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 (Figure 1). 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.

NAP- mode of action

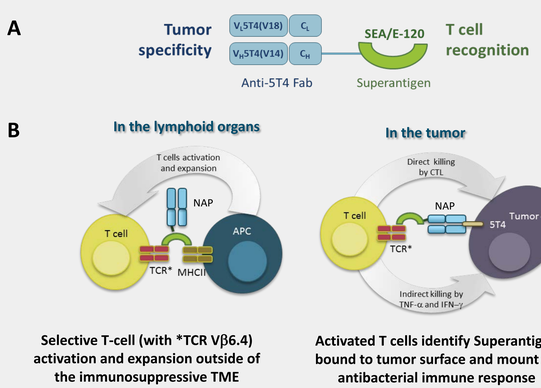

Figure 1: Naptumomab estafenatox(NAP) is a fusion protein that consist of genetically engineered Superantigens (Sag) linked to Fragment antigen binding (Fab) moieties directed to tumor-associated antigens. NAP only binds and activates T cells that contain certain TCR β variable (TRBV) regions, e.g. TCR Vβ6.4 (TRBV 7-9) . **B.** NAP activates and expands T cells (TCRVβ6.4) inside the lymphoid tissues, which infiltrate the tumor and mediate killing of the cancer cells. **C**. Selective activation and enrichment of TCR Vβ6.4 T cells following addition of NAP *in vitro*. Flow cytometry analysis demonstrated a ten-fold enrichment of TCR Vβ6.4 T cells, following activation with NAP (day 7). αCD3&CD28 activation did not change TCR Vβ6.4 percentage (baseline~6%).

Results

CAR-T cells activated by NAP are significantly more potent compared to CAR-T cells activated by αCD3/CD28

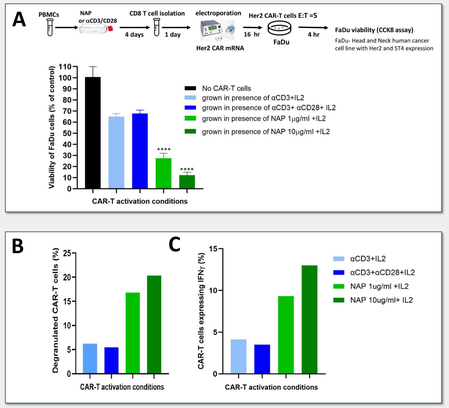

Figure 2: A. The viability of FaDu cultured with CAR-T cells, prepared following the specified activation methods, was quantified. CAR-T activated by NAP demonstrated superior killing potency compared to the standard activation method. B, C. Activation markers of CAR-T following incubation with FaDu cells were quantified by flow cytometry. In accordance with the killing assay, CAR-T produced following activation with NAP presented increased degranulation (B) and IFNγ production (C) indicating enhanced potency.
FaDu- Head and neck cancer cell line expressing Her2 and 5T4.
Mean ± SD; n=4 per group; ****< 0.0001 vs. αCD3 or αCD3&CD28 (One way ANOVA). The results are representative of at least three independent experiments.

Expansion of CAR-T cells in the presence of NAP results in a significantly higher expression of activation markers compared to αCD3&CD28

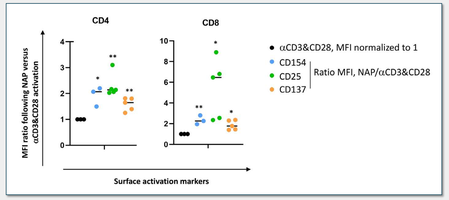

Figure 3. Following activation, CAR-T cells surface markers: CD154(CD40L), CD25 and CD137 were quantified by flow cytometry. The MFI values of these markers following αCD3&CD28 stimulation were normalized to 1. Ratio of expression following NAP vs. αCD3&CD28 was calculated and displayed. Production of the CAR-T in the presence of NAP resulted in a significantly higher expression of those activation markers compared to using αCD3&CD28, indicating on enhanced potency.
Mean ± SD; n=3 or 5 different donors per group; *< 0.05 or **< 0.01 vs. αCD3&CD28 reference normalized to 1 (*t test*).
Each surface marker is represented by a different color. Each dot represents a different donor.

