REFERENCES

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539 HIGH DIMENSIONAL ANALYSIS OF THE HUMAN LYMPH NODE DURING MELANOMA PROGRESSION REVEALS SHIFTS IN MYELOID CONTENT THAT RELATE TO DIFFERENTIAL T CELL CONTENT

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Background The sentinel lymph node (SLN) in melanoma represents the crossroads of the initiation of effector T cell responses and of lymphatic metastasis of the primary tumor. As such, alterations in the human LN immune cell network during melanoma progression are of particular interest for the development of effective immunotherapeutic approaches for each stage of disease.

Methods We used mass cytometry (CyTOF) and multiparameter flow cytometry to characterize the alterations in the major immune populations in the human LN. We included LN derived from healthy donors (n=10), tumor-negative (SLN, n=7) and tumor-positive SLN (SLN+, n=3) and LN metastatic samples (n=4).

Results Our results show that melanoma progression in the LN is accompanied by increased relative frequencies of myeloid cells, B cells and NK cells whereas T cell rates are significantly decreased. More specifically, for the myeloid cells we observed a decrease in frequencies of migratory cDC subsets and of LN-resident cDC and macrophage subsets in the SLN accompanying early melanoma development and metastasis. In fully metastatic LN from patients with advanced melanoma, a clear predominance of inflammatory, monocyte-derived subsets was observed. Simultaneously with this shift in myeloid subsets, an increase in CD4+ Tregs and CD8+ effector T cell subsets became apparent with metastatic progression in the LN. Both Tregs and CD8+ effector T cells in LN metastases were further characterized by relatively high expression of PD-1 and TIGIT immune checkpoints.

Conclusions The changes observed in the myeloid compartment accompanying metastatic progression in melanoma-draining LN, were found to be related to the shifts in lymphocytic subsets and their differentiation and activation state. Our results provide insights into the steady-state immune characteristics of the healthy human LN and identify all the changes that accompany melanoma progression through the different stages and give important clues about possible therapeutic interventions, aiming at immune potentiation of the SLN.

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540 TRANSCRIPTIONALLY DEFINED IMMUNE LANDSCAPE IN HUMAN GLIOMAS

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Background Brain immunity is largely myeloid cell dominated rather than lymphoid cells in healthy and diseased state including malignancies of glial origins called as gliomas. Despite this skewed myeloid centric immune contexture, immune checkpoint and T cell based therapeutic modalities are generalizably pursued in gliomas ignoring the following facts i) T cells are sparse in tumor brain ii) glioma patients are lymphopenic iii) gliomas harbor abundant and highly complex myeloid cell repertoire. We recognized these paradoxes pertaining to fundamental understanding of constituent immune cells and their functional states in the tumor immune microenvironment (TIME) of gliomas, which remains elusive beyond a priori cell types and/or states.

Methods To dissect the TIME in gliomas, we performed single-cell RNA-sequencing on ~123,000 tumor-derived sorted CD45+ leukocytes from fifteen genomically classified patients comprising IDH-mutant primary (IMP; n=4), IDH-mutant recurrent (IMR; n=4), IDH-wild type primary (IWP; n=3), or IDH-wild type recurrent (IWR; n=4) gliomas (hereafter referred as glioma subtypes) and two non-glioma brains (NGBs) as controls.

Results Unsupervised clustering analyses delineated predominately 34-myeloid cell clusters (~75%) over 28-lymphoid cell clusters (~25%) reflecting enormous heterogeneity within and across glioma subtypes. The glioma immune diversity spanned functionally imprinted phagocytic, antigen-presenting, hypoxia, angiogenesis and, tumoricial myeloid to classical cytotoxic lymphoid subpopulations. Specifically, IDH-mutant gliomas were predominantly enriched for brain-resident microglial subpopulations in contrast to enriched bone barrow-derived infiltrates in IDH-wild type especially in a recurrent setting. Microglia attrition in IWP and IWR gliomas were concomitant with invading monocyte-derived cells with semblance to dendritic cell and macrophage like transcriptomic features. Additionally, microglial functional diversification was noted with disease severity and mostly converged to inflammatory states in IWR gliomas. Beyond dendritic cells, multiple antigen-presenting cellular states expanded with glioma severity especially in IWP and IWR gliomas. Furthermore, we noted differential microglia and dendritic cell inherent antigen presentation axis viz, osteopontin, and classical HLAs in IDH subtypes and, glioma-wide non-PD1 checkpoints associations in T cells like Galectin9 and Tim-3. As a general utility, our immune cell deconvolution approach with single-cell-matched bulk RNA sequencing data faithfully resolved 58-cell states which provides glioma specific immune reference for digital cytometry application to genomics datasets.

Conclusions Altogether, we identified prognosticator immune cell-signatures from TCGA cohorts as one of many potential immune responsiveness applications of the curated signatures for basic and translational immune-genomics efforts. Thus, we not only provide an unprecedented insight of glioma TIME...
but also present an immune data resource that can be exploited for immunotherapy applications.

**Ethics Approval** The brain tumor/tissue samples were collected as per MD Anderson internal review board (IRB)-approved protocol numbers LAB03-0687 and, LAB04-0001. One non-tumor brain tissue sample was collected from patient undergoing neurosurgery for epilepsy as per Baylor College of Medicine IRB-approved protocol number H-13798. All experiments were compliant with the review board of MD Anderson Cancer Center, USA.

