Naptumomab Estafenatox Induces T cells Tumor Recognition, Turning anti-PD-1 Unresponsive "Cold" Tumors into "Hot" Responsive Tumors

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Introduction

Tumor recognition is a key factor in checkpoint inhibitors (CPI) efficacy and acquired resistance. Lack or loss of tumor antigens expression or inefficient presentation prevents tumor cell recognition by T cells, inhibiting CPI anti-cancer effect. Naptumomab estafenatox (Nap) is a tumor-targeted superantigen (TTS) protein that increases tumor recognition by both coating tumor cells with bacterial-derived superantigens (SAg) as well as selectively expanding T cells lineages that can recognize it. Nap consists of a genetically engineered SAg, staphylococcal enterotoxin A (SEA/E-120), linked to a fragment antigen binding (Fab) moiety directed to the 5T4 oncofetal tumor-associated antigen expressed on many tumors (Fig. 1).

Here we present new pre-clinical results of synergistic anti-tumor effect of Nap (or its murine equivalent TTS) with anti-PD-1, under conditions which mimic poor tumor recognition.
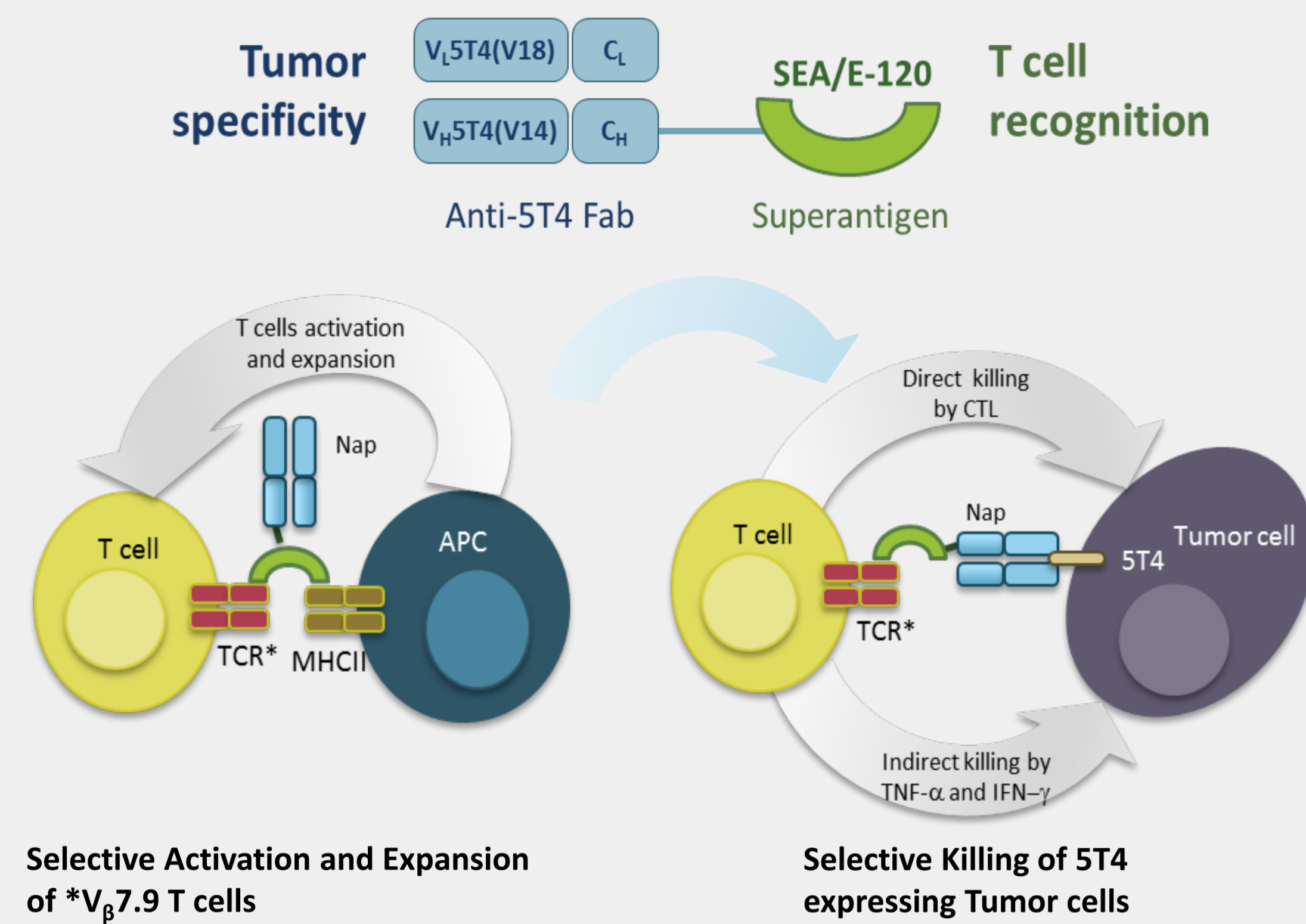

Figure 1: Naptumomab estafenatox (Nap).
Nap is a recombinant fusion protein that consists of an anti-tumor Fab moiety, targeting the 5T4 glycoprotein (expressed on a large number of solid tumors), genetically fused to the SEA/E120, a modified superantigen (SAg) which mediate the activation and expansion of Vβ7.9 positive T cells.

