208 T-SIGN VECTOR-MEDIATED ANTIGEN "SPRAY PAINTING" AND TUMOR MICROENVIRONMENT (TME) REPROGRAMMING TO ENABLE CAR-T CELL TARGETING OF SOLID TUMORS

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Background Multiple barriers in the tumor microenvironment (TME) have hampered development of CAR-T cell therapies for solid tumors. These challenges include an immunosuppressive TME, poor trafficking of CAR-T cells to the tumor and shortage of highly expressed tumor-specific antigens. We recently demonstrated that Tumor-Specific Immuno-Gene (T-SIGn) viral vectors encoding immunostimulatory cytokines, costimulators and chemokines can reprogram the TME towards a pro-inflammatory phenotype resulting in a markedly increased therapeutic efficacy of CAR-T cells in a A549 human tumor xenograft and lung metastasis model.[1] Here we further explored the potential of the T-SIGn platform in combination with CAR-T cell therapy by developing and characterizing a T-SIGn viral vector that simultaneously expresses immunostimulatory cytokines/chemokines and a secreted bispecific protein incorporating a CAR-T cell target antigen. This secreted ligand binds to ("spray paints") tumor cells to enable recognition by CAR-T cells.

Methods We used a T-SIGn virus (NG-1125) expressing a secreted anti-HER2-CD19 fusion protein (saH2-19), as a model "spray paint" antigen, encoded together with IFNa and CXCL9 as example cytokine/chemokines. *In vitro,* human tumor cell lines were used to assess the ability of T-SIGn viruses to induce tumor-specific expression and activity of saH2-19 as CAR-T cell target antigen. We quantified T-SIGn vector-encoded CD19 expression on tumor cell surfaces using flow cytometry and CAR-T mediated killing via xCELLigence. *In vivo*, expression of CD19 fusion protein and transferred CD19 CAR-T cells in tumors were assessed by flow cytometry analysis and immunohistochemistry of A549 tumor xenografts.

Results Using *in vitro* human cell culture models, the NG-1125 vector led to efficient expression of saH2-19 on tumor cell surfaces, both on vector-infected and non-infected or non-permissive cells. This enabled effective antigen-specific tumor cytotoxicity by CD19-specific CAR-T cells. Using an *in vivo* human A549 lung tumor xenograft model adoptively transferred with human CD19-specific CAR-T cells, NG-1125 induced tumor-specific CD19 expression on both vector infected and non-infected cells (demonstrating antigen "spray painting") together with accumulation of activated T cells. This accumulation of T cells was not seen with a vector only expressing the saH2-19 transgene.

Conclusions Together, our data provide a proof of concept that T-SIGn vectors can be designed to deliver TME-modifying immunomodulators together with "spray paint" antigens that effectively enable tumor cell recognition and destruction by CAR-T cells specific for target antigens not endogenously expressed by tumors. Further studies are exploring the full potential of this "spray painting" approach to enable CAR-T cell therapy for of solid tumors.

REFERENCE

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