Targeting Highly attenuated IL18 (HT18) to PD-1: A Next Generation Checkpoint Inhibitor with Enhanced Anti-tumor Activity

Xueyuan Zhou, PhD[1]; Jeffrey Takimoto, PhD[1]; Sunny Yoon, BA[1] ; Felix Geyer, MS[2] ; Harald Kolmar, PhD[2] & Brian Rabinovich, PhD[1]
[1] Fuse Biotherapeutics. Boston, U.S.A.; [2] Technical University of Darmstadt, Germany

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Background

IL-18 is a danger induced cytokine that serves to promote the activity and survival of antigen presenting cells, NK cells and subsets of T cells. The cytokine is strongly regulated by a negative feedback loop that includes the induction of IL-18BP and IL-37, which act to bind IL-18 and IL-18Rα, respectively.

In the tumor microenvironment (TME), the IL-18Rα/β complex is expressed on PD-1$^+$ progenitor exhausted T cells that are responsive to PD-1 antagonism. Targeting an IL-18BP resistant variant of IL-18, which is attenuated for safety, but retains activity when brought into close proximity of the IL-18 receptor complex is hypothesized to maintain survival of the αPD-1 responsive T cell pool. Thus, we developed a first-in-class variant of IL-18 termed "HT18" fused to an αPD-1 antibody (αPD1-HT18$_{cis}$). HT18 is 10,000-fold attenuated but retains nearly full activity of native cytokine when delivered to PD-1$^+$ cells.

Findings

- HT18 did not bind IL-18BP nor IL-18Rα in trans but can bind strongly to the IL-18Rα/β complex
- Targeting HT18 to PD-1 augmented PD-1 induced T cell mediated proinflammatory cytokine release in-vitro
- Strong efficacy was observed with αPD1-HT18$_{cis}$ in multiple aggressive mouse tumor models including a complete response (CR) rate ranging from 60%-100% without weight loss or other signs/symptoms of toxicity
- Immune memory and evidence supporting epitope spreading was observed in both mouse tumor models that induce both weak and strong emergency myelopoiesis
- TGI was associated with about a 6-fold increase in TIL numbers relative to the PD-1 antibody benchmark with significant increases in CD8$^+$ T cells, effector and central memory T cells, reduced tumor associated macrophages (TAM) and skewing towards a proinflammatory anti-tumor cellularity

Unique mechanism renders HT18 active when targeted to PD-1$^+$/IL-18R$^+$ cells but 10,000-fold attenuated in circulation

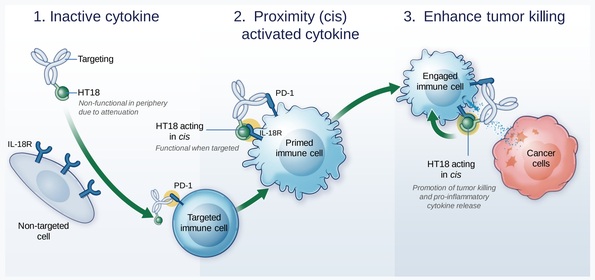

Figure 1. αPD1-HT18$_{cis}$ is 10,000 less active than IL-18 unless bound to cells that express both PD-1 IL-18R. In the tumor, PD-1$^+$/IL-18R$^+$ T cells are functional and respond to PD-1 checkpoint inhibition.

IL-18 preserves and augments tumor reactive T cells that are functional and responsive to PD-1 checkpoint inhibition

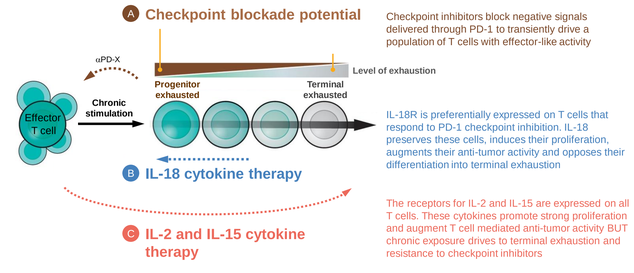

Figure 7. Response of T cell subsets to checkpoint inhibition, IL-2, IL-15 and IL-18. Only IL-2 and IL-15 have evolved to both promote and limit immune responses. IL-18 is a "danger signal" that has evolved to preserve immune cell activity

αPD1-HT18$_{cis}$ induced both memory and epitope spreading in immunogenic colon carcinoma model

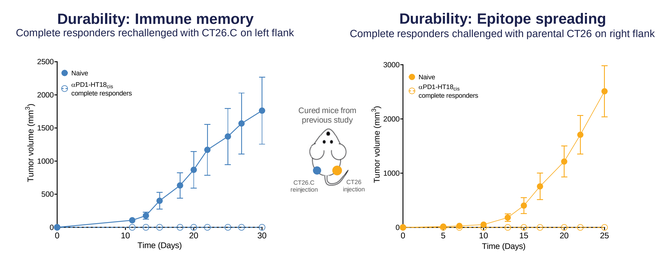

Figure 12. BALB/c mice that harbored CT26.C tumors and experienced a CR following treatment with αPD1-HT18$_{cis}$ were rechallenged with CT26.C after 65 days on the left flank and parental CT26 after 70 days on the right flank.
As a means of comparison, a group of 5 naïve mice were subjected to the equivalent tumor inoculation

