Selective T cell Redirection Proteins (STR) Enhance the Anti-Tumor Activity of Checkpoint Inhibitors (CPIs) and can Lead to Long-Lasting Immunity Against the Tumor

Meir Azulay, Sveta Lifshits, Eitan Shany, Adam Friedmann, Gunnar Hedlund and Michal Shahar

NeoTX Therapeutics LTD, Rehovot, Israel

Abstract

Background

Tumor-targeted superantigens (TTS) such as Naptumomab Estafenatox (Nap) are fusion proteins that consist of genetically engineered Superantigens (Sag) linked to Fragment antigen binding (Fab) moieties directed to tumor-associated antigens. Unlike CD3-based T cell redirection approaches (e.g. BiTEs) which bind and activate all T cells, TTS only bind and activate subsets of T cells that contain certain TCR β variable (TRBV) regions, e.g. TRBV 7-9 [1] and are thus defined as STR. We previously reported the synergistic anti-tumor effect of combining CPI with our lead STR compound, Nap (5T4 targeted Sag) or its murine surrogate protein [2]. Here, we present new pre-clinical data showing that STR not only enhances the anti-tumor effect of CPIs, but also stimulates the overall immune response that could lead to long term immunity against the tumor.

Methods

The combination of Nap with PD-L1 inhibitor (durvalumab) was tested *in vitro* against high (MDA-MB 231) and low (RKO) 5T4-expressing cancer cell lines in the presence of human PBMCs. For the *in vivo* studies, mice bearing hEpCAM transfected MC38 tumors were treated with TTS (consisting of a Fab against hEpCAM), an anti-PD-1 antibody, or the combination. Tumor growth and survival were assessed and tumor recurrence following re-challenge was evaluated.

Results

Combination of Nap with durvalumab had synergistic anti-tumor effect against both high and low 5T4-expressing cancer cell lines. Concomitant treatment of MC38-hEpCAM tumor bearing mice with TTS and anti-PD-1 achieved complete tumor rejection in 4 of 10 mice and significantly prolonged survival and delayed outgrowth of tumors compared to the monotherapies. All cured mice rejected re-challenge with MC38-hEpCAM and parental MC38 tumors, indicating long-term memory responses.

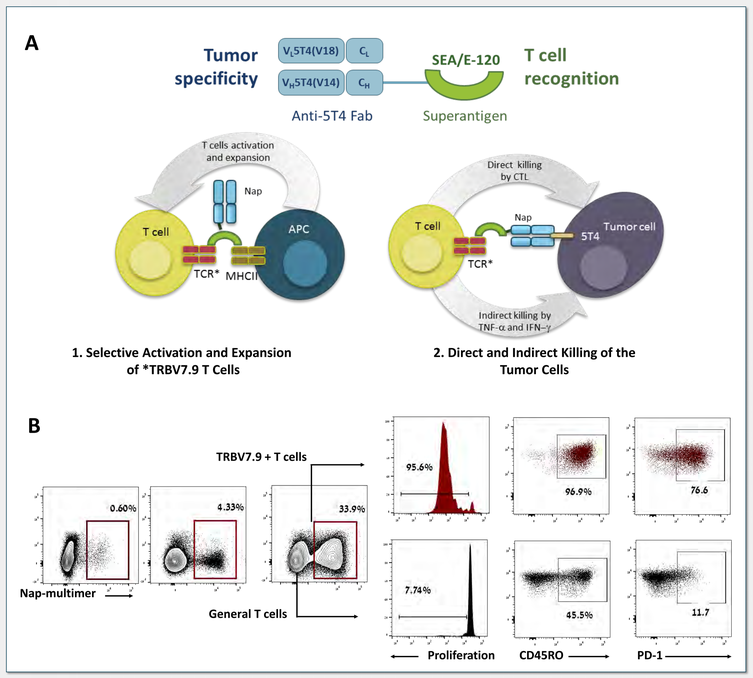

Figure 1: Naptumomab estafenatox (Nap). A. Nap is used in cycles of four once-daily intravenous injections. Early in the cycle, the TRBV7.9 T lymphocytes are activated, proliferate and differentiate into effector cells (1), which later localize to the tumor and mediate their antitumor functions (2). **B.** Selective activation, proliferation and differentiation of TRBV7.9 T cells following addition of Nap in vitro. FACS analysis of Nap-biotin/SA-PE multimeric complex binding to TRBV7.9 T lymphocytes derived from fresh human PBMC of healthy donor on day 0 (~4% of total CD3+ cells) and on day 4 following activation with Nap (selective expansion of ~30% of total CD3+ T cells).