Nap is used in cycles of four to five once-daily intravenous injections. In the first phase of a cycle, the Vβ7.9 T lymphocytes are activated, proliferate and differentiate into effector cells, which later in the cycle localize to the tumor and mediate their antitumor functions.

In vitro Results

Nap Turns a Cold (i.e. non-responsive to anti-PD1 antibody) Tumor into a Hot, Responsive Tumor

In vitro studies with Nap investigated the effect of co-culturing SAg activated T cells with HCC827 NSCLC or MDA-MB-231 Triple-Negative Breast Cancer cells. Co-cultures of T cells with either cancer cell line resulted in an increase in tumor staining for PDL-1, which was further elevated in the presence of Nap, probably due to increase in IFNγ secretion (Fig. 2).
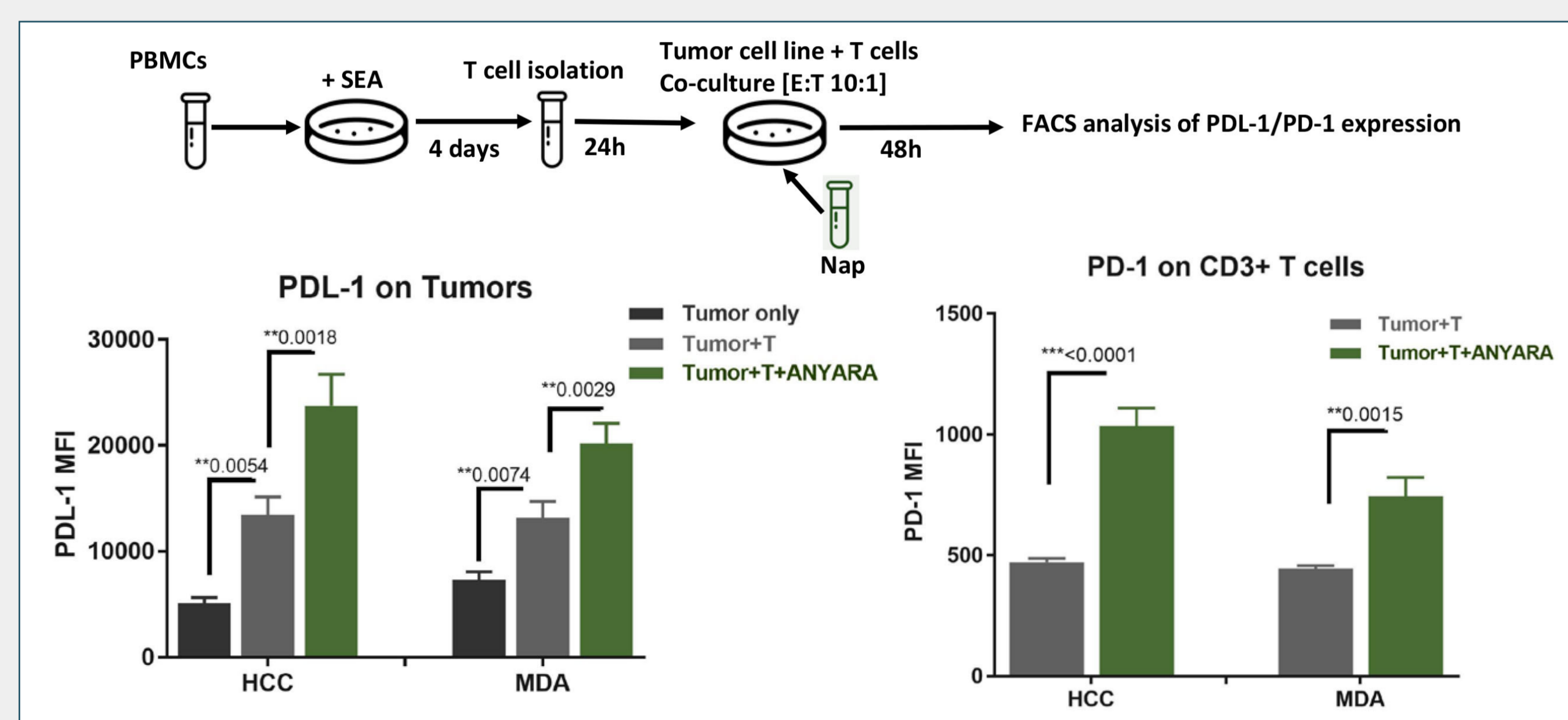

Figure 2: Nap Increases PD-1 Expression on T cells and PDL-1 on Tumor Cells. SEA activated T cells were cocultured with HCC827 and MDA-MB-231 tumor cell line for 24hr and the expression of PD-1 on T cells and PDL-1 on tumor cells was assessed by FACS. n=3; Mean±SD.

