclitaxel treatment were improved for patients with high CAV1/2 expression.
- Higher CAV2 expression is associated with worse DFS and OS in all patients.
- For patients who receive paclitaxel-based treatment, high CAV1/2 expression is associated with worse DFS and OS.
- For patients who received nab-paclitaxel-based treatment, no significant differences in DFS and OS based on CAV1/2 expression were noted.
- Taken together, these findings suggest that CAV1/2 expression may offset the negative prognostic factor associated with higher CAV2 expression in patients treated with nab-paclitaxel regimens by enhancing the efficacy of treatment, perhaps through increased nab-paclitaxel endocytosis/transcytosis. These results are hypothesis generating and further data are needed.

Table 1. Multivariate regression models with interaction test between CAV1/CAV2 and HR status

<table>
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<th>CAV-2</th>
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<tr>
<td>TNBC</td>
<td>OR/HR (95%CI)</td>
<td>OR/HR (95%CI)</td>
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<td>HR-positive</td>
<td>0.85 (0.31-2.33)</td>
<td>0.69 (0.28-1.70)</td>
</tr>
<tr>
<td>TNBC</td>
<td>OR/HR (95%CI)</td>
<td>OR/HR (95%CI)</td>
</tr>
<tr>
<td>HR-positive</td>
<td>1.33 (0.48-3.68)</td>
<td>0.67 (0.26-1.70)</td>
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Table 2. Multivariate regression models with interaction test between arm (nab-paclitaxel vs paclitaxel) and CAV1/CAV2

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>CAV-1</th>
<th>CAV-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>low</td>
<td>OR/HR (95%CI)</td>
<td>OR/HR (95%CI)</td>
</tr>
<tr>
<td>high</td>
<td>0.94 (0.38-2.34)</td>
<td>4.92 (1.70-14.22)</td>
</tr>
<tr>
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<td>0.94 (0.38-2.34)</td>
<td>4.92 (1.70-14.22)</td>
</tr>
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</table>

Figure 1. Study design

Figure 2. DFS with nab-paclitaxel vs paclitaxel according to CAV1

Figure 3. OS with nab-paclitaxel vs paclitaxel according to CAV1

Figure 4. OS according to CAV1 (A) and CAV2 (B)

Figure 5. OS according to CAV1 (A) and CAV2 (B)

Figure 6. DFS with nab-paclitaxel vs paclitaxel according to CAV1

Figure 7. OS with nab-paclitaxel vs paclitaxel according to CAV1

References


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The genetic analysis was financed by the University Hospital Cologne.

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Presented at ASCO Annual Meeting 2019, Chicago, USA.
Background

Immunosurveillance suppression, evasion, or avoidance, has emerged as a key targetable hallmark of cancer, driven by e.g. checkpoint expression, T-cell exhaustion, and immunosuppressive tumor microenvironment (TME). Many of these processes generate defined combinations of immune-cell infiltrates at the tumor site, which can be detected by immunohistochemistry (IHC), CyTOF, or more recently can be inferred from gene-expression deconvolution. While significant work has been done to study gene signatures in the TME, the clinical relevance of such immune-cell gene signature on therapy has not been studied to a great extent.

We investigate the hypothesis that the individual patterns of immune-cell signatures determine the clinical behavior of BC, in particular response to neoadjuvant chemotherapy.

Figure 2. Immune scores in the GeparSepto cohort
A) Clustered immune activity scores for 279 GeparSepto patients. B) Proportions of GeparSepto considered high (>1sd from mean), normal (within 1sd of mean), and low (<1sd from mean) for comparison to reference breast cancer samples.

Figure 4. Follicular helper cells (TFH) are associated with differential survival
A) TFH score was significantly predictive on paclitaxel-based Tx for OS, DFS, and pCR in multivariate models B) Mosaic plot showing enrichment of high TFH score within patients that had pCR (p=0.0012).

