SUCCESSFUL IMMUNOTHERAPY OF THE BREAST CANCER METASTATIC DISEASE IN MICE USING A PHARMACEUTICAL TLR4-AGONIST INDUCES SYSTEMIC ANTI-TUMOR T CELL RESPONSE AND LONG-TERM T CELL MEMORY

E. Ushakova, E. Lebedeva, A. Pichugin, R. Aitaulla Khanov. NRC Institute of Immunology FMBA of Russia, Moscow, Russian Federation

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Background A study of the anti-tumor T-cell response and immunological memory following successful 4T1 breast cancer immunotherapy with the combination of surgical resection of the primary tumor and subsequent macrophage/dendritic cell reprogramming using injections of the pharmaceutical TLR4-agonist.

Materials and Methods 15,000 cells of the 4T1 mouse breast carcinoma inoculated subcutaneously into BALB/c mice generated solid tumors and metastatic disease ended by the death of all the tumor-bearing animals during 30-40 days. Surgical resection of the primary tumor was performed on day 11. Pharmaceutical TLR4-agonist (Immunomax®) administered intraperitoneally in dose of 14 μg every 2-3 days, in total seven injections per course. Sorted macrophage/dendritic cells reprogramming was examined by RT-PCR. Tumor-reactive IFN-γ-secretory T cells were counted using ELISPOT in ex vivo co-cultures of sorted CD4 T cells or CD8 T cells with the tumor lysate-loaded syngeneic dendritic cells or alive 4T1 tumor cells. Sorted CD8 effector T cell cytotoxicity was measured in their co-culture with different numbers of 4T1 target cells.

Results Using a combination of surgical resection of the primary 4T1 tumor and immunotherapy with the pharmaceutical TLR4-agonist for the treatment of metastatic disease in BALB/c mice a complete recovery of 20-30% mice was achieved. The complete responder mice effectively generated CD4 T cells and CD8 T cells, which specifically respond to 4T1 tumor antigens by IFN-production and kill 4T1 tumor cells.

Conclusions These results support the use of NCV delivered by DNA-EP with αCTLA-4 and suggest a new combined therapy for clinical testing.

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COMBINED PHARMACOLOGICAL TARGETING OF ADENOSINE 2A- AND 2B-RECEPTOR ENHANCES CAR T CELL FUNCTION

1M Seifert*, M Bermebake 1, B Cadilha 1, J Jobst, J Dörött, T Lorenzini, D Dhoquina, J Zhang, Y Zhang, U Schindler, S Endres, 1,1,5 Kobold, 1Division of Clinical Pharmacology, Department of Medicine IV, University Hospital, Ludwig Maximilian, Munich, Germany; 2Independent scientist with past affiliation Arcus Biosciences, Inc., 3928 Point Eden Way, Hayward, CA, USA; 3German Center for Translational Cancer Research (DKTK), partner site Munich, Germany

Background Despite remarkable response rates mediated by anti-CD19 chimeric antigen receptor (CAR) T cells in selected B cell malignancies, CAR T cell therapy still lacks efficacy in the vast majority of tumors. A substantial limiting factor of CAR T cell function is the immunosuppressive tumor microenvironment. Among other mechanisms, the accumulation of adenosine within the tumor can contribute to disease progression by suppressing anti-tumor immune responses. Adenosine 2a- and 2b-receptor (A2a and A2b)-mediated cAMP build up suppresses T cell effector functions. In the present study we hypothesize, that combination therapy with the selective A2a/ A2b dual antagonist AB928 (etrudemadenant) enhances CAR T cell efficacy.

Materials and Methods Second generation murine (anti-EPCAM) and human (anti-MSLN) CAR constructs, containing intracellular CD28 and CD3ζ domains, were fused via overlap extension PCR cloning. Murine or human T cells were retrovirally transduced to stably express the CAR constructs. A2a/ A2b signaling in CAR T cells was analyzed by phosho-specific flow cytometry of CREB (pS133)/ATF-1 (pS63). CAR T cell activation was quantified by flow cytometry and enzyme-linked immunosorbent assay (ELISA) of IFN-γ, IL-2 and TNF-α. CAR T cell proliferation was assessed by flow cytometry. CAR T cell cytotoxicity was assessed by impedance based real-time cell analysis.