The viability of HCC827 or MDA-MB-231 cells co-cultured with the SAg activated T cells was examined in the presence or absence of the PD-1 inhibitor, Nap or the combination. Anti-PD-1 alone had no effect on T cell mediated cytotoxicity, whereas Nap induced a significant cytotoxic effect, with the combination of anti-PD-1 plus Nap producing the most significant reduction in tumor cell viability (Fig. 3).

Method:
To test the cytotoxicity mediated by Nap, cells were co-cultured with SEA activated T cells with or without 0.1μg/ml of Nap or in combination with 0.2μg/ml of Pembrolizumab, for 48hr. Following removal of non-adherent T cells, tumor cell viability was measured using a colorimetric assays for the determination of the number of viable cells (Fig. 3).
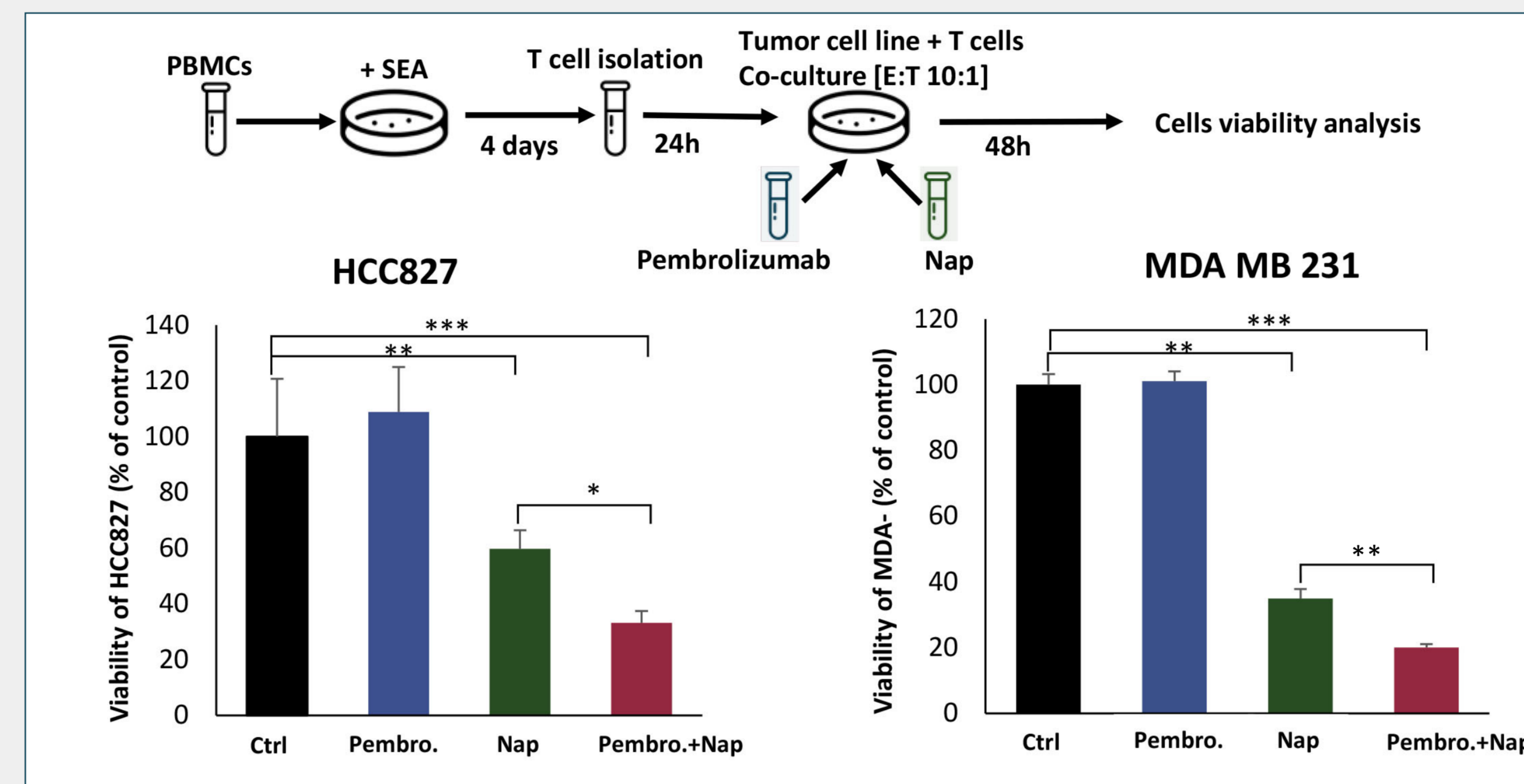

Figure 3: Combination of Nap and αPD-1 (pembrolizumab) shows Synergistic Killing Effect against EGFR mutant NSCLC and TNBC cell lines. HCC827 –EGFR mutant NSCLC cell line; Mean ± SD (n=3-6/group); * p = 0.024, ** p = 0.013; *** p= 0.0018. MDA-MB-231- TNBC cell line; Mean ± SD (n=4/group); ** p = 0.0001, *** p<0.0001.