Safe and effective delivery of IL-18 to PD-1$^+$ T cells necessitates resistance to IL-18BP and attenuation

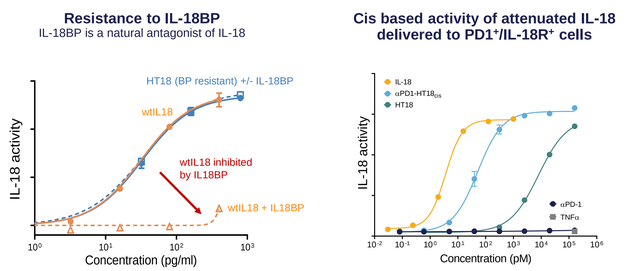

Figure 2. Impact of 100-fold excess IL-18BP on activity of wtIL-18 and HT18 activity in HEK-Blue IL-18 reporter assay

Figure 3. Impact of targeting HT18 to PD-1$^+$ HEK-Blue-IL-18. Exemplary test articles normalized across >5 experiments

αPD1-HT18$_{cis}$ but not αPD-1-IL-18 results in strong tumor efficacy without weight loss

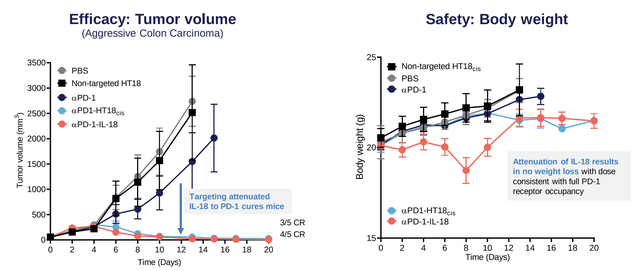

Figure 8. C57BL/6 mice were injected subcutaneously with MC38i, an aggressive MC38 clone. When mean tumor volume ranged between 75-100 mm^3, mice were randomized into groups of 5 and treated with PBS, αPD-1, αPD1-HT18$_{cis}$, or αPD-1-IL-18 Days 0 and 4 at 15mg/kg

αPD1-HT18$_{cis}$ induces strong efficacy and memory in an aggressive model of melanoma

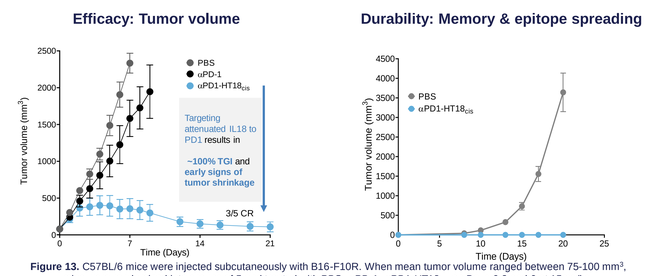

Figure 13. C57BL/6 mice were injected subcutaneously with B16-F10R. When mean tumor volume ranged between 75-100 mm^3, mice were randomized into groups of 5 and treated with PBS, αPD-1, αPD1-HT18$_{cis}$ on Days 0,3 and 6 at 15mg/kg.
Mice that experienced a CR following treatment with αPD1-HT18$_{cis}$ were rechallenged with B16-F10 after 60 days

Affinity of IL-18 and HT18 for IL-18Rα and the IL-18Rαβ complex

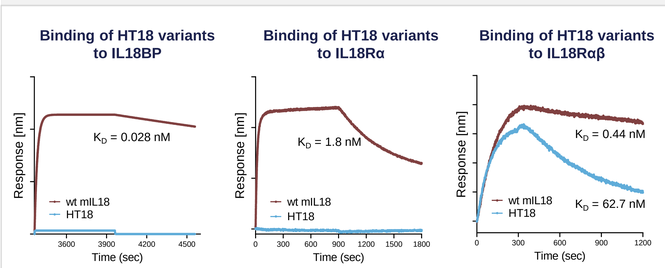

Figure 4. Equilibrium dissociation constants (KD) of monovalent human Fc fusions of wt-mIL-18 and HT18 for mIL-18BP, IL-18Rα and IL-18Rαβ. For mIL-18BP, human Fc fusions of wt-mIL-18 and HT18 were immobilized on AHC biosensors.
For IL-18Rα and IL-18Rαβ mouse Fc fusions of IL-18Rα or IL-18Rαβ were immobilized on AMC biosensors.

