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

Jéssica Kamiki, Patrícia António, Pedro Noronha, Carolina Condeço, Georgia Paraschoudi, Eric De Sousa, Andreia Maia, Mineia Castro-Martin, Antonio Beltran, Carlos Carvalho, Joana Lérias, Markus Maue. Champalimaud Centre for the Unknown, Lisbon, Portugal

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) outcome\(^1\) 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 microbial 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.\(^5\)

The 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 present in primary and metastatic cancer lesions.\(^3\)\(^,\)\(^4\) The fungal mycobiome promotes pancreatic oncogenesis via activation of MBL, Nature 2019;574:264–267.


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.

REFERENCES


B-CELL-BASED VACCINATION ELICIT POTENT IMMUNITY AGAINST GLIOBLASTOMA

Catalina Lee-Chang*, Jason Miska, David Hou, Aida Rashidi, Peng Zhang, Rachel Burga, Craig Horbinski, Roger Stupp, Maciej Lesniak. Northwestern University, Chicago, USA

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 IFNgamma 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 (TNFaLpha). 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 IFNgamma receptor (IFNgR) 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

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BVax generation. (A) BVax are generated from 4-1BBL-expressing B cells obtained from glioma-bearing mice secondary lymphoid organs or GBM patient peripheral blood. After magnetic isolation, B cells are activated with B-cell survival factor BAFF, CD40 agonist and IFNgamma. Cells are pulsed with tumor lysate before intravenous injection. BVax effector function as APC. (B) BVax were tested for their ability to uptake AlexaFluor488-conjugated OVA [BVax (OVA)], and (C) present SIINFEKL peptide via MHC class I (H-2Kb). Surface transport of the H-2Kb + SIINFEKL complex was inhibited using Brefeldin A (BFA). Shown, a representative experiment of 3 independent experiments. Bars represent 15 mm (fluorescence images) and 50 mm (fluorescence and bright light image). (D) BNaive, BNaive + IFNg, BVax and dendritic cells (DCs) were pulsed with OVA and tested for their ability to promote OT-I CD8+ T-cell activation assessed by cell proliferation (expansion index, X-axis) and intracellular expression of GzmB (Y-axis). (E) OT-I CD8+ T cells cultured with BNaive, BVax pulsed with OVA and isotype control, BVax (OVA) + IC, or with MHC class I blocking Ab, BVax (OVA) + anti-H2Kb, and tested for their cellular expansion. Statistical significance is depicted as ns: no statistically significant, *p<0.05, **p<0.01, ***p<0.001.

Abstract 167 Figure 1 BVax generation and its effector function as APC

Abstract 167 Figure 2 BVax therapeutic effect

promotes both the survival and functionality of CD8+ T cells (figure 2B and C). A combination of radiation, BVax, and PD-L1 blockade conferred tumor eradication in 80% of treated tumor-bearing animals (figure 2D and E). This treatment elicited immunologic memory that prevented the growth of new tumors upon subsequent re-injection in cured mice (figure 2D and E). GBM patient-derived BVax were successful in activating autologous CD8+ T cells; these T cells showed a strong ability to kill autologous glioma cells. In addition to the role in activating CD8+ T cells, BVax produce mainly IgGs able to react to tumor cells (figure 4A) and tumor-associated antigens (figure 4B). Treatment of glioma-bearing mice with BVax-derived IgG elicited extended animal survival (figure 4C). Statistical analysis: Data are shown as mean±SD for a continuous variable and number (percentages or optical density measures) for a categorical variable. Differences between two groups were analyzed by Student’s t-test. Differences among multiple groups were evaluated using one-way ANOVA with post hoc Tukey’s test followed by post hoc Dunn’s multiple tests as appropriate. Survival curves were generated via the Kaplan-Meier method and compared by log-rank test and multiple comparisons were adjusted using the Bonferroni method. All the tests are two-sided and p-values or Benjamini-Hochberg adjusted false discovery rates less than 0.05 were considered as significant. Statistical analyses were performed using SAS9.4 and GraphPad Prism7.03.

Conclusions 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.