Synergistic Anti-tumor Effect of Nap with Anti PD-L1 Against both High and Low 5T4-expressing Cancer Cell Lines

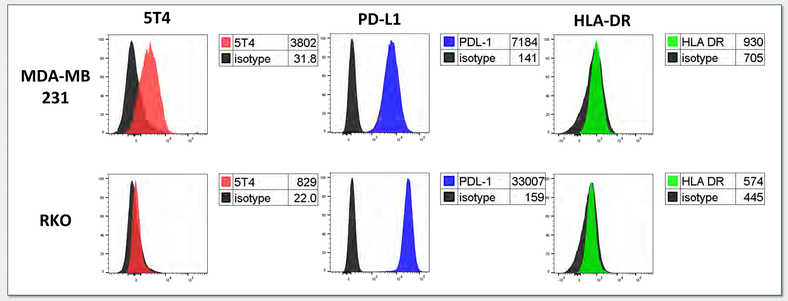

Figure 2: The expression of 5T4, PD-L1 and HLA-DR on RKO (colorectal cancer) and MDA-MB-231 (TNBC) human tumor cell lines was assessed by flow-cytometry. The results show that both cell lines express high levels of PD-L1 while a differential 5T4 expression profile was shown, as MDA-MB-231 cells express high 5T4 levels and low 5T4 expression was detected on RKO cells. HLA-DR was undetectable on both tumor cell line.

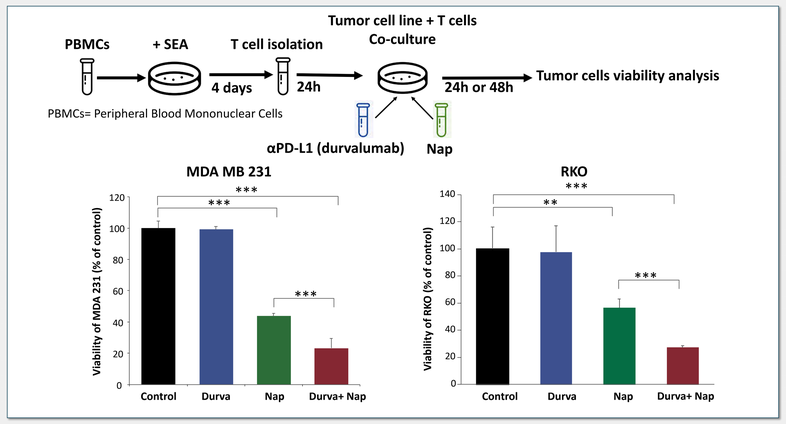

Figure 3: The viability of MDA-MB-231 or RKO cells co-cultured with the SAg activated T cells was examined in the presence or absence of the PD-L1 inhibitor (durvalumab), Nap or the combination. Anti-PD-L1 alone had no effect on T cell mediated cytotoxicity, whereas Nap induced a significant cytotoxic effect, with the combination of anti-PD-L1 plus Nap producing the most significant reduction in tumor cell viability of both high and low level 5T4 cell lines.
RKO- colorectal cancer cell line; MDA-MB 231- TNBC cell line. Mean ± SD; n=4 per group. ** p<0.005, *** p<0.0005. The results are representative of at least three independent experiments

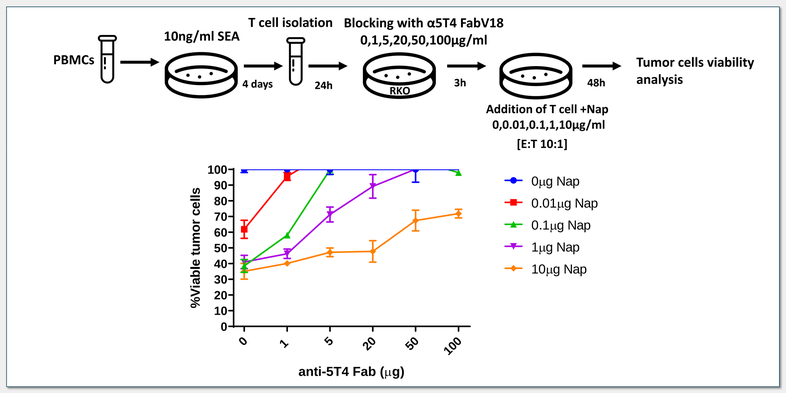

Figure 4: Blocking of 5T4 with anti-5T4 Fab inhibited the anti-cancer effect of Nap against the 5T4 low RKO cell line showing that Nap activity is mediated through the 5T4 antigen and is effective also against 5T4 low expressing tumors.

