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MUCOSAL-ASSOCIATED INVARIANT T-CELLS (MAIT) IN PANCREATIC CANCER

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Background Immunotherapy has changed the standard of care for multiple cancers; however, its efficacy is limited. Chemotherapy and radiation had little effect in pancreatic ductal adenocarcinoma (PDAC) outcome¹ in patients with metastatic disease, hence the urgency for new effective courses of treatment. Increasing evidence suggests mucosal-associated invariant T-cells (MAIT) play a role in anti-cancer T-cell responses, by recognizing transformed cells or bacterial products. MAIT respond towards microbial antigens and vitamin derivatives, produce pro-inflammatory cytokines^{2 3} and have been found present in primary and metastatic cancer lesions.^{3 4} Long-term survival PDAC patients present a unique microbiome pattern. In contrast, some microbial species may promote oncogenesis.⁵ ⁶The focus of this project is the characterization of MAIT as immune effector cells in PDAC specimens.

Methods We performed a retrospective analysis of long-term survivors (LTS) and short-term survivors (STS) patients with pancreatic cancer associating clinical endpoints with the presence of MAIT infiltration in the tumor tissue using immunofluorescence staining for MR1 (MHC class I-related gene, a MAIT ligand receptor), CD3 and TCR Vα7.2 (frequently reported chain in MAIT). Tumor infiltrating lymphocytes (TILs) were expanded and tested for recognition of microbial products presented to TILs or to PBMCs defined by cytokine production (ELISA), cytotoxicity (CD107a induction assay), CD69 or 4-1BB upregulation (flow cytometry). Reactive MAIT will be molecularly defined by deep TCR (T-cell receptor) sequencing which allows to 'back-trace' MR1 reactive TIL in the tumor specimen. The complex interaction of microbial antigen presentation from freshly harvested tumor specimens to TILs is being optimized for Nanolive technology that allows to follow live cell interactions for several days.

Results TIL reactivity directed against microbial products from different bacterial species was detected by IFN-γ production and CD69 upregulation in responder TILs. A broader panel of TILs is currently being tested against bacterial species. TCRs will undergo laser microdissection for subsequent TCR repertoire sequencing. A more pronounced MAIT infiltration in close vicinity to tumor cells in LTS compared to STS is being studied, further supporting the anti-tumor role of MAIT.

Conclusions MAIT cells may exhibit anti-tumor properties, based on cytokine production and cellular marker activation. TCRs directed against cancer cells can serve as viable blueprints to engage with MR1 on PDAC recognizing tumor-associated targets or microbial products that elicit IFN-γ production. This allows to explore MAIT TCRs for adoptive therapies or distinct microbial species that drive clinically relevant responses.

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

Consent For each patient, written informed consent and approval by the Ethical Committee of the Champalimaud Foundation will be obtained. The study will be in compliance with the Declaration of Helsinki.

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B-CELL-BASED VACCINATION ELICIT POTENT IMMUNITY AGAINST GLIOBLASTOMA

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Background Despite the tremendous effort in basic, translational and clinical research, the standard-of-care of patients with glioblastoma (GBM) has been virtually unchanged for the past two decades, aside from tumor-treating fields. GBM is one of the immunologically 'coldest tumors' where T-cell exclusion is at its maximum, and myeloid infiltration predominates. This is due to profound immunosuppression, the metabolically hostile microenvironment, and the low mutational burden of these tumors. Together, these barriers have hindered the development of effective immunotherapies. With the goal of exploring ways to boost anti-GBM immunity, we developed a B-cell-based vaccine (BVax) that consists of 4-1BBL+ B cells activated with CD40 agonism and IFNγ stimulation.

Methods Studies on B-cell-driven inflammation have identified a subset of B cells expressing the co-stimulatory marker 4-1BBL (or CD137L) capable of enhancing CD8+ T-cell anti-tumor cytotoxicity. Such activation was achieved through multiple mechanisms, including antigen presentation, T-cell co-stimulation (4-1BBL and CD86), and cytokine production (TNFα). Thus, 4-1BBL+ B cells could be utilized to boost anti-tumor CD8+ T-cell response. In order to stabilize their antigen presentation function in-vivo and avoid potential immunosuppressive functions, we activated 4-1BBL+ B cells using CD40 and IFNγ receptor (IFNγR) ligation (designated as BVax, figure 1A), both of which were effective to enhance B-cell-mediated antigen presentation (figure 1B-E). In the present study, we explored the ability of BVax to inhibit GBM growth by promoting tumor-specific CD8+ T-cell immunity and production of tumor-reactive antibodies. BVax's therapeutic effectiveness was examined both alone and in combination with radiation and checkpoint blockade.