Abstract 167 Figure 1 BVax generation and its effector function as APC

BVax therapeutic effect

BVax promote survival and activation of CD8+ T cells. (A) Rag1 deficient mice were challenged intracranially with CT2A cells. Nine days after, mice received intravenously and concomitantly both Cell Tracker red CMPTX BVax (red cells) and CellTracker green CMFDA-labeled CD8+ T cells (green). BVax and CD8+ T-cell splenic localization were analyzed by fluorescent microscopy. Bars represent 100 um (left image, 20x magnification) and 50 um (right image, 63x magnification). (B) B-cell deficient (B KO) mice were challenged intracranially with GL261-OVA. Nine days after mice received intravenously BNaive or BVax pulsed with OVA protein. A group of BVax (OVA) was pretreated with pertussis toxin (PTX) before injection (n=4 mice/group). Seven days after the cell adoptive transfer, SIINFEKL-specific CD8+ T cells were analyzed in the tumor-bearing brains by flow cytometry using SIINFEKL-tetramer. (C) B KO mice were challenged intracranially with CT2A cells. Nine days after mice received intravenously BNaive and BVax pulsed with CT2A tumor lysates pretreated with or without PTX (n=5 mice/group). Seven days after the cell adoptive transfer, CD8+ T cells were evaluated for the intracellular expression of GzmB and IFNγ in the tumor-bearing brain, blood, and dCLN. BVax potentiate the therapeutic effect of combined RT+CD8+ + PD-L1 blockade. (D) Irradiated CT2A-bearing mice received vehicle (Mock, black line), 3 injections of anti-PD-L1 (dotted black line), 3 injections of CD8+ T cells and anti-PD-L1 (gray line), or 3 injections of pulsed BVax + CD8+ T cells, and anti-PD-L1 (pink dotted line). A non-irradiated group was kept as control (No RT, dashed black line). Seventy-five days after tumor challenge (arrow), surviving mice were re-challenged with CT2A cells in the left hemisphere, opposite of the initial tumor injection site. (E) Long-term survivors (LTS) were sacrificed and checked for the presence of tumor mass using H&E staining. Non-tumor-bearing but skull drilled (no tumor) and age-matched CT2A-bearing mice (Control) were used as controls. Sections were performed as depicted in the cartoons. For LTS treated with BVax and CD8+ T cells (LTS-BVax+CD8), brains were sectioned both in the right hemisphere (1st site of injection, LTS-BVax+CD8 R) and left hemisphere (2nd site of injection – rechallenge, LTS-BVax+CD8 L). H&E sections images are representative of 3 LTS-BVax+CD8, 2 control and 1 no tumor brains. Bars represent 5 mm. (F) The same brains utilized in (E) were used to stain for infiltrating CD8+ T cells. Representative images of one LTS-R section where the choroid plexus, site of injection, the pons (arrows) and the cerebellum (arrows) are magnified. For the top image, bar represents 2.5 mm. For magnified images bars represent 100 um. (G) Freshly dissected brains from no tumor (n=2), control (n=3), LTS that only received CD8+ T cells and PD-L1 blockade (LTS-CD8, n=4) and LTS-BVax+CD8 (n=5) mice were analyzed for lymphocytes phenotype. Statistical significance is depicted as ns: no statistically significant, *p<0.05, **p<0.01, ***p<0.001.

Ethics Approval All human samples (tumor, peripheral blood, and frozen tissue) were collected by the Nervous System Tumor Bank at Northwestern University (NSTB) under the institutional review board (IRB) protocol N° STU00202003. All animal experimentation protocols are approved by the Institutional Animal Care and Use Committee (IACUC) under protocol # IS00002459 at Northwestern University.
Abstract 167 Figure 3 GM patient-derived BVax

GM patient-derived BVax promote anti-tumor CD8+ T cells. (A) Paired fresh peripheral blood and tumor were collected from newly diagnosed GM 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 GM IDH WT (case NU 02120, B) and recurrent GM 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) obtainment. 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 samples 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. 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.

Acknowledgements In conclusion, BVax tackles GBM immuno-surveillance 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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