Combination of STR with CPI Induced a Prolonged and Protective Immune Response Against Tumor

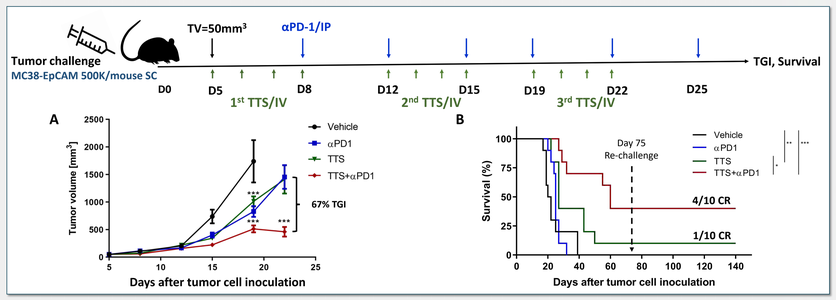

Figure 5: Combination of murine STR (TTS) and anti-PD-1 significantly inhibited tumor growth and increased survival of MC38 tumor bearing mice. Anti-PD-1 monotherapy had limited effect on MC38-hEpCAM tumor. Although TTS alone was able to inhibit tumor growth to the same extent as anti-PD-1 treatment, the combination with anti-PD-1 was significantly more effective in reducing tumor burden and prolonging median survival. In addition, 40% and 10% of the Mice from the combination group and TTS alone group (respectively) had complete response (CR).
A. The mean tumor volume of at least 8 mice/group ±SE. At day 19- ***p<0.0001 treatment vs. control. At day 22- ***p<0.0001 combination vs. TTS or anti-PD1 alone. TGI= tumor growth inhibition.
B. Median survival time of each treatment group. *p=0.02, **p=0.006, ***p=0.0002. n=10 per group. Mice were re-challenged on day 75 (Figure 6).
TTS- tumor-targeted superantigens ,a mouse version of STR (C215Fab-SEA).

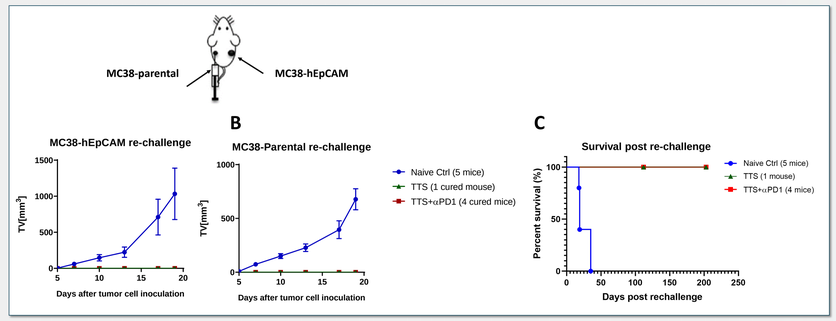

Figure 6: Murine STR (TTS) induced protective immune response against tumor re-challenge. 50 days following last treatment in the MC38 study, mice in complete remission and naïve control mice were challenged with MC38-hEpCAM and MC38-parental tumor cells as follow: 500K MC38-hEpCAM tumor cells were injected SC into the right flank and 500K MC38 parental tumor cells were injected SC into the left flank. The mean tumor volume of the MC38-hEpCAM (**A**) and MC38-parental tumors was measured(**B**) and the Survival duration (**C**) was determined.
While 100% of the naïve mice developed flank tumors on both sides, all the pre-treated mice completely rejected the second tumor challenge (**A and B**). All the naïve mice died by day 35 of the study whereas 100% of the pre-treated mice lived for at least 203 days post re-challenge with no recurrence of the tumors (**C**). These results indicate that the cured pre-treated mice have long-term immunologic memory against not only the MC38-hEpCAM tumor but also against the parental MC38 tumor.

Conclusion

Our studies show that combination of CPI with STR overcomes the limited effect of CPI monotherapy regardless of tumor antigen expression level. In addition, our *in vivo* studies demonstrate that the combination of STR with CPI may lead to long term durable responses not possible in most patients receiving single agent CPI therapy. Moreover, the ability of these "cured mice" to reject tumor re-challenge suggests that STRs cause release of secondary antigens that prime subsequent immune responses.
Taken together, our data suggests that combining anti-PD(L)1 with STR may be a promising therapeutic strategy for patients with solid tumors.
Clinical phase 1b trial is expected to be initiated in Q4 2019 investigating the combination of Nap with durvalumab in subjects with selected advanced or metastatic solid tumors [NCT03983954].

References

1. Hedlund G, Eriksson H, Sundstedt A, et. al. The Tumor Targeted Superantigen ABR-217620 Selectively Engages TRBV7-9 and Exploits TCR-pMHC Affinity Mimicry in Mediating T Cell Cytotoxicity. PLoS One. 2013; 8(10): e79082
2. Azulay M, Lifshits S, Friedmann A, et al. Naptumomab Estafenatox induces T cell recognition, turning anti-PD-1 unresponsive "cold" tumors into "hot" responsive tumors. Cancer Research. Jul 2018, 78 (13 Supplement) abstract # 2712 AACR Annual Meeting 2018; Chicago, IL; DOI: 10.1158/1538-7445.AM2018-2712.