Key Findings
- Natural killer (NK) cells (71%), and regulatory T-cells (70%) were found elevated in this cohort.
- Stimulatory T-cell signatures were high in approximately half of the population including Th2 (53%), effector-memory (53%), follicular helper (51%), Th1 (41%), and Gamma-delta T-cells (39%).
- While cytotoxic CD8+ T-cell signature was high in only 19% of patients, the signature of CD56dim cytotoxic subset of NK cells was high in 48% of patients.
- The most infrequently detected gene signatures were for innate response cell types: mast cells (8%), Macrophages (11%), immature dendritic cells (11%) and neutrophils (12%).
- 16 signatures were significantly differentially activated in TNBC compared to hormone-receptor positive (HR+) patients (p<0.05 respectively).
- 6/9 signatures more active in TNBC are associated with innate immune response (e.g. eosinophils and dendritic cells). Elevated NK CD56dim, Th1, and activated dendritic cell (ADC), signatures were associated with grade 3 tumors as well as with elevated levels of KI67 (p=0.0001 respectively).
- The most predictive TME signature for paclitaxel-based therapy was T follicular helper cells (TFH).

Conclusions
Whole-transcriptome sequencing in breast cancer FFPE core biopsies from clinical cohorts can be used to identify immune-cell signatures. Specifically, adaptive immunity through NK rather than T-cell response appears prevalent in high-risk BC. The patterns of these immune signatures, in particular the presence of T follicular helper cells, reflect the clinical behavior of breast cancer and might be used to identify tumors with an increased response rate to neoadjuvant chemotherapy.

References

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Tumor immune markers such as tumor infiltrating lymphocytes (TILs) or expression-based profiles have been associated with both response to neoadjuvant chemotherapy and prognosis in early breast cancer (BC) patients. Here we analyzed breast tumors to test if RNAseq-based classification of BC tumors is predictive of clinical outcome. We performed a retrospective analysis of 279 HER2-negative patients with complete response (pCR) and 350 patients with non-pCR from the neoadjuvant GeparSepto (GSP) trial (NCT01588452) and the adjuvant PIBO study (PIBO 10-03). Post-doc Session 6: Landscape of immune cell infiltration in early high-risk breast cancer (BC): relevant clinically-relevant enrichment of immune subpopulations.

**Background**

The G7 trial was a two-arm trial comparing nab-paclitaxel to solvent-based paclitaxel in 1200 early high-risk BC patients. All patients received epirubicin plus cyclophosphamide before surgery in addition to the taxane paclitaxel or nab-paclitaxel [1,2]. Presented here is a retrospective-prospective analysis of 279 HER2-negative patients with sufficient remaining tissue for additional analysis. Deep whole-genome RNAseq (~200,000 reads per tumor) was performed. Immune activity classification was provided by ImmunityBio (Culver City, CA) by comparison expression of 23 immune-cell-specific gene signatures as described by Binda et al. [3] to those from a reference population of 1467 similarly-profiled unscreened tumor samples from a large tumor database (NanoString, Culver City, CA).

Unsupervised Hierarchical clustering of inferred immune activities classified the patients into 3 distinct groups termed "hot," "warm," and "cold" clusters. Logistic regression analysis based on age, trial arm, tumor nodal status, Ki-67, hormone-receptor (HR) status and immune activity cluster (hot/warm vs. cold) as independent variables was performed to predict pCR (p=0.0105). Cox regression analysis with the same covariates was also performed to predict disease-free survival (DFS) and overall survival (OS).

