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 I = 5 (9%) patients, stage IIA = 33 (63%) patients, stage IIB = 9 (17%) patients, stage III = 6 (11%) patients. Stage grouping without gland involvement = 34 (64%). Stage grouping included stage IIB = 9 (17%) patients, T2 = 41 patients (77%) and T3 = 3 patients (6%).

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.
intermediated by the less exhausted, GZMK-enriched CD8 population (figure 3C-D). GZMK also discriminated between 2 MAIT populations. GZMK-enriched cells had increased expression of the stem-like T cell transcription factor TCF7, and the T cell memory transcription factor EOMES. GZMK expression was associated with improved survival in de novo TCGA AML cohort (p=0.0017). scTCR clonotype assessment revealed shared clonotypes with the terminally effector CD8 CTL cells following PD-1 blockade. Following treatment, novel clones represented 38.7% (39/101) of total clones, followed by contracted clones (32.6%) and expanded (28.7%) clones (figure 3E-F). However, 76.9% and 72.4% of novel and expanded clones were contributed by the responders. Non-responders contributed only 5% and 3.4% of the novel and expanded clones, respectively.

Conclusions

Chr7/7q loss is associated with resistance to PD-1 blockade. CD8 cells exist in a continuum in BMs of patients with AML and GZMK expression identifies a stem-like, memory T cell subset. The subverted T cells can be reinvigorated via PD-1 blockade and induce responses in AML driven via novel and expanded clones demonstrating AML T cell plasticity and adaptability. Further functional characterization of GZMK expressing lymphocytes in mediating antileukemic responses is underway.

Ethics Approval

The study was approved by IRB at MD Anderson Cancer Center

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HIGH THROUGHPUT TISSUE IMAGING AND PHENOTYPING BY STREAMLINED 8-PLEX STAINING AND ANALYSIS OF COMPLEX TMA SAMPLES

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Conclusions

In this study, we highlighted the benefits of using a combination of well-characterized TMAs, a fast, optimized 8-plex miHCC protocol, and a detailed analysis pipeline to characterize the immune-response in a broad range of cancer types and samples, leading to a better understanding of the TME as well as a streamlined workflow for further translational studies.

Results

Immune cell counts and phenotypes were identified using automated analysis for cores within the tumor and within the tumor margin using a panel characterizing a range of immune cell populations, and compared across each tissue type. Deep phenotyping was performed for each core to identify unique profiles for each tissue type, with a workflow optimized for high-throughput analysis of rich-content TMAs.

Methods

Each slide comprised 144 cores (1 mm) and included duplicate cores for each case (1 from invasive margin; 1 from tumor center) from 11 different tumor types including breast cancer (ER+, Her2+, TNBC), NSCLC (squamous, adenocarcinoma), SCLC, CRC, pancreatic, gastric, hepatic and esophageal cancers. TMA sections were stained using the UltiMapper I/O Immuno8 panel, which includes markers for CD3, CD4, CD8, FOXP3, CD68, PD-1, PD-L1, and a pan-CK/SOX10 cocktail as a tumor indicator. The stained TMAs were scanned at 20X magnification on a fluorescence whole slide scanner. To provide accurate marker colocalization data, marker images were aligned using the UltiStacker software, using the nuclear