

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

Marice Alcantara*, Dayson Moreira, Chia-Yang Hung, Chunsong, Dongfang Wang, JoAnn Hsu, Sumanta Pal, Marcin Kortylewski. *City of Hope, Duarte, CA, USA*

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 Luminex 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-I 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 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.

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EXPANSION OF CYTOTOXIC NK CELLS FROM PBMCS USING INDIVIDUALIZED CYTOKINE COMBINATION

Andreia Maia *, Joana Lérias, Markus Maeurer, Mireia Castillo-Martin. *Champalimaud Centre for the Unknown, Lisboa, Portugal*

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