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

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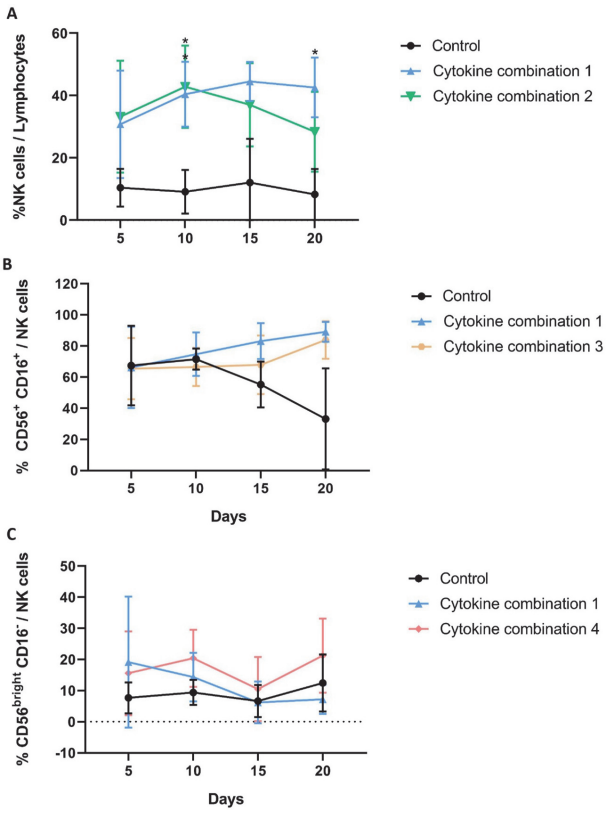
Background Adoptive immunotherapy relies on the use of T-cells to target tumour cells, through Major Histocompatibility Complex (MHC) Class I recognition.[1] However, many tumours display alterations in the MHC-I pathway, a well-described immune evasion mechanism.[2] Natural Killer (NK) cells recognize 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 blood[4] 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



Abstract 542 Figure 1 Representative percentage of NK cells in total lymphocytes (A), CD56+CD16+ subpopulation in total NK cells (B), and CD56brightCD16- subpopulation amongst total NK cells (C) at different time points (5, 10, 15 and 20 days) expanded from PBMCs
* p-value < 0.05

CD107a-expressing cells, more than the CD56+CD16+, the most cytotoxic subpopulation of NK cells.

Conclusions This work shows that we are able to grow and efficiently expand NK cells from PBMCs with different cytokine combinations leading to clinically relevant NK cell numbers as well as cytotoxic functions. This enables to produce NK cell products for therapy and as recipients for transgenic tumor antigen-specific receptors.

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Ethics Approval This study was approved by the Champalimaud Foundation Ethics Committee and by the Ethics Research Committee of NOVA Medical School of NOVA University of Lisbon.

Consent Written informed consent was obtained from the blood donors to use their samples for research purposes.

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543 NATURAL KILLER CELLS RESTRICT THE GROWTH OF LIVER METASTASES IN NUDE HOSTS

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Background Cancer patients with liver metastases have limited treatment options, especially as only 15–20% are eligible for curative-intent surgical resection.[1] Unfortunately, liver metastases also seem to be poorly responsive to immune checkpoint inhibitors (ICI)].[2 3] It could be that the unique immunological hallmarks of the liver, including resident macrophages and significant numbers of NK and NKT cells, create a tumor microenvironment that is best suited to alternative forms of immunotherapy that do not rely exclusively on ICI.

Methods We investigated how the presence of T, natural killer (NK), and NKT cells impact overt liver metastases using a model in which tumor cells are delivered to the liver via intraportal injection to hosts that were either wiltype, nude, or nude with NK-depletion. NK cell depletion was achieved via administration of anti-asialo GM1 antibody 2 days before tumor cell injection and for the duration of the experiment until endpoint at 6 weeks post tumor cell injection, with NK cell depletion confirmed by flow cytometry. Tumors were assessed histologically.

Results Using the portal vein model in female nulliparous mice, overt liver metastasis incidence was about 30% across 2 different mammary tumor cell lines. The incidence rose to 80–100% when tumor cells were delivered to hosts in the post-wean window (referred to as involution hosts), mirroring increased breast cancer metastasis to the liver observed in postpartum breast cancer patients.[4] Conversely, when tumor cells were delivered to nude hosts, either nulliparous or involution stages, the incidence of metastases dropped to 0–10%. Importantly, tumor cells injected into the mammary gland of nude mice grew robustly with 100% take. Nude hosts lack T cells and NKT cells; however, NK cells are present. Furthermore, the liver is enriched for NK cells, whilst the mammary gland has few NK cells.[5] We hypothesized that NK cells, when in the background of T- and NKT-cell depletion (i.e. nude host), restrict outgrowth of mammary tumor cells in the liver. Six weeks after portal vein injection of mammary tumor cells to nude hosts we find increased incidence of metastasis in the NK-depleted group compared to isotype control, as well as increased number of metastases per mouse.

Conclusions Our data suggest that NK cells play an important role in controlling liver metastases in nude hosts, and that NK activity in wild type hosts is insufficient to control liver metastases. Increasing NK cell cytotoxic activity could be an effective immunotherapy strategy to control liver metastases.

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