In vivo Results

Synergistic Anti-tumor Activity of Tumor-Targeted Superantigen (TTS) and Anti-PD1 in the Poorly-Immunogenic B16 Melanoma Model

Similar to the *in vitro* studies, the *in vivo* mouse studies using the B16 low immunogenic tumor model showed limited or no effect of anti-PD-1 monotherapy. Although TTS alone increased T cells activation and infiltration into the tumor, the combination with anti-PD-1 was significantly more effective in increasing serum cytokines, increasing the CD8:CD4 ratio in the tumor, reducing tumor burden and prolonging median survival (Fig. 4, 5, 6, 7).

Method:
C57BL/6 mice were inoculated i.v. with 175K EpCam-tranfected murine B16-F10 (B16-EpCam) melanoma cells to induce lung tumors. For the *in vivo* studies, a murine version of Tumor-Targeted Superantigen (TTS) directed to EpCam Ag was used - C215Fab-SEA. Mice were treated with 4 daily injections of C215Fab-SEA (0.5 μg/mouse) i.v. A Rat IgG2a anti-mouse PD-1 antibody or the isotype was administered i.p. at 200 μg/injection twice a week for 3 weeks. For survival studies, mice were treated with 2 cycles of C215Fab-SEA as described in Figure 5.
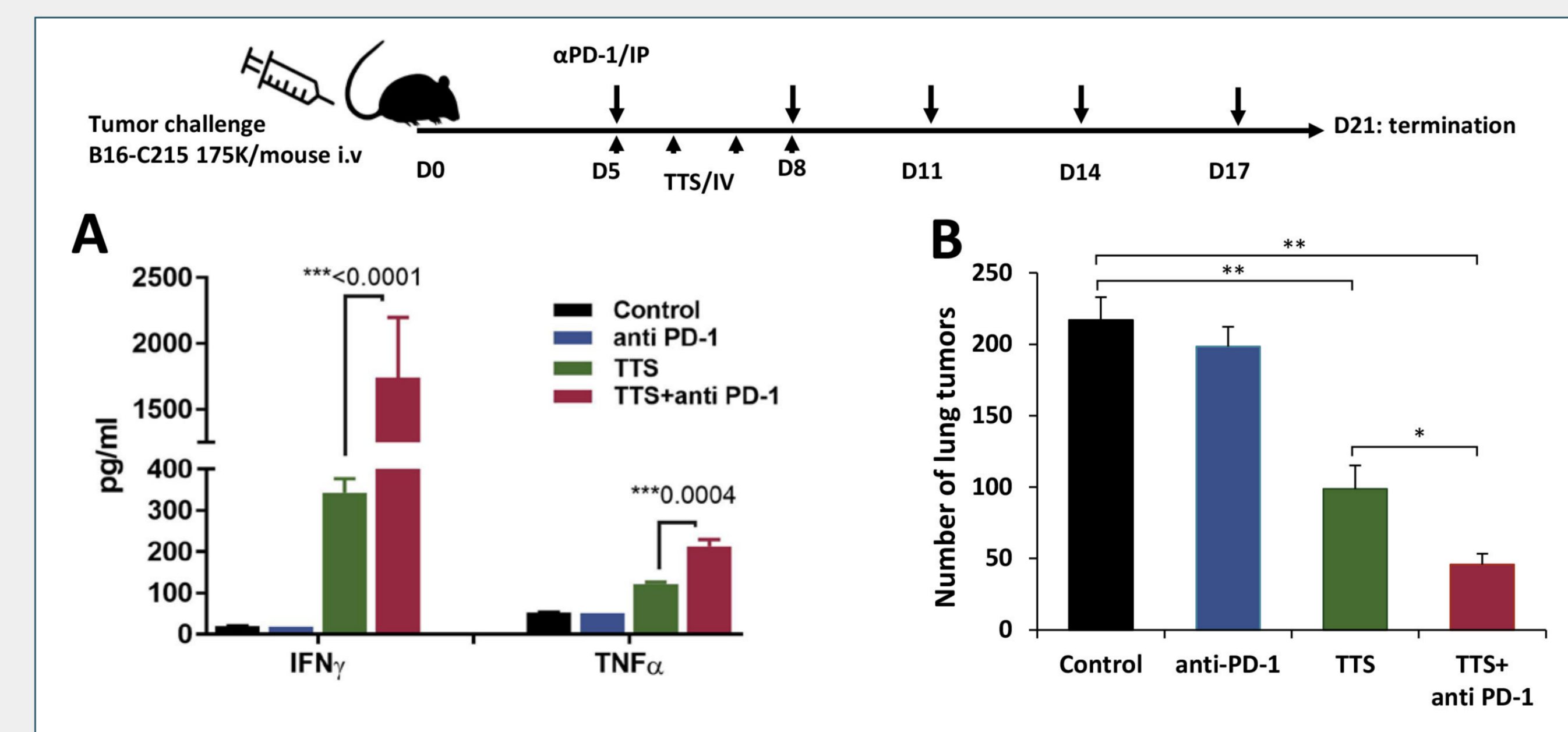