Results AB928 protected murine CAR T cells from cAMP response element-binding protein (CREB) phosphorylation in the presence of stable adenosine analogue 5'-N-ethylcarboxamidoadenosine (NECA). NECA inhibited antigen-dependent CAR T cell cytokine secretion in response to four murine tumor cell lines. CAR T cell-mediated tumor cell lysis as well as proliferation were decreased in the presence of NECA or adenosine. Importantly, AB928 fully restored CAR T cell cytotoxicity, proliferation, and cytokine secretion in a dose dependent manner. Further, AB928 also restored antigen dependent cytokine secretion of human CAR T cells in the presence of NECA.
Conclusions Here we used the A24/A28 dual antagonist AB928 to overcome adenosine-mediated suppression of CAR T cells. We found that AB928 enhanced important CAR T cell effector functions in the presence of the adenosine analogue, suggesting that combination therapy with AB928 may improve CAR T cell efficacy. This study was limited to in vitro experiments. To confirm the relevance of our findings, this combination therapy must be further investigated in an in vivo setting.

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Combining RIG-I-Targeted Immune Activation with CAR T Cell Therapy Induces Efficient Tumor Control in Murine Pancreatic Cancer Models

AM Senz*, SL Formisano, B Cadilha, T Lorenzini, S Endres, S Kobold, M Schnurr, UM König, Klinikum der university München, München, Germany

Background The efficacy of chimeric antigen receptor (CAR) T cells against solid tumors remains unsatisfactory due to impaired trafficking of the CAR T cells into the tumor microenvironment (TME) and the presence of immunosuppressive factors and cells. 5'- triphosphate double-stranded RNA (3p-RNA) is recognized by the intracellular pattern recognition receptor retinoic acid-induced gene I (RIG-I). RIG-I activates a downstream signaling cascade, triggering the expression of type I interferons (IFN), proinflammatory cytokines and chemokines enhancing immune surveillance in the TME. We hypothesized that priming the TME with RIG-I ligands increases the efficacy of CAR T cell therapy.

Materials and Methods T110299 pancreatic tumor cells (derived from a genetically-engineered Kras and p53 mutant murine PDAC model) were engineered to express murine epithelial cell adhesion molecule (EpCAM) and used to induce subcutaneous or orthotopic tumors in C57BL/6J female mice. Mice bearing T110299 EpCAM⁺ tumors were treated with intratumoral or i.v. injections of 3p-RNA followed by i.v. injection of syngeneic murine T cells that were retrovirally transduced to express anti-EpCAM CARs. Three days after CAR T cell injection, immune cell composition and CAR T cell infiltration in the TME were assessed by flow cytometry. Additionally, tumor growth and survival were monitored.

Results Intratumoral injections of 3p-RNA reshaped the myeloid immune compartment in the TME by significantly reducing suppressive polymorphonuclear-MDSC and macrophages while increasing Ly6Chigh inflammatory monocytes. Moreover, antigen-presenting cells, such as dendritic cells and macrophages, were activated as evidenced by increased MHC-I expression levels. This was paralleled by a significant increase in the infiltration of CAR T cells into the TME in the combination therapy group. Interestingly, anti-EpCAM CAR T cells alone failed to control the tumor growth of T110299 EpCAM⁺ tumors, while monotherapy with 3p-RNA slightly delayed tumor growth in the subcutaneous model. Combination of 3p-RNA with anti-EpCAM CAR T cells induced a significant clinical benefit with tumor regression in 50% of the treated mice in the subcutaneous tumor model and prolonged survival in an orthotopic model.

Conclusions Remodeling the immunosuppressive TME using RIG-I ligands is a promising strategy for overcoming therapeutic resistance of CAR T cells in solid tumors, such as pancreatic cancer.