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 (17%), 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%), stage II = 33 (63%) patients, stage III = 9 (17%) patients, stage IV = 6 (11%) patients. The median Ki-67 was 45% (5 – 90%). The median density of CD3 CT cells was 1190 mm² (range 34 – 4614), CD3 IM was 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 TILs 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 = 12% (CD3 IM: 12% vs CD3 CT: 30%) and CD8 = 30% (CD8 CT: 30% vs CD8 IM: 12%) patients at pre- and post- treatment, demonstrating significant 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 high-

Abstracts

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

1Bernardo Rapoport, 2Jerôme Galon, 3Simon Naylor, 4Aurelie Fugon, 5Isabelle Boquet, 6Marine Martel, 6Mlienik, 7Teresa Smit*, 8Carol Ann Benn, 5Farhana Moosa, 9Liezl Heyman, 10Ronald Anderson. 1University of Pretoria, Pretoria, South Africa; 2Laboratory of integrative cancer immunology, Paris, France; 3Drs Gritzman and Thatcher Inc Laboratories, Johannesburg, South Africa; 4HalsDiaX, Paris, France; 5The Medical Oncology Centre of Rosbank, Johannesburg, South Africa; 6Head of Netcare breast care Centre, Johannesburg, South Africa

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

Hussein Abass*, Dapeng Hao, Katarzyna Tomczak, Praveen Barrodia, Jin Seon Im, Patrick Reville, Zoe Alaniz, Wei Wang, Ruiping Wang, Gheath Al-Atrash, Koichi Takahashi, Jing Ning, Maomao Ding, Jairo Matthews, Latasha Little, Jianhua Zhang, Sreyoshi Basu, Marina Konopleva, Guillermo Garcia-Manero, Michael Green, Padmanee Sharma, James Allison, Steven Kornblau, Linghua Wang, Naval Daver, Andrew Futreal. The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Background Allogeneic stem cell transplantation can cure relapsed/refractory (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 MALT (figure 3A-B). Among CD8 cells, we identified a unique GZMK-enriched population that was high-

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

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0744

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

Amanda Bares, Marie Cumberbatch, Lorcan Sherry, Christopher Womack, Milan Bhagat, Angela Vasaturo, Angela Vasaturo*, Ultivue, Inc, Cambridge, MA, USA; TriStar Technology Group, LLC, Washington DC, DC, USA; OracleBio, North Lanarkshire, UK

Conclusions In this study, we highlighted the benefits of using a combination of well-characterized TMAs, a fast, optimized 8-plex mIHC 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, 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