Figure 4A: Enhanced T cell activation in the mice serum following TTS and anti-PD-1 combination. On day 8 post tumor inoculation, mice were treated as indicated, and 4hrs later, blood was collected from vena cava and cytokines were detected in samples of isolated serum by cytometric bead array. Mean±SEM (n=3/group)

Figure 4B: Combination of TTS with anti-PD-1 significantly reduces the number of lung metastases. On day 21 post tumor inoculation, mice were sacrificed and the lungs were removed. After fixation in Bouin's solution, the numbers of lung tumors were counted. Mean±SEM (n=7-8/group); *p<0.05, **p<0.001
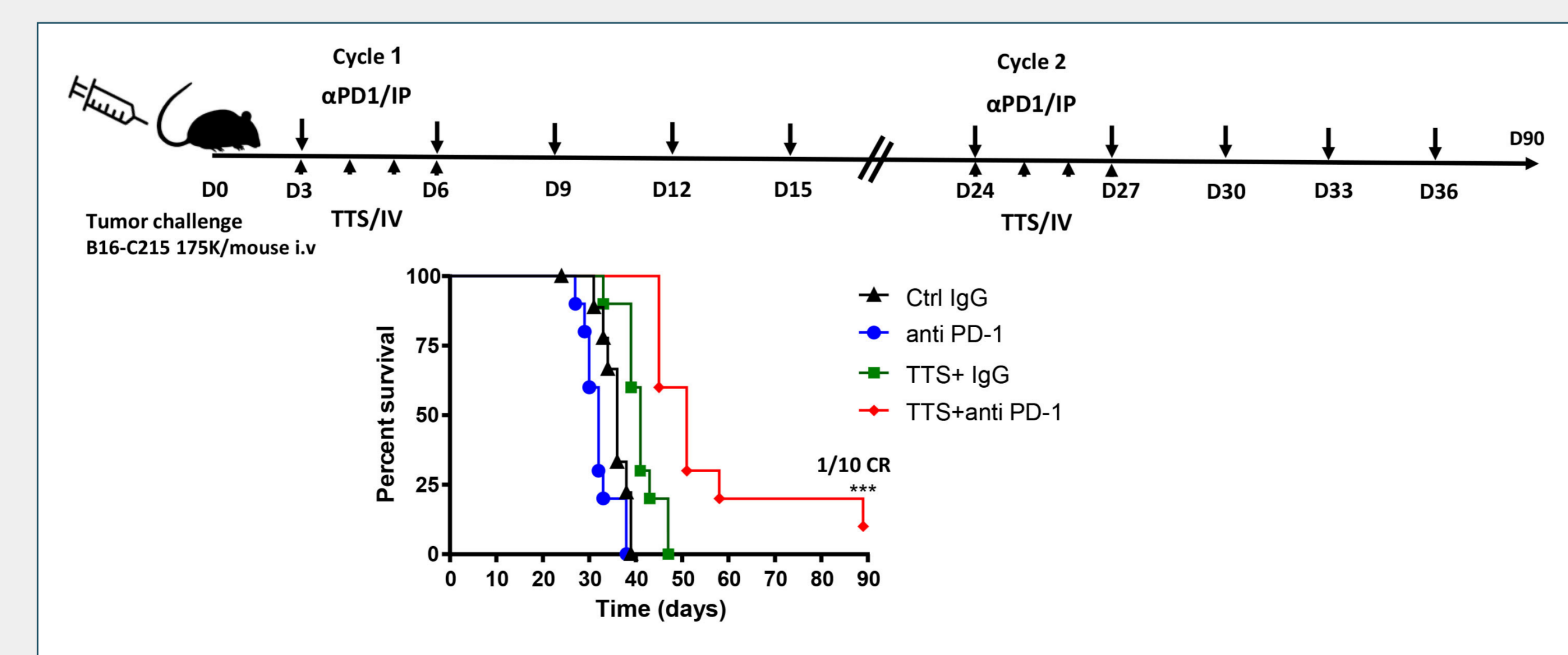