**Patients and Methods**

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**Conclusion**

- Of the 279 patients, 67 had a pCR (24%). The analyzed subset was similar to the main HER2-negative population (p=0.99), with a slightly increased proportion of HER2-ER+PR+HR+ patients. Patients with a "hot/warm" or "cold" immune activity classification had a pCR in 30% and 13% of the cases, respectively. The odds-ratio of the multivariate logistic regression analysis was 2.17 (95% CI: 1.00-4.71, p=0.0512).
- With regard to DFS and OS, T follicular helper cell, B-cell, and T-cell signatures seemed to play a prominent role, as the hazard ratios (also "hot/warm" vs. "cold") for the multivariate analyses were 0.39 (95% CI: 0.28-0.54, p<0.0007) and 0.34 (95%CI: 0.16-0.72, p=0.0045), respectively.
- Within the 23 individual immune-cell-specific gene signatures, CD8+ Natural Killer (NK), CD8 T helper T-cells, and CD8+ T-cell signatures seemed to be closely associated with achievement of a pCR.

RNAseq-based decoupling of immune-cell activity was corroborated by IHC-based TIL scoring, as reported previously by others [4]. Immune-hot/warm patients had more intratumoral lymphocytes compared to cold tumors (mean: 11.6% vs. 4.9%, p<0.0001).
- Specific adaptive immune gene signatures (i.e. CD8+ T-cell signature, CD8+ NK, and Th1) were moderately correlated with the percentage of TILs (rho correlation coefficients from 0.42 to 0.53).

**References**

The GeparNovo (G9) trial showed that an addition of anti-PD-L1 antibody durvalumab to neoadjuvant antihuman-tyrosine kinases chemotherapy resulted in a numerical increase in pCR rate of 53% vs 44%; p=0.287 compared to placebo in primary TNCB (Figure 1, Table 1). Somatic mutations in malignant cells manifest over the evolutionary history of a tumor. Reports in selected tumor types suggest that the tumor mutational burden (TMB) may predict clinical outcomes on immune-checkpoint inhibitors (ICIs). The clinical relevance of TMB in breast cancer has not been studied widely. Here, we investigated the hypothesis that TMB response to PCI. The GeparNovo trial showed that the tumor mutational burden (TMB) was comparable in the immune cell infiltration in peripheral blood of triple negative breast cancer patients undergoing neoadjuvant therapy - derived from the prospectively randomized GeparNovo trial

Whole exome sequencing was conducted on patient-matched fresh-frozen core biopsies and blood samples with Illumina (n=149714). SNVs and indels were called with MuTect; purCN was used for copy number calls. Mutational signatures were identified as described by Alexandrov et al. [5].

Table 1. Baseline characteristics

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<th>Parameter</th>
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<td>&lt;145 mut/MB</td>
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Figure 1: G9 study design

Patients with triple-negative (G9 + CTCA) breast cancer were randomized to receive antihuman-tyrosine kinase- and tocilizumab-based chemotherapy or with or without the PD-L1 inhibitor durvalumab. The window phase (4 weeks) was closed after an amendment.

Figure 2: Genomic landscapes of G9 and TCGA

Conclusions

- The main genetic alterations were in TP53, c-MYC, PTEN
- Results were comparable between G9 and TCGA
- TMB may predict pCR in primary TNBC, but no dependency onICI treatment was found

References

1. Lobit S et al. Annals Oncol 2019
3. Goodman AM et al. Mol Cancer Ther 2017

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Presented at ASCO Annual Meeting 2019, Chicago, IL, USA, May 31 - June 4 The trial was financially supported by AstaZeneca and Celgene
Figure 2. Genomic landscapes of G9 and TCGA

- **Copy number variation (% patients)**
  - TCGA
  - G9

Genes: ARID1A, ATM, BRCA1, BRCA2, CCNE1, c-MYC, NOTCH1, PIK3CA, PTEN, TP53
Figure 3. Median TMB in subgroups

Median TMB in subgroups:
- Overall:
- Age: <40 vs. >=40
- APOBEC sign.: low vs. high
- Alexandrov-3: low vs. high
- Durva sign.: wt vs. mut
- HRD sign.: wt vs. m/d
- DDR sign.: wt vs. m/d
- GFR sign.: wt vs. m/a
- BRCA2: wt vs. mut
- BRCA/ATM: wt vs. mut
- TP53: wt vs. mut
- Cell cycle sign.: wt vs. m/a/d
- ARID1A: wt vs. mut

