743 HIGH LEVELS OF STROMAL TUMOR INFILTRATING LYMPHOCYTES, CD3, CD8 CELLS & IMMUNOSCORE® ARE ASSOCIATED WITH PATHOLOGICAL CR AND TIME TO PROGRESSION IN TNBC PATIENTS UNDERGOING NEO-ADJUVANT CHEMOTHERAPY

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Background The presence of high levels of stromal tumor infiltrating lymphocytes (TILs) has been associated with better prognosis in early triple-negative breast cancer (TNBC). The Immunoscore® (IS) is a prognostic tool, which categorizes the densities of spatially positioned CD3 and CD8 cells in both invasive margins (IM) and the center of the tumor (CT), yielding a five-tiered classification (0–4). High IS values have been reported to predict improved outcomes in colorectal cancer.

Methods The cohort consisted of 52 TNBC patients (pts) who previously received neo-adjuvant anthracycline and taxane based chemotherapy. Quantitative analysis of the immune cells was carried out using a computer-assisted image analysis in different tumor locations for CD3 and CD8 T-cell markers. Additionally, we measured stromal TILs according to the internationals TILs working group. Pre-treatment tumor samples were immune-stained for CD3 and CD8 T-cell markers and stromal TILs. The relationship between various clinical pathological factors including tumor size, glands, stage and immune factors were analyzed by Chi2 and Fischer exact test. The log-rank test and the Kaplan Meyer methods were used to estimate relapse free survival.

Results The median age of the patients was 50 years (27–84 years). Tumor sizes were categorised as T1 = 9 patients (17%), T2 = 41 patients (77%) and T3 = 3 patients (6%). Patients with positive glands = 19 (36%) patients and patients without gland involvement = 34 (64%). Stage grouping included stage 1 = 5 (9%), stage IIA = 33 (63%) patients, stage IIB = 9 (17%) patients, stage III = 6 (11%) patients. The median Ki-67 was 45% (5 – 90%). The median density of CD3 CT cells = 1190 mm² (range 34 – 4614), CD3 IM = 1855 mm² (range 57 – 6190), CD8 CT 508 mm² (range 17 – 2486) and CD8 IM 805 mm² (range 90 – 3156). The median percentage of stromal TIL's was 5% (0 – 60%). Patient with an IS of 0 = 4 patients (8%), IS 1 = 3 (5%), IS 2 = 20 patients (38%), IS 3 = 24 patients (45%) and IS 4 = 2 patients (4%). The pathological complete response (pCR) rate of the entire cohort was 62%. A positive correlation was found between TILs and CD3 CT (R = 0.641, p < 0.0000), CD8 CT (R = 0.5623, p < 0.0000), CD3 IM (R = 0.6099, p < 0.0000), and CD8 IM. (R = 0.5010, p < 0.0010). TILs correlated with immunoscore (R = 0,3603, p < 0.0087). There was no correlation between TILs and Ki-67 (R = 0.1497, p < 0.2943). On univariate analysis, factors associated with higher pCR included nodal status (positive = 42,11% vs negative = 73,53% (p<0,02362) and Ki67 <40%= 33,33% vs =40% = 76,47% (p<0,00235). A high density of CD3 (> than 1100 mm²) and CD8 (> than 400 mm²) positive T-cells in the CT was associated with higher pCR (CD3 CT: 30% vs 70%, p=0.00489 and CD8 CT: 30% vs 70%, p=0.03344). Analysis of CD3 (> than 1200 mm²) (CD3 IM: 12% vs 88%, p=0.0.02367) and CD8 in the IM (> than 550 mm²) was also significant for an association with pCR (CD8 IM:23% vs 77%, p=0.03). High IS (3+4= 73%) vs intermediate (2=55%) vs low (0+1=43%) showed a numerical difference, however, did not reach a statistical significance with pCR (p=0.111). Analysis of TILs = 20% showed a pCR of 76% compared to patients with TILs < 20% with a pCR of 54% (p < 0.12295). A Ki67 =40% was associated with pCR of 76% compared to patients with Ki67 < 40% with a pCR 33% (p < 0.00235).The median time to progression of the patients not attaining a pCR was 1600 days, compared to patients who did attain a pCR with a median PFS not reached yet, but exceeds 1800 days. The median time to progression of patients with Ki67 < 40% was 1700 days while the patients with Ki67 =40% has not been reached yet (p < 0.03). At 1800 days, 95% of patients with CD3 > 1100 mm² did not relapse, compared to 75% patients with CD3 = 1100 (p < 0.03).

Conclusions This exploratory study shows that analysing CD3 and CD8 in the centre of the tumor and invasive margin might be more sensitive than examination of TILs in TNBC patients.

Ethics Approval The study was approved by Pharma-Ethics - (Institution's Ethics Board), approval number 170516563.

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744 SINGLE CELL PROFILING OF ACUTE MYELOID LEUKEMIA (AML) AND ITS MICROENVIRONMENT REVEALS A CD8 CONTINUUM AND ADAPTABLE T CELL PLASTICITY IN RESPONSE TO PD-1 BLOCKADE-BASED THERAPY

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Background Allogeneic stem cell transplantation can cure relapsed/refractor (R/R) AML via grafted T cells versus leukemia effect, but not a viable option to many patients. By combining azacitidine with nivolumab, we harnessed T cell activity and demonstrated 33% response rates. The tumor microenvironment (TME) factors impacting response and resistance to PD-1 blockade-based treatment in AML are unknown.

Methods We performed single cell RNA sequencing (scRNA-seq) on 113,394 bone marrow (BM) cells, paired with >30,000 single cell T cell receptor (scTCR) repertoires, from 8 pre- and 14 post- azacitidine/nivolumab treatment aspirates of 8 R/R AML patients (median age 73 years; 3 responders; 3 non-responders; 2 stable disease) (figure 1).

Results Inferred copy number loss of chromosome 7/7q (chr7/7q) by scRNAseq was associated with resistance to azacitidine/nivolumab (figure 2A), which was validated in a larger cohort based on clinical karyotyping (figure 2B). There was significant enrichment (q<0.005) for IFNg pathway in chr7/7q. We identified marked variation in the T cell components across AML patients at pre- and post- treatment, demonstrating significant dynamic changes in CD4, CD8 and non-classical T cells populations, including MAIT (figure 3A-B). Among CD8 cells, we identified a unique GZMK-enriched population that was highest at pretreatment in responders. Pseudotemporal trajectory analysis revealed a continuum of CD8 cell states,