αPD-1-IL-18 induced far greater serum IFNγ but weaker immune memory than αPD1-HT18$_{cis}$

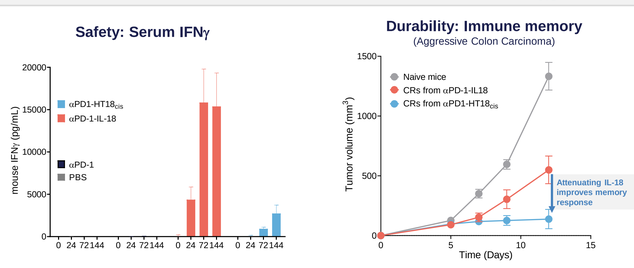

Figure 9. C57BL/6 mice were injected subcutaneously with MC38i. Serum was collected at 0, 24, 72 and 144 hours after the Day 0 treatment and mouse IFNγ measured

Figure 10. C57BL/6 mice that harbored MC38i tumors and experienced a CR following treatment with αPD1-HT18$_{cis}$ or αPD-1-IL-18 were re-challenged with MC38i after 90 days

αPD1-HT18$_{cis}$ induces strong immune infiltration in aggressive murine melanoma tumors

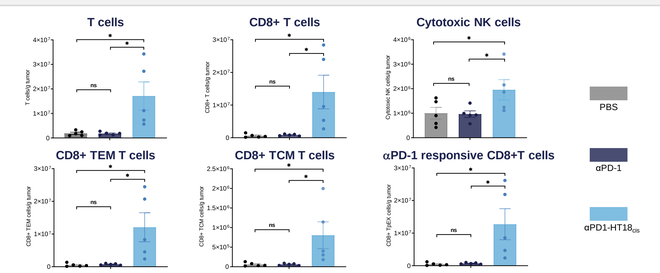

Figure 14. C57BL/6 mice harboring B16-F10R tumors were treated on Days 0 and 3. Mice were euthanized on Day 5, tumors collected and dispersed into singe cell suspensions for flow cytometric assessment of TIL (CD45$^+$ cells). TEM and TCM were defined CD44$^+$/CD62L$^-$ and CD44$^+$/CD62L$^-$, respectively within the CD8$^+$ pool. αPD-1 responsive CD8$^+$ T cells were defined as PD1$^+$/TCF1$^+$ cells within the CD8$^+$ pool

αPD1-HT18$_{cis}$ augments T cell activity

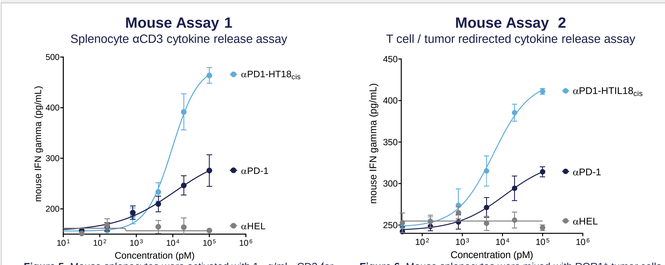

Figure 5. Mouse splenocytes were activated with 1 μg/ml αCD3 for 48hr, washed and re-activated with 100 ng/ml αCD3 in the presence of indicated test articles.
mIFNγ in the supernatant was measured after 48hr

Figure 6. Mouse splenocytes were mixed with ROR1$^+$ tumor cells at an E:T of 30:1 in the presence of the 20% maximum activity of a ROR1 x mCD3 bispecific antibody and the indicated test articles.
mIFNγ in the supernatant was measured after 72hr

αPD1-HT18$_{cis}$ induced 100% cure rate in immunogenic colon carcinoma model

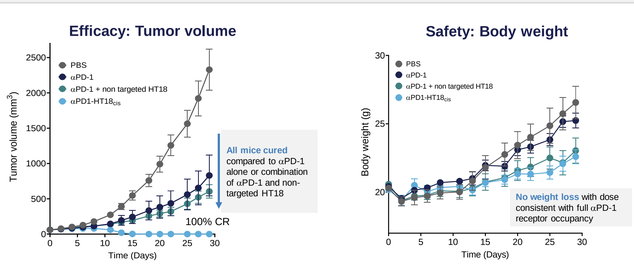

Figure 11. BALB/c mice were injected subcutaneously with CT26.C. When mean tumor volume ranged between 75-100 mm^3, mice were randomized into groups of 5 and treated with PBS, αPD-1, αPD1-HT18$_{cis}$, or the combination of αPD-1 + isotype control-HT18 on Days 0,4 and 8 at 15mg/kg

TAM and inflammatory ratios in aggressive melanoma tumor model

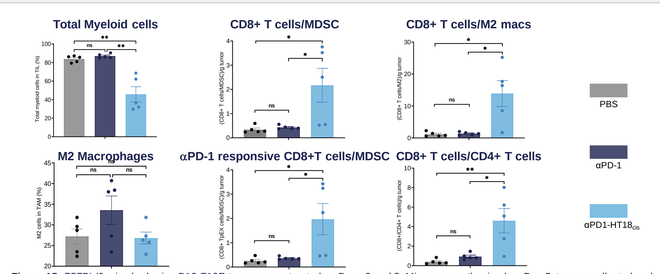

Figure 15. C57BL/6 mice harboring B16-F10R tumors were treated on Days 0 and 3. Mice were euthanized on Day 5, tumors collected and dispersed into singe cell suspensions for flow cytometric assessment of TIL (CD45$^+$ cells). All myeloid subsets were gated on CD11b.
Within that pool, MDSC were defined as Ly6G$^+$/Ly6C^{mid}+Ly6G-/Ly6C^{high} and M2 macrophages as F480$^+$/CD206$^+$