576 TUMOR TARGETED SUPERANTIGEN (TTS), NAPTUMOMAB ESTAFENATOX (NAP), ENHANCES CAR-T CELLS POTENCY AND CAN BOOST CAR-T EFFICACY AGAINST SOLID TUMORS

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Background CAR-T therapy has limited efficacy against solid tumors due to low trafficking to the tumor, limited cell expansion in patients, tumor antigen heterogeneity, and an immunosuppressive microenvironment. TTS are fusion proteins that consist of genetically engineered Superantigens (Sag) linked to Fragment antigen binding (Fab) moieties directed to tumor associated antigens. It was previously shown that TTS selectively activates a subset of T cells [1], turns "cold tumors hot" [2] and, in preclinical models, can lead to long-term memory responses [3]. Here we present preclinical data demonstrating that the lead TTS compound, NAP (5T4 targeted Sag), enhanced the efficacy of CAR-T treatment against tumor cells in vitro, suggesting that NAP may overcome current CAR T limitations.

Methods Her2-CAR-T cells were produced in the presence of NAP or CD3&CD28, and their potency was evaluated against the FaDu cell line and by measurement of T cell activation markers (CD25, CD137, IFN-gamma, CD107a). The expression of memory markers (CCR7, CD45RA/CD45RO, CD95) and Th1 polarization (transcription factors) of the resulted CAR-T cells were analyzed by staining with specific antibodies. The combined potency of NAP with CAR-T was also tested in vitro against the FaDu cell line (which expresses both Her2 and 5T4 antigens). The chemotactic activity of T cells was assessed using a chemotactic chamber.

Results PBMCs grown in the presence of NAP, in comparison with PBMCs grown in the presence of CD3&CD28 antibodies, resulted in the production of more potent CAR-T cells as measured both by killing assay using the FaDu cell line and by INFγ production, activation markers and T cell degranulation. Central memory (CM) percentages were increased and Th1 polarization was significantly more prominent after NAP stimulation. Following incubation with NAP, the chemotaxis towards the tumor cells was significantly enhanced. Finally, combination of CAR-T and NAP resulted in a synergistic killing effect of the tumor cell line.

Conclusions Our studies show that NAP generates more potent CAR-T cells and acts synergistically with CAR-T against tumor cell lines in vitro. The ability of NAP administration to activate T cells outside of the immunosuppressive microenvironment (in the lymphoid organs), promote T cell infiltration into the tumor and induce long-term memory responses, strongly suggests that combination of CAR-T cells with NAP may overcome the limited effect of CAR-T therapy against solid tumors. NAP is currently being evaluated in clinical studies in combination with durvalumab [NCT03983954] and docetaxel [NCT04880863].

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