Figure 5: Combination of TTS and anti-PD-1 significantly increased survival of tumor bearing mice. The median survival time of the combination of TTS +anti PD-1 was significantly longer than TTS +IgG, ***p=0.0004. One mouse from the combination group had complete response (CR). n=10 per group.
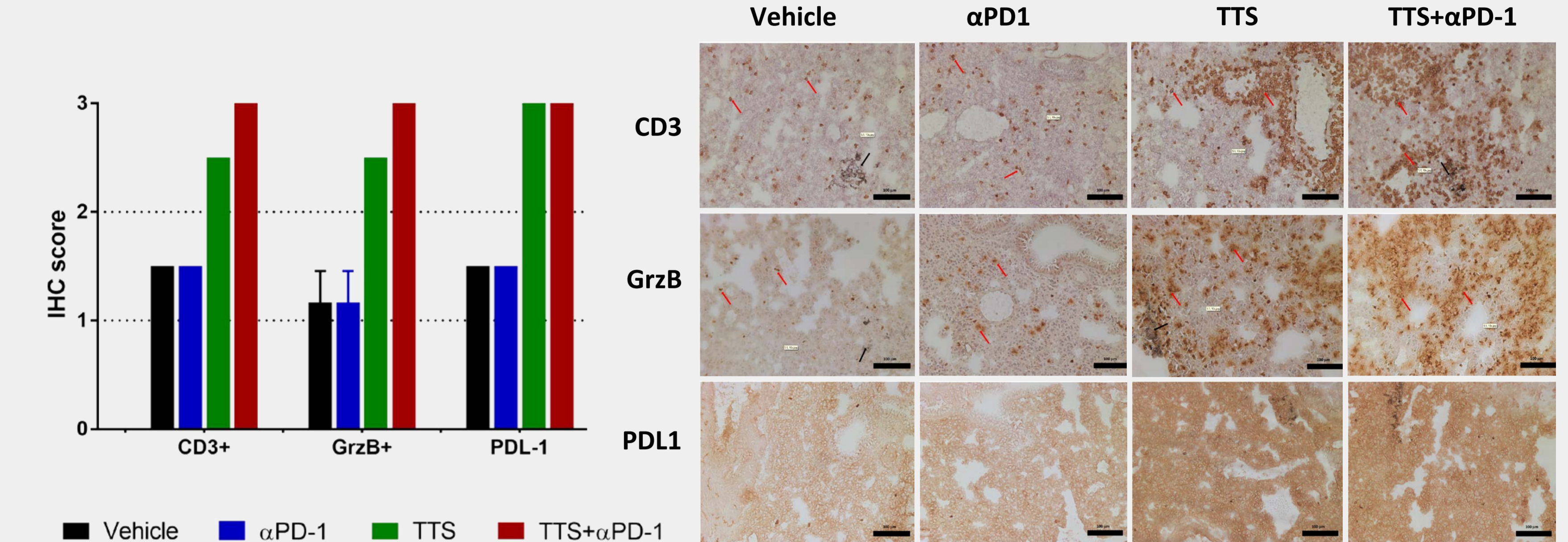

Figure 6: Combination of TTS and anti-PD-1 results in increased T cell infiltration and PDL-1 upregulation in lung metastases. Frozen sections of lung metastasis from the study described in Figure 4 where analyzed with a Leica DMRX microscope. Representative photos of IHC single staining. Single labeling, CD3 and GranzymeB (GrzB); Graph shows IHC score, no positive cells (0), few positive cells (1+), moderate numbers of positive cells (2+) or high numbers of positive cells (3+). PDL-1 showed positive staining in large part of the lung and was judged as weak staining in lung (1+), moderate staining in lung (2+) or strong staining in lung (3+). Mean±SD (n=3/group).
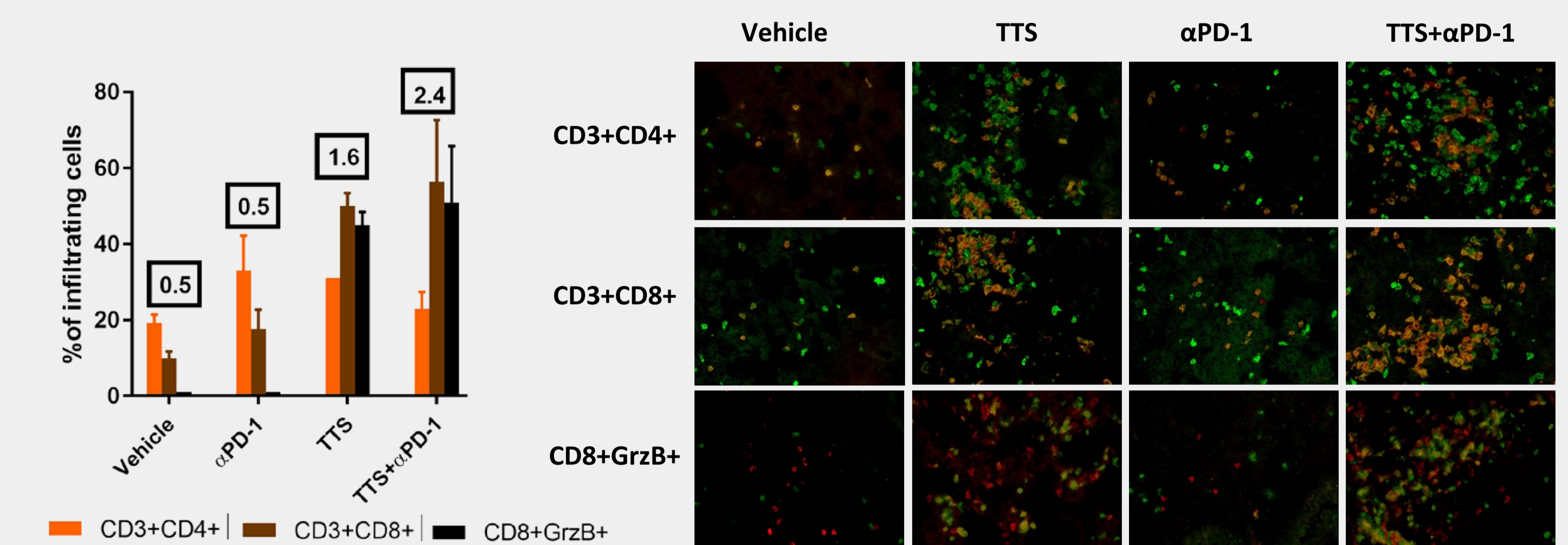