Results BVax migrate to key secondary lymphoid organs and are proficient at antigen cross-presentation (figure 2A), which

Abstract 167 Figure 3 GBM patient-derived BVax

GBM patient-derived BVax promote anti-tumor CD8+ T cells. (A) Paired fresh peripheral blood and tumor were collected from newly diagnosed GBM patients (n=4). BVax were generated and pulsed with tumor lysates and co-cultured with autologous eFluor450-labeled CD8+ T cells. CD8+ T-cell activation was assessed by cell proliferation (eFluor450 fluorescence dilution measured as expansion index) and intracellular expression of GzmB. (B and C) Paired samples from primary GBM IDH WT (case NU 02120, B) and recurrent GBM IDH WT (NU02265, C). BVax-activated autologous CD8+ T cells were obtained as shown in (A) and tested for their ability to kill autologous glioma cells. Cell killing measurement were taken periodically for 12.5 hours using the IncuCyte S3 Live Cell Analysis System. Statistical significance is depicted as ns: no statistically significant, *p<0.05, **p<0.01, ***p<0.001.

Abstract 167 Figure 4 BVax produce anti-tumor IgG

BVax produce tumor-reactive antibodies with therapeutic effect. (A) Schema of BVax-derived serum immunoglobulin (Ig) obtention. BNaive, BAct and BVax-derived IgG were tested for IgG subtype (top) and their reactivity to B KO tumor bearing-brains (bottom). Top: Diagram representing the distribution of different Ig subtypes from serum antibodies derived from BNaive, BAct and BVax. Ig subtype measurement of serum samples was performed by ELISA, and mean total Ig concentration is shown in the bottom of the diagram (mg/ml). The experiment was performed in 7 mice/group. Bottom: B-cell subsets IgG reactivity was measured by immunofluorescence. Serum samples were incubated on tumor-bearing brains sections from B KO. Binding IgG was detected using anti-mouse IgG Cy5 (red) secondary antibody. Nuclei was detected using DAPI (blue), and myeloid cells were evaluated by using anti-mouse CD11b AF488 antibody (green). Bars represent 100 mm. Shown, a representative experiment of serum obtained in 4 mice/group, performed twice independently. (B) BNaive, BAct and BVax were generated from GL261 overexpressing ovalbumin (GL261-OVA) tumor-bearing mice. B cells were allowed to produce antibodies in GL261-OVA-bearing B KO. Serum samples were collected and IgG were purified and tested for their reactivity against OVA peptide SIINFEKL by ELISA. Semi-quantitative measurement is shown as optical density (O.D). Serum from B-cell deficient mice and C57BL/6 SIINFEKL-immunized mice were used as negative and positive control respectively (n=4/group). (C) Purified IgG were tested for their therapeutic effect in the CT2A model. IgG were delivered intracranially for 3 consecutive days (12.5 mg/mouse/injection). Untreated mice (black line) were used as controls. Experimental groups received either BNaive-derived IgG (BNaive IgG, blue line) or BVax-derived IgG (BVax IgG, pink line). The experiment was performed using n=10 mice/group. Statistical significance is depicted as ns: no statistically significant, *p<0.05, **p<0.01, ***p<0.001.

Acknowledgements In conclusion, BVax tackles GBM immunosurveillance escape by using both cellular (CD8+ T-cell activation) and humoral (anti-tumor antibody production) immunity. Our study provides an efficient alternative to current immunotherapeutic approaches that can be readily translated to the clinic.

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A NOVEL PROSTATE-RESTRICTED TUMOR-ASSOCIATED ANTIGEN: A POTENTIAL THERAPEUTIC TARGET

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Background Prostate cancer is the second leading cause of cancer related death in men in the United States, mainly due to disease progression to metastatic castration-resistant prostate cancer (mCRPC). Although immunological treatment with the FDA-approved vaccine sipuleucel-T extends survival for 2–4 months by targeting the prostate-restricted antigen PAP, the identification of more immunogenic tumor-associated antigens (TAAs) continues to be an unmet need.

Methods We evaluated the differential expression profile of the subset of epithelial cells reported to give rise to CRPC from mice following an androgen deprivation/repletion cycle. The expression levels of a set of androgen-responsive genes was further evaluated in prostate, brain, colon, liver, lung, and skin normal tissues from murine and human databases. The expression of a novel prostate-restricted TAA was then analyzed in primary tumors across all human cancer types in The Cancer Genome Atlas (TCGA). Finally, the immunogenicity of this novel prostate-restricted TAA was evaluated in vitro by autologous co-culture assays with cells from healthy donors and in vivo by antibody profiling (PhIP-Seq) in the sera of a cohort of prostate cancer patients treated with AR blockade alone or in combination with the cell-based vaccine GVAX.

Results Here, we discovered a set of androgen-responsive genes exclusively expressed by the putative cell-of-origin for prostate cancer. We confirmed prostate-restricted enrichment of these androgen-responsive genes in normal tissues from murine and human databases. Among these prostate-restricted genes, we identified PAP, PSA, and a novel non-mutated TAA. This novel TAA was confirmed to be expressed in prostate cancer. Furthermore, its expression was associated with survival in patients with primary prostate cancer. Interestingly, we found that pro-inflammatory activated TBET+ EM CD8 and CD4 T cells were expanded by moDCs pulsed with our novel TAA to a greater extent than moDCs pulsed with either PAP or PSA were used. An IgG antibody response to this novel TAA was detected in 30% of vaccinated patients, while fewer than 8% of vaccinated patients developed antibody responses to PSA or PSMA.

Conclusions Taken together, these results suggest we have found a novel immunogenic prostate-restricted TAA that represents a promising therapeutic target for treating mCRPC.

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