

tumor vaccine (GVAX) given intradermally on days 6, 9, and 12. Tumors were harvested for single-cell RNA sequencing (scRNAseq) or flow cytometry using panels designed to interrogate the activation and differentiation landscape of infiltrating T cells.

**Results** Combined  $\alpha$ CD25/GVAX therapy resulted in three different clinical response patterns - no response, partial response, and secondary resistance, with characteristic immune phenotypes. Amongst partially responsive tumors, about 90% of them relapsed between day 35–45. Reasoning that loss of immune control precedes clinical progression, we characterized the evolution of the immune landscape in pre-relapse tumors. We analyzed stable, partially responding tumors on days 28, 35, and 47. Over time, we found a decrease in the abundance of 4–1BB+TIM-3+TCF7- CD8+ effector memory T cells and Ki67+ CD4 effector cells (Teffs). In parallel, non-activated TCF7+ T cells rose in abundance. Treg abundance also recovered over time. ScRNAseq and scTCRseq analyses of pre-relapse and relapse tumors revealed that non-activated CD4 Teffs accumulating at relapse were transcriptionally equivalent to their activated counterparts at pre-relapse except for the expression of activation related genes. Overlaps were found in CDR3 usage between CD4 activated and non-activated populations at both pre-relapse and relapse, suggesting that the accumulating non-activated CD4 cells had been deactivated. In contrast, little overlap in CDR3 usage was found between CD8 activated and non-activated populations at relapse, indicating that the accumulating non-activated CD8 cells had been replaced by new, non-reactive clones. Additionally, we observed that in pre-relapse tumors, the percentage of Fas+ cells in activated Teffs is higher than that in non-activated Teffs. Blocking Fas/FasL interactions with an  $\alpha$ FasL antibody synergized with  $\alpha$ CD25 on stable tumors to prevent relapse.

**Conclusions** Combined Treg depletion/whole tumor vaccine therapy is effective in a poorly infiltrated B16 model. Most mice that achieve partial response eventually relapse, mimicking what is often seen in human disease. By characterizing the evolution of the immune landscape within partially controlled tumors, we revealed that progression is associated with a loss of immune fitness characterized by deactivation and death of activated infiltrating Teffs.

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## Plenary symposium 12: combination therapy (with a special focus on local IO)

12.03

### ISB 1442, A FIRST-IN-CLASS CD38 AND CD47 BISPECIFIC ANTIBODY INNATE CELL MODULATOR FOR THE TREATMENT OF CD38 POSITIVE HEMATOLOGICAL MALIGNANCIES

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**Background** ISB 1442 is a fully human first-in-class 2+1 biparatopic bispecific antibody targeting CD38 x CD47 using the BEAT<sup>®</sup> 2.0 (Bispecific Engagement by Antibodies based on the TCR) platform to target CD38 and CD47 as a treatment for CD38+ malignancies. ISB 1442 is designed with a bi-paratopic anti-CD38 arm that strongly binds two CD38 epitopes on tumor cells which do not functionally compete with daratumumab. The anti-CD47 arm is made of a single Fab designed to block interaction between CD47 and the signal-regulatory protein alpha (SIRP $\alpha$ ) with low affinity. This approach enables CD47 binding only of proximal receptors on the same cell via avidity-induced binding of CD38 on tumor cells which is expected to induce minimal unintended effects on red blood cells (RBC) compared to anti-CD47 monoclonal antibody (mAb). The Fc portion of ISB 1442 is engineered to enhance antibody dependent cell phagocytosis (ADCP), antibody dependent cell cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC).

**Materials and Methods** ISB 1442 was tested for its capacity *in vitro* to induce ADCP, ADCC and CDC across a broad range of tumor cell lines expressing different levels of CD38 and CD47 relative. To assess the complex mechanisms of action of ISB 1442 in a single system, a multiple mode of action of killing (MMoAK) assay was established to allow for simultaneous killing by natural killer cells (ADCC), autologous macrophages (ADCP), and complement from human serum (CDC). *In vivo*, ISB 1442 was assessed in therapeutic tumor models, expressing high or low CD38 level, of subcutaneously established xenograft in CB17/SCID mice. On-target specificity was evaluated *in vitro* in human whole blood assays.

**Results** *In vitro*, ISB 1442 exhibited higher killing potency compared to daratumumab across a range of CD38-expressing tumor cells. Additionally, ISB 1442 showed *in vitro* tumor killing potency through phagocytosis comparable to anti-CD47 (5F9) mAb, acting mostly through ADCP. In the CDC, ADCC and MMoAK assays, ISB 1442 exhibited tumor cell killing that was twice as high as daratumumab in MM cell lines. *In vivo*, ISB 1442 induced higher tumor growth inhibition than daratumumab. ISB 1442 did not cause any detectable RBC depletion or binding to RBC suggesting a more favorable on-target specificity profile in humans as compared to anti-CD47 (5F9) mAb.