Expansion in the presence of NAP results in increased central memory differentiation

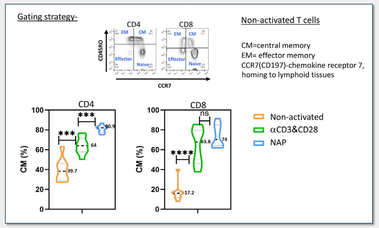

Figure 4. Analysis of T cell subsets was performed by flow cytometry. The markers CCR7 and CD45RO were used to divide the T cells into subsets (Gating strategy).
Analysis of 8 donors showed significantly higher percentage of CM CD4 T cells and similar percentage of CM CD8 T cells following NAP stimulation compared to the standard activation method. This indicates preferred memory differentiation following NAP activation and suggests also better persistence of these T cells *in vivo*.
Mean ± SD; n=8 different donors per group; ***p< 0.001, ****p< 0.0001 (*t test*).

Expansion in the presence of NAP results in Th1 polarization

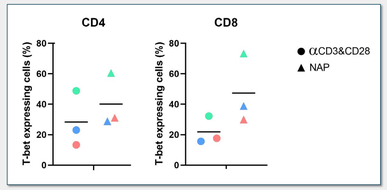

Figure 5: Th1/Tc1 polarization was quantified by flow cytometry according to the expression of the Th1 master regulator, the transcription factor T-bet. CAR-T produced following activation with NAP showed higher percentage of T-bet positive cells, for both CD4 and CD8, compared to the standard activation method, indicating stronger polarization towards Th1/Tc1. This may serve as an explanation for the enhanced killing potency of the CAR-T cells produced following NAP activation.
Each symbol represents a stimulation type, and each color represents a different donor. A total of three donors are shown.

NAP enhances T cell chemotaxis

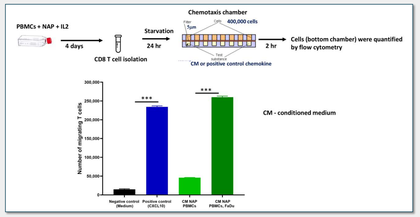

Figure 6: The chemotactic activity of T cells was quantified using a standard chemotactic chamber and the migrating cells were quantified by flow cytometry.
Negative control medium did not induce significant migration of T cells, while the chemokine, CXCL10, resulted in a ten-fold enhanced migration. Conditioned medium (CM) from immune cells incubated with NAP enhanced chemotaxis compared to the negative control. Interestingly, CM from FaDu cancer cells and immune cells incubated with NAP was much more chemotactic and resulted in an additional 5-fold increase in T cell migration.

This represents what could potentially occur in the tumor microenvironment (TME) where NAP interacts with immune cells and cancer cells and induces the production of chemokines, which would drive the infiltration of T cells into the TME and thereby boost the immune response.
Mean ± SD; n=2; ***< 0.001(*t test)*.

Combination of CAR-T with NAP results in synergistic killing of the tumor cells

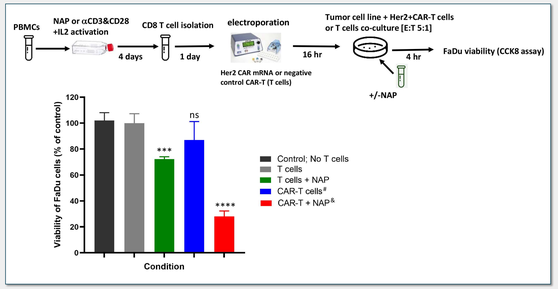

Figure 7: The viability of FaDu co-cultured with CAR-T cells (low expression) or T cells was examined in the presence or absence of NAP. Control of T cells, which were activated with NAP and then incubated with FaDu cells only (without NAP) did not kill the target cells. CAR-T cells alone had no effect on tumor cells viability, whereas NAP induced a significant cytotoxic effect, with the combination of CAR-T plus NAP producing the most significant reduction in tumor cell viability. # CAR-T cells activated by αCD3&CD28 ; &CAR-T cells activated by NAP; T cells, No NAP - were activated by NAP and incubated with FaDu cells, without NAP. FaDu- Head and neck cancer cell line expressing Her2 and 5T4.
Mean ± SD; n=4 per group; *** p= 0.0007 vs. control; **** p< 0.0001 vs. all test groups. The results are representative of at least three independent experiments (One way ANOVA).

Conclusion

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].

References

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