**Consent** Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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### Abstracts

**541 INVESTIGATING MYELOID DERIVED SUPPRESSOR CELLS (MDSCS) AND OLIGONUCLEOTIDE BASED TARGETING OF STAT3 IN RENAL CELL CARCINOMA**

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**Background** Recent advancements in the treatment of renal cell carcinoma (RCC) using immune checkpoint inhibitors (ICI) against PD1 or CTLA-4 receptors have improved survival rates in patients. However, more than half of RCC patients does not respond to anti-PD-1/-CTLA-4 combination immunotherapy. Thus, we decided to investigate mechanisms underpinning the resistance to ICI at the cellular and molecular levels.

**Methods** We utilized multicolour flow cytometry and Lumexin assays to investigate patient peripheral blood and used syngeneic mouse models to determine the efficacy of oligonucleotide based targeting of STAT3.

**Results** First, we characterized immunosuppressive myeloid cell populations, T cell subsets and immune biomarkers in blood samples from RCC patients with advanced stage IV disease, undergoing anti-PD-1/-CTLA-4 (nivolumab/ipilimumab) combination therapy. Results of our multicolor flow cytometry and plasma analysis suggested that ICI therapy is associated with a significant almost 15-fold increase of polymorphonuclear MDSCs (PMN-MDSCs) in the peripheral blood of RCC patients over the course of 3 therapeutic cycles. Notably, we found that PMN-MDSCs showed high levels of activated Signal Transducer and Activator of Transcription 3 (pSTAT3) and a significant increase its downstream target Arginase-1 between cycle 1 and cycle 8 of treatment (P=0.0008). The pSTAT3/ARG-1 signaling is known for promoting tumor immune evasion, thus strongly suggesting that immature PMN-MDSCs are potentially involved in limiting outcome of ICI therapy in RCC patients similar as shown before in other genitourinary cancers such as prostate and bladder cancers. We recently developed a strategy to target STAT3 selectively in tumor-associated myeloid cells using STAT3 antisense oligonucleotide (STAT3ASO) conjugated to immunostimulatory CpG oligodeoxynucleotides acting as targeting moiety. In our initial efficacy studies, we assessed activity of three versions of CpG-STAT3ASO conjugates with various chemical modifications, such as 2′-O-methyl- or locked nucleic acid, in a syngeneic bladder tumor model (MB49). MB49 cancer cells were subcutaneously injected into two flanks of male C57BL/6 mice and treated every second day with 5 mg/kg of various CpG-STAT3ASO injected intratumorally into one of the tumor sites. All CpG-STAT3ASOs inhibited tumor cell growth in both treated and distant tumors in comparison to controls. The immunohistochemical analysis indicated an increase in the percentage of CD8+ T cell with reduction of regulatory T cells within CpG-STAT3ASO treated tumors in comparison to controls, suggesting activation of CD8 T cell-mediated antitumor immunity.

**Conclusions** Overall, our preliminary results suggest that immune suppressive pSTAT3+/ARG-1+ PMN-MDSCs accumulate in patients with RCC undergoing ICI combination therapy, which may potentially contribute to resistance to ICIs. Targeting STAT3 signaling in the RCC-associated myeloid cells using CpG-STAT3ASO may provide a potential novel strategy for augmenting immune checkpoint therapies.

**542 EXPANSION OF CYTOTOXIC NK CELLS FROM PBMCS USING INDIVIDUALIZED CYTOKINE COMBINATION**

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**Background** Adoptive immunotherapy relies on the use of T-cells to target tumour cells, through Major Histocompatibility Complex (MHC) Class I recognition.1 However, many tumours display alterations in the MHC-I pathway, a well-described immune evasion mechanism.2 Natural Killer (NK) cells recognize transformed cells independently from the presence of MHC-I and may be a reliable therapeutic option for patients with altered tumour MHC-I expression. The source of NK cells may be autologous or allogeneic and NK cells are also clinically relevant recipients of transgenic receptors (TCRs or antibodies) targeting tumour cells. NK cells have been categorized according to their CD56 and CD16 surface expression into different subpopulations: cytotoxic (CD56+CD16+) and regulatory (CD56brightCD16-).3 Expanding cytotoxic NK cells is challenging, since the frequency of NK cells is low in peripheral blood4 and there is also – at this point – not an optimal expansion protocol available. The goal of this project is to determine the best cytokine combination that facilitates expansion of cytotoxic NK cells that either target tumor cells directly or serve as recipients for transgenic receptors.

**Methods** Peripheral Blood Mononuclear Cells (PBMCs) were extracted using Ficoll methodology from blood donors and cultured in T25 flasks with Cell Genix Medium supplemented with 10% human serum and antibiotics. NK cells were expanded supplemented with feeder cells (ratio 1:1) and different cytokine combinations (1000 U/mL of IL-2, 10 U/mL of IL-12, 180 U/mL of IL-15 and/or 1 U/mL of IL-21) during 20 days. The immunophenotype of expanded NK cells was analyzed at days 0, 5, 10, 15 and 20 by flow cytometry. The cytotoxicity of NK cells was measured by a CD107a Assay or by a Total Cytotoxicity and Apoptosis Assay at days 10 and 20. Thirteen different cytokine combinations were tested.

**Results** 4/13 cytokine combinations produced a statistically significant increase of the absolute number of NK cells with a higher percentage of cytotoxic NK cells (figure 1). However, induction of cytotoxicity was not associated with a strong NK cell expansion. The regulatory NK cells subset (CD56brightCD16-) showed the highest percentage of