Figure 7: Combination of TTS and anti-PD-1 results in increased CD8+ T cell infiltration. Frozen sections of lung metastasis from the study described in Figure 4 where analyzed with a Leica DMRX microscope. Double labeling; CD3 and GrzB marked in green, CD4 and CD8 marked in red. Merged markers appears as Orange. CD3+CD4+ and CD3+CD8+; positive cells were counted directly from observation in the microscope using the 40x lens within a frame of 300 x 230um and were used to determine CD8:CD4 ratio (□). Granzyme B+ CD8+ calculation was made from overlay photographs. Mean±SD (n=3/group).

Conclusion

Our studies show that combination of anti-PD-1 with tumor-targeted superantigens overcomes the limited effect of anti-PD-1 monotherapy in low immunogenic tumor models *in vitro* and *in vivo*.

This novel combination may offer clinical benefit for CPI unresponsive patients, particularly in cases where tumor recognition is lost or restricted. Clinical phase 1-2 trials are planned with the combination of naptumomab estafenatox and anti-PD-1/PDL-1 blockade.

Tumor recognition is essential for anti-PD-1/PDL-1 activity
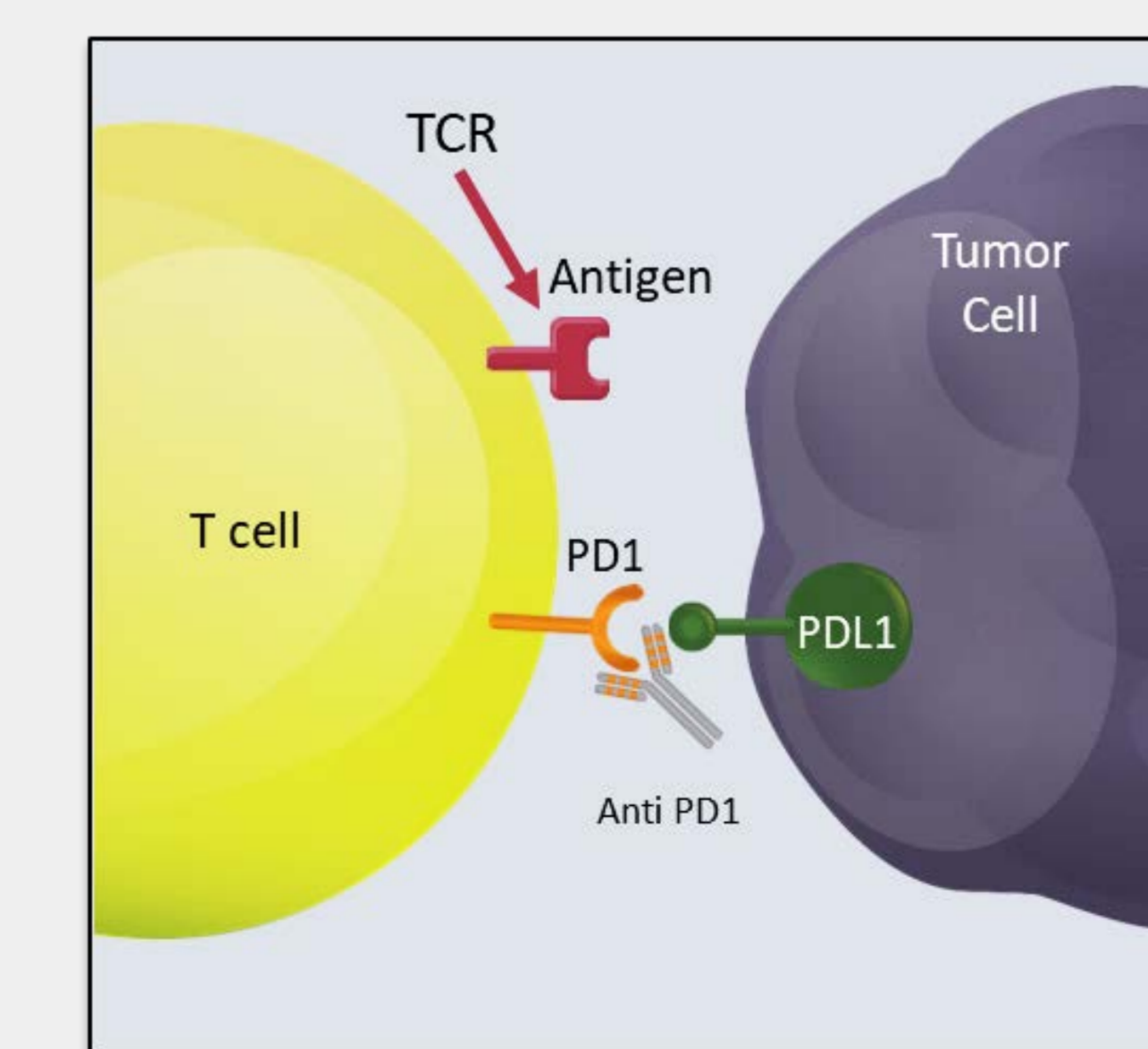
No T cell activation without recognition signal

