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 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, 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-CX/CDX10 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
Background

Multiplex immunohistochemistry (mIHC) and associated data analysis methods are rapidly becoming invaluable tools to improve our understanding of the complex tumor micro-environment (TME) and accelerate the discovery of novel immunotherapy targets. These techniques can enable the accurate phenotyping of the immune response and checkpoint expression in the spatial context of the tumor. The goal of this study was to identify the populations of immune cells (T-cytotoxic, T-helper, T-reg, and macrophages), their functional status, as well as their interactions with the tumor, in a range of samples and indications using a carefully designed multi-tumor Tissue Micro-Array (TMA) set of 2 slides from TriStar.

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NON SMALL-CELL LUNG CANCER CELLS AND CANCER-ASSOCIATED FIBROBLASTS DRIVE MACROPHAGE POLARIZATION IN A NOVEL CO-CULTURE MODEL

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Background

The plasticity of macrophage phenotype within the tumor microenvironment (TME) correlates with prognosis in non-small cell lung cancer (NSCLC). M2-like macrophages promote immunosuppression and facilitate tumor progression, while M1-like macrophages may drive an inflammatory antitumor immune response. Through a novel co-culture model comprised of cancer cells, cancer-associated fibroblasts (CAFs), and macrophages, we investigated whether NSCLC oncogene-type impacts macrophage phenotype and postulated that the immunosuppressive activity of macrophages is mediated through tumor-secreted soluble molecules. If identified and inhibited, these may re-sensitize cancer cells to immune surveillance and enhance antitumor immunity.

Methods

We developed an in vitro co-culture system (patient-derived NSCLC cells, human CAFs, and mouse macrophages) to interrogate impact of NSCLC cells and CAFs on macrophage phenotype. Expression of salient macrophage genes (i.e. ARG1, NOS2, IL-1β, IL-6, CHIL-3, SOCS3) was investigated through species-specific qPCR. Whole-genome RNA sequencing (RNAseq) in select cases was conducted and cytokine arrays measuring expression of 40 inflammatory cytokines were performed. Positive controls included stimulation of macrophages with LPS and IL-4.

Results

More than 70 NSCLC cell lines were characterized in the co-culture assay. Three highly reproducible clusters of macrophage phenotypes were identified: high Arginase (immunosuppressive), high IL-1β (inflammatory) and high SOCS3 (inflammatory, involved in JAK-STAT3 pathway) (figure 1). 3-4

Abstract 746 Figure 1 Three macrophage phenotypes induced in co-culture

Heatmap of mRNA expression from mouse macrophages co-cultured with human NSCLC cells and CAFs. mRNA expression of salient mouse macrophage genes depicted (x-axis) for each NSCLC cell line co-culture (y-axis).

Abstract 746 Figure 2 Macrophage phenotype independent of oncogenotype

Percentage of mutations of known human NSCLC oncogenes per mouse macrophage phenotype cluster.

Major oncogenotypes (i.e. KRAS, TP53, STK11, EGFR, BRAF mutation) did not correlate with macrophage phenotype (figure 2). Analyses of differences between the 3 clusters is ongoing. 10 exemplar NSCLC lines representing each of these 3 clusters were selected for RNA sequencing (mouse genes) and cytokine array protein (human) profiling. Across all clusters, we found suppression of macrophage endocytosis pathways and activation of scavenger receptor A (SRA) signaling, reflecting an M2-like phenotype. 5 We also observed increased expression of human IL-6, IL-8, and MCP1, which are implicated in suppression of innate immune sensing of tumor cells (figure 3). RNAseq of CAF lines demonstrated mixed inflammatory and myofibroblastic phenotypes (figure 4), with increased expression of genes associated with macrophage recruitment and activation including: IL-6, CSF-1, CXCL6, CCL2, and CCL7. 6