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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.1 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.

Disclosure Information
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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 let 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 SIRPα receptor expressed on myeloid cells, key effector cells in anti-GD2 based immunotherapy. Binding of CD47 to SIRPα 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/SIRPα axis. These bifunctional antibodies recognize GD2 and contain the extracellular SIRPα domain that is able to block CD47.

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