Nap induces tumor recognition, turning cold tumors into hot, responsive tumors
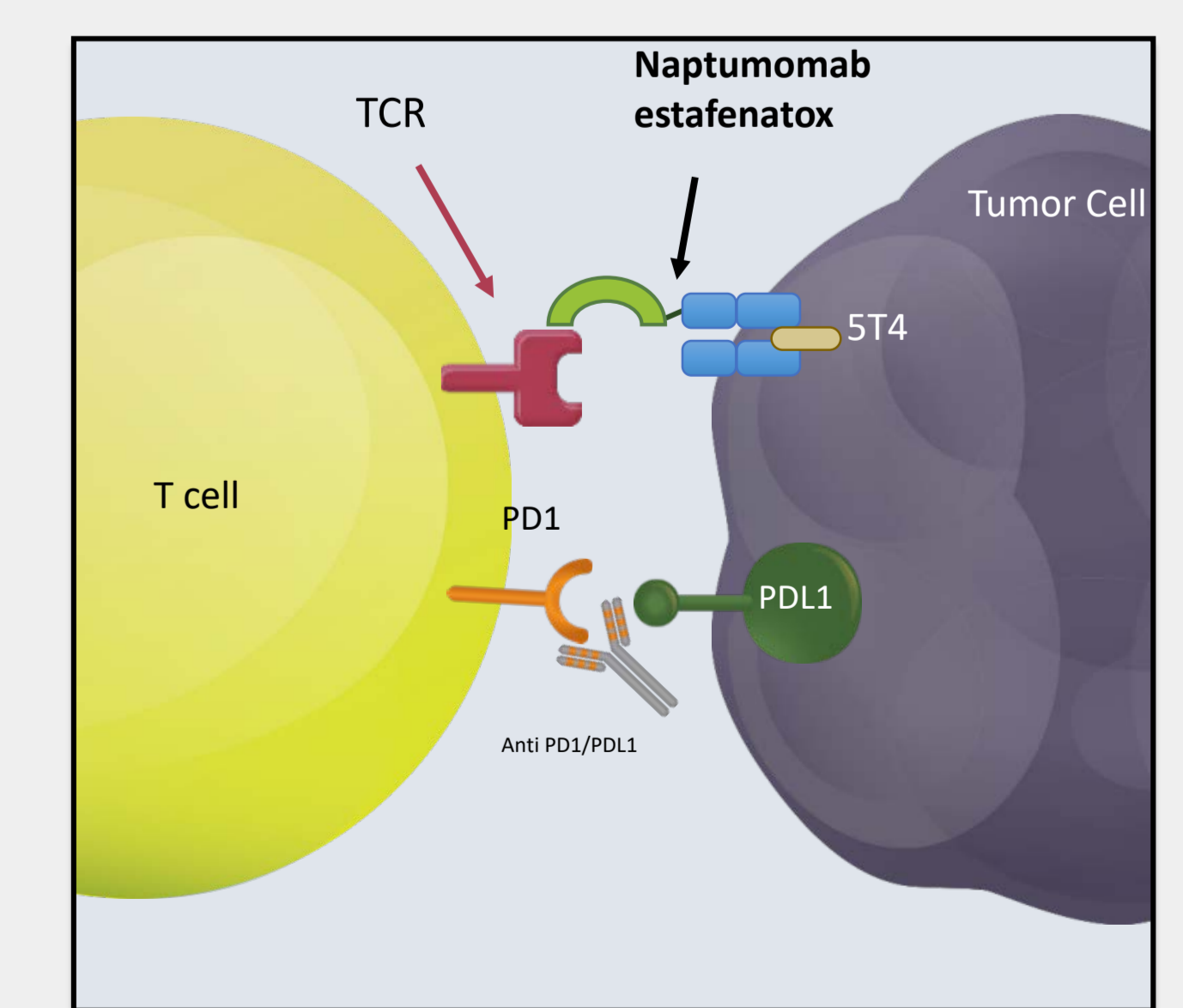
Recognition signal enables T cell activation

References

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