Sign.: signature; Durva sign.: durvalumab-response related genes BRCA2, NFE2L2, ARID1A, NOTCH1; HRD sign.: 16 genes related to HRD; DDR sign.: 36 genes without BRCA from DDR pathway; GFR sign.: growth factor receptor genes EGFR, FGFR1, FGFR2, FGFR4, IGF1R, KIT, c-MET; BRCA/ATM: BRCA1, BRCA2, ATM; cell cycle sign.: CDKN2A, RB1, CDK4, CDK6, CCNE1, c-MYC; low: below median; high: above median; wt: wildtype; mut: mutated; m/d: mutated or deleted; m/a: mutated or amplified; m/a/d: mutated, amplified or deleted.
Figure 4. Response to treatment

| TMB low: below 66.7% percentile; TMB high: above 66.7% percentile; p-values from multivariate logistic regression models |
|---|---|---|
| both arms | durvalumab | placebo |
| 38% | 40% | 37% |
| 58% | 63% | 52% |
| p=0.0067 | p=0.0284 | p=0.2320 |
Background: The GeparNuevo trial is a randomized, double-blind, multi-center phase II trial of neoadjuvant therapy in patients with early-stage triple-negative breast cancer (TNBC) investigating the role of durvalumab, an anti-PD-L1 antibody, which blocks PD-L1 binding to PD1 and CD80 in addition to standard anthracycline/taxane- based chemotherapy (Loidl S et al. JCO 2016; 36, 15, suppl.104).

Aim: Determination whether there is a link between the tumor mutational burden (TMB) and composition, frequency and function of blood immune cells in patients of the GeparNuevo trial as well as with the pathological complete response (pCR).

Table 1. Data of patients evaluated by flow cytometry (N=101)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TMB monitoring</th>
<th>Both</th>
<th>All patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>window treatment</td>
<td>63</td>
<td>53</td>
<td>116</td>
</tr>
</tbody>
</table>

Table 2. Panel of antibodies used for blood immune monitoring

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Staining Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3</td>
<td>surface staining</td>
</tr>
<tr>
<td>CD4</td>
<td>intracellular staining</td>
</tr>
<tr>
<td>CD8</td>
<td>surface staining</td>
</tr>
</tbody>
</table>

Figure 2. TMB subcohorts

- Tumor biopsy (TMB): Shown are patients who underwent pCR as well as pathological complete response (pCR).

Figure 3. TMB parameters

Correlation between continuous TMB and T cell-related markers of enrollment was evaluated. Shown are the Spearman correlation coefficient (rs) and its 95% CI for selected variables having a significant correlation to TMB in cell number (%blood); T effector memory (Teff); T effector cells (Teff).

Figure 4. TMB dichotomized cohort

- Changes in total population have the same role, but opposite effects when combined with CD83 or HLA-DR expression.

Table 3. Odds ratios (OR) and 95% confidence intervals (CI)

Conclusions:

- The median TMB in patients from the GeparNuevo trial is 1.52 mut/Mb, the patients with immunomonitoring are a representative subcohort.
- Patients with higher TMB have better pathological responses.
- TMB negatively correlates with the absolute number of CD8+ T cells, but positively with the percentages of memory cells.
- Many T cell biomarkers interact with the TMB to predict pCR.
- Biomarkers changes along treatment have opposite effects in the TMB dichotomized cohort, except for CD45RA CD8+ T cells at endpoint (T4):
  - Changes in total population have the same role, but opposite effects when combined with CD38 or HLA-DR expression.

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HERZLICHEN DANK!

- Durch Follow-up und Rekrutierung ermöglichen Sie klinisch relevante Erkenntnisse!