



**Abstract 542 Figure 1** Representative percentage of NK cells in total lymphocytes (A), CD56<sup>+</sup>CD16<sup>+</sup> subpopulation in total NK cells (B), and CD56<sup>bright</sup>CD16<sup>-</sup> 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<sup>+</sup>CD16<sup>+</sup>, 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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## 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.<sup>1</sup> Unfortunately, liver metastases also seem to be poorly responsive to immune checkpoint inhibitors (ICI).<sup>2, 3</sup> 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 wildtype, 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.<sup>4</sup> 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.<sup>5</sup> 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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**544** **MULTIOMIC CHARACTERIZATION OF T-CELL POPULATIONS AT THE SINGLE-CELL LEVEL UTILIZING SENSITIVE DEXTRAMERS AND BD<sup>®</sup> ABSEQ ON THE BD RHAPSODY™ SINGLE-CELL ANALYSIS SYSTEM**

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**Background** Adoptively transferred antigen-specific T cells have shown great efficacy in treatment of some virus-associated diseases and malignancies. A major driver of the development of adoptive T-cell therapy has been our ability to successfully characterize the functional status and antigen specificity of T cells. However, this has been limited by inefficient detection of antigen-specific T cells possibly due to their low frequency and low binding affinities to known MHC-peptide complexes.

**Methods** Here, we aim to combine two powerful technologies, advanced dCODE™ Dextramer<sup>®</sup> from Immudex and single-cell multiomics analysis using the BD Rhapsody™ Single-Cell Analysis system, to detect and characterize disease-specific CD8+ T cells within thousands of PBMCs.

**Results** Currently, we are able to identify over 350 mRNAs alongside a panel of over 20 BD<sup>®</sup> AbSeq cell surface protein markers which can be associated with T cell activation states. These data can be used to define T-cell phenotypes alongside antigen specificity of enriched CD8+ Dextramer(R)+ cells from a PBMC population.

**Conclusions** his study outlines our ability for high-resolution T-cell profiling that has broader implications and utility in immuno-oncology, infectious diseases and autoimmunity.

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**545** **TUMOR-SPECIFIC CYTOLYTIC CD4 T CELLS MEDIATE PROTECTIVE IMMUNITY AGAINST HUMAN CANCER**

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**Background** CD4 T cells have been implicated in cancer immunity for their helper functions. However, their direct cytotoxic potential remains elusive in cancer patients. Here, we aimed at assessing the presence, rate and cytotoxic function of tumor-specific Th-CTX directly in cancer patients.

**Methods** We capitalized on published single cell transcriptomic analyses of patient samples, integrated with the direct phenotypic and functional characterization of clonal, tumor-specific CD4 T cell populations, using peptide-MHC class II multimers

and a novel high-throughput single-cell cytotoxicity assay in picowell arrays. The direct tumor cell killing by cytolytic tumor-specific CD4 T cells in the arrays was monitored in a high-throughput manner by combining multi-channel time-lapse microscopy with deep neural networks.

**Results** By mining single-cell RNA-seq datasets of tumor infiltrating lymphocytes, we identified CD4 T cells displaying cytotoxic phenotypes in different human tumors. The cytolytic CD4 T cells formed a distinct cluster and expressed genes related to classical cytotoxic functions, largely resembling CD8 T cell gene profiles. Using the peptide MHC class II multimer technology, we confirmed directly ex vivo the presence of cytolytic tumor antigen-specific CD4 T cells, both in the circulation and in the tumors of patients. We performed an integrated phenotypic and functional characterization of cytolytic tumor-specific CD4 T cells, down to the single cell level, through a high-throughput nanobiochip consisting of massive arrays of picowells with sub-nanoliter volumes and machine learning. We demonstrated a direct, contact-dependent, granzyme-dependent cytotoxic activity against tumor cells, with delayed kinetics compared to classical cytotoxic lymphocytes. Lastly, we discovered that this cytotoxic activity was at least in part dependent on the expression of SLAMF7, a homophilic receptor known to regulate NK cell activity.

**Conclusions** Our work provides a deep characterization of human Th-CTX in cancer and supports their role in tumor immunity. Moreover, our results showing that agonistic engagement of SLAMF7 enhances the cytolytic capacity of tumor-specific CD4 T cells, suggests that targeting these cells might prove synergistic with the use of other immunotherapies in cancer patients.

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**546** **THE DIFFERENTIATION STATUS OF SYSTEMIC PD1+ CD8 T CELLS IS ASSOCIATED WITH FAVORABLE OUTCOME TO PD1 BLOCKADE THERAPY IN NON SMALL CELL LUNG CANCER**

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**Background** Non small cell lung cancer is one of the cancer types where Immune checkpoint blockade has demonstrated unprecedented clinical efficiency. However, only a fraction of patients benefit from such therapy; factors determining this response are yet to be elucidated. Here, we investigated whether the differentiation status of circulating CD8 T cells might be associated with outcome of PD1 blockade therapy in NSCLC.

**Methods** We used multi-parameter flow cytometry to study CD8 T cell differentiation states in NSCLC patients at baseline and to examine the effects of blocking the PD1/PDL1 pathway on those cells.

**Results** We found that responders to PD1 blockade therapy has more peripheral PD1+ CD8 T cells with an early-like differentiated status at baseline and that this phenotype is associated with longer survival. Moreover, PD1 blockade induced reinvigoration is mostly observed in cells with this with an early-like differentiated status.

**Conclusions** An early like differentiation status of peripheral CD8 T cells is associated with favorable outcome of PD1 blockade immunotherapy

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