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

Disclosure Information F. Palombo: A. Employment (full or part-time); Significant; Neomatrix biotech. **E. Salvatori:** A. Employment (full or part-time); Significant; Takis biotech. **L. Lione:** A. Employment (full or part-time); Significant; Takis biotech. **M. Compagnone:** A. Employment (full or part-time); Significant; Neomatrix biotech. **A. Conforti:** A. Employment (full or part-time); Significant; Evvivax. **L. Aurisicchio:** A. Employment (full or part-time); Significant; Neomatrix biotech, Takis biotech, Evvivax.

P08.04 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

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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 IFNγ-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 in ex vivo co-cultures. The T-cell response is systemic, as tumor-specific T cells accumulate in the spleen. The second or third inoculation of the 4T1 tumor is accompanied by a complete absence of tumor growth in 50% and inhibition of tumor growth in the rest of the immune mice. An accumulation of significant numbers of T cells that respond to 4T1 tumor antigens by IFNγ-secretion, as well as of CD8 T cells that kill 4T1 tumor cells in a cytotoxic test was found in the secondary (tertiary) tumors, as well as in the draining lymph nodes. Immunological memory in complete responder mice that recovered due to the treatment with resection of the primary tumor and immunotherapy with a 4T1-agonist persisted for a long time (maximum observation period of 260 days).

Conclusions Macrophage/dendritic cell reprogramming with the TLR4-agonist for the post-resectional immunotherapy of 4T1 breast cancer metastatic disease induce tumor-specific CD4 and CD8 T cell responses and T-cell mediated long-living immune memory.

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P08.05 COMBINED PHARMACOLOGICAL TARGETING OF ADENOSINE 2A- AND 2B-RECEPTOR ENHANCES CAR T CELL FUNCTION

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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 ($A2_A$ and $A2_B$)-mediated cAMP build-up suppresses T cell effector functions. In the present study we hypothesize, that combination therapy with the selective $A2_A$/$A2_B$ dual antagonist AB928 (etrumadenant) 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. $A2_A$/$A2_B$ signaling in CAR T cells was analyzed by phospho-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 $A2_A/A2_B$ 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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P08.06 COMBINING RIG-I-TARGETED IMMUNE ACTIVATION WITH CAR T CELL THERAPY INDUCES EFFICIENT TUMOR CONTROL IN MURINE PANCREATIC CANCER MODELS

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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 tranduced 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.

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P09 Young researcher session

P09.01 THE USE OF FDA APPROVED JAK, MTOR AND SRC INHIBITORS TO REGULATE T CELL-BISPECIFIC ANTIBODY-INDUCED CYTOKINE RELEASE WHILE NOT PREVENTING T CELL CYTOTOXICITY

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Background T cell bispecific antibodies (TCBs) are potent T cell engagers, harboring a 2+1 format with one binder to the CD3ϵ chain and two binders to specific tumor antigens. Crosslinking of CD3 with tumor antigens triggers T cell activation and proliferation, cytokine release and tumor cell killing. TCB treatment is sometimes associated with safety liabilities due to on-target on-tumor or on-target off-tumor cytotoxicity and cytokine release. Off-tumor activity of the TCB may occur if the targeted tumor antigens are expressed on healthy cells, which may potentially result in tissue damages and compromise the patient's safety. Patients treated with TCBs may also experience a Cytokine Release Syndrome (CRS), characterized by fever, hypotension and respiratory deficiency and associated with the release of pro-inflammatory cytokines such as IL-6, TNF-α, IFN-γ, and IL-1β. Tyrosine kinases such as Src, mTOR and JAK1/2 are involved in downstream signaling pathways after engagement of the T cell receptor.

Materials and Methods 52 FDA approved kinase inhibitors were screened in the presence of T cells activated on CD3 coated plates, mimicking TCB stimulation. Src, mTOR and JAK inhibitors were selected based on their capacity to prevent both, cytokine release and T cell proliferation. Using an *in vitro* model of target cell killing by human peripheral blood mononuclear cells stimulated with TCBs, we validated the effects of mTOR, JAK and Src kinase inhibitors on TCB-induced T cell activation, tumor cell killing and cytokine release. *In vivo*, the effect of mTOR, JAK and Src kinase inhibitors on TCB-induced cytokine release was confirmed in humanized NOD scid gamma (NSG) mice engrafted with human hematopoietic stem cells and treated with CD19-TCB.