**Conclusions** We report a novel approach for the treatment for CD38 positive hematologic malignancies by co-targeting CD38 and CD47 using a first in class multispecific antibody. Based on its unique design and multiple mechanisms of action, ISB 1442 is anticipated to enhance antitumor activity in patients relative to anti-CD38 mAbs by overcoming primary and acquired tumor escape mechanisms of resistance.

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#### 12.04 RE-DIRECTING CAR-T CELLS AGAINST SOLID TUMORS USING T-SIGN-MEDIATED ANTIGEN DELIVERY AND TUMOR MICROENVIRONMENT REPROGRAMMING

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**Background** Chimeric antigen receptor (CAR) T cell therapy has proven highly effective in the treatment of hematologic malignancies, however multiple barriers in the tumor microenvironment (TME) reduce its efficacy against solid tumors. Such barriers include an immunosuppressive TME, poor CAR-T cell trafficking and shortage of highly expressed tumor-specific target antigens. We recently demonstrated that Tumor-Specific Immuno-Gene (T-SiGn) viruses encoding multiple immunostimulatory mediators can reprogram the TME towards a pro-inflammatory phenotype, resulting in a markedly increased therapeutic efficacy of anti-EGFR and anti-HER2 CAR-T cells in an A549 human tumor xenograft and metastasis model.<sup>1</sup> Here we further explored the potential of T-SiGn platform combination with CAR-T cells by developing different T-SiGn viral vectors for the simultaneous tumor-specific expression of CAR-T cell target antigens and immunostimulatory molecules.

**Materials and Methods** We used *in vitro* human tumor cell lines to assess the ability of T-SiGn viruses to induce expression of a range of chimeric CAR-T cell target antigens. Using CD19 as a model antigen, we quantified virus-encoded CD19 expression on tumor cell surface using flow cytometry. *In vivo*, T-SiGn-dependent tumor-specific CD19 expression was assessed by flow cytometry analysis of tumor cell suspensions from A549 subcutaneous lung tumor xenografts in NSG mice.

**Results** In multiple *in vitro* human cell culture models, T-SiGn virus infection led to efficient expression of chimeric CD19 antigen on the tumor cell surface that enabled effective antigen-specific tumor cytotoxicity by anti-CD19 CAR-T cells. T-SiGn viruses with enhanced activity were also successfully generated by encoding immunostimulatory chemokines and cytokines together with chimeric CD19, enabling simultaneous tumor cell-specific CD19 antigen expression and enhancement of CAR-T recruitment and activity. *In vivo*, T-SiGn-dependent CD19 expression was demonstrated in intravenously dosed A549 human tumor xenografts, enabling future studies to optimize T-SiGn co-therapy with CAR-T cells directed against antigens otherwise not expressed in the tumor.

**Conclusions** Together, our data provide a proof of concept that T-SiGn viruses can re-direct CAR-T cells to act against solid tumors by enabling tumor-specific expression of cognate target antigens that are not endogenously expressed by the tumor cells. Further studies are ongoing to explore the full potential of synergistic combination of T-SiGn viruses and T cell therapy against solid tumors.

#### REFERENCE

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#### 12.05 TUMOR TARGETED INNATE IMMUNE CHECKPOINT BLOCKADE FOR THE TREATMENT OF NEUROBLASTOMA USING BIFUNCTIONAL ANTIBODIES

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**Background** Neuroblastoma is a childhood malignancy characterized by high expression of the disialoganglioside GD2, which is ranked in the top 15 of prioritized cancer antigens. Its prominent tumor specific expression has led to the development of anti-GD2 immunotherapy, resulting in improved patient survival. Some patients, however, still progress highlighting the need for improvement of the existing therapy. Innate immune checkpoints, like CD47, are a relatively new group of potential targets to stimulate the anti-tumor immune response. CD47 is a ubiquitously expressed protein overexpressed on tumor cells and the ligand for the SIRP $\alpha$  receptor expressed on myeloid cells, key effector cells in anti-GD2 based immunotherapy. Binding of CD47 to SIRP $\alpha$  prevents tumor cell phagocytosis and therefore serves as a *don't eat me signal*, providing the tumor with a mechanism to evade destruction and processing by antigen presenting myeloid cells (APCs). Targeting of CD47, however, has proven challenging as its ubiquitous expression on healthy cells forms an antigen sink.

**Approach** To improve anti-GD2 immunotherapy, we developed bifunctional antibodies able to target neuroblastoma and locally interfere with the CD47/SIRP $\alpha$  axis. These bifunctional antibodies recognize GD2 and contain the extracellular SIRP $\alpha$  domain that is able to block CD47.

**Results and Conclusion** *In-vitro* we found that the bifunctional antibody constructs bind tumor cells and block CD47 in a