Poster nr. 940

SOT201, a novel cis-acting PD-1/IL-15 mutein-based immunocytokine that reinvigorates anti-tumor immunity qualitatively superior to PD-1/IL2v-based IL-2/15Rβγ agonism

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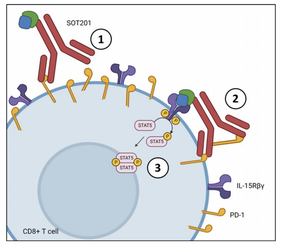
Introduction

Background: SOT201 is a novel cis-acting immunocytokine consisting of a humanized, Fc-silenced anti-PD-1 monoclonal antibody (mAb) fused to an attenuated human IL-15 and the IL-15Rα sushi+ domain. SOT201 spatio-temporally reinvigorates PD-1$^+$ CD8$^+$ tumor infiltrating lymphocytes (TILs) via cis activation and concomitantly activates innate immunity by IL-15-mediated signaling via the IL-2/IL-15Rβγ.
Methods: Human PBMC and cell lines were used to evaluate cis/trans activity of SOT201. Anti-PD-1 mAb responsive (MC38, CT26) and resistant (B16F10, CT26 STK11 KO) mouse tumor models were used to determine the anti-cancer efficacy of SOT201. The immune cells responsible for anti-tumor efficacy were analyzed via scRNAseq and flow cytometry. The expansion of tumor antigen-specific CD8$^+$ T cells, adoptively transferred ovalbumin-primed OT-I CD8$^+$ T cells by SOT201 together with memory CD8$^+$ T cell generation *in vivo* was determined by flow cytometry.
Results: SOT201 delivers attenuated IL-15 to PD1$^+$ T cells via cis presentation, reinvigorates exhausted human T cells and induces a higher IFN-γ production than pembrolizumab *in vitro*. Mouse surrogate mSOT201 administered as a single dose exhibits strong anti-tumor efficacy with several complete responses in all tested mouse tumor models. In MC38 colorectal tumors the treatment with mSOT201 expands predominantly exhausted T cells (Tex) with a better effector profile than anti-PD-1 mAb or the IL-15 mutein bearing immunocytokine lacking PD-1 targeting (hPD1-mSOT201). Importantly, mSOT201 reactivates effector Tex more effectively resulting in higher cytotoxicity, lower exhaustion and lower immune checkpoint transcriptional signatures in comparison to mPD1-IL2v, a 50fold more active PD1-targeted immunocytokine signaling via the same IL-2/15Rβ. This correlates with a higher rate of complete responses and relative number of tumor antigen-specific CD8$^+$ T cells in the MC38 tumor model induced by mSOT201 and compared to mPD1-IL2v. Similarly, mSOT201 stimulated stronger expansion of adoptively transferred ovalbumin-primed CD8$^+$ T cells than mPD1-IL2v, concomitantly limiting the peripheral CD8$^+$ T cell sink which led to the development of memory CD8$^+$ T cells *in vivo*.
Conclusions: SOT201 represents a promising therapeutic candidate targeting preferentially PD-1$^+$ TILs with a balanced cytokine activity for reviving Tex in tumors. SOT201 is currently being evaluated in the Phase I clinical study VICTORIA-01 (NCT06163391) in advanced metastatic cancer patients.

SOT201

Delivering attenuated IL-15Rα/IL-15 to PD-1$^+$ CD8$^+$ TILs via cis presentation



1. High copy number of PD-1 promotes the binding of a high number of SOT201 molecules to CD8$^+$ TILs via its PD-1 binding activity
2. Interaction of PD-1 tethered SOT201 with multiple IL-15Rβγ on TILs results in strong signaling and stimulation
3. Strong stimulation via IL-15Rβγ results in strong anti-tumor efficacy

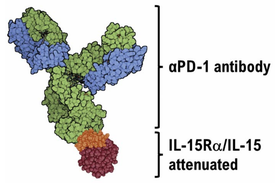
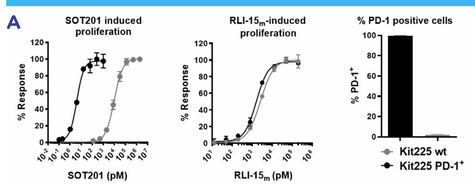


Figure 1: Cis-acting SOT201 blocks PD-1/PD-L1, enhances IFN-γ production and reinvigorates exhausted human T cells in vitro.



B

Cell population	EC50 [nM] PD-1$^+$	EC50 [nM] PD-1$^-$	Fold increase PD-1$^+$ EC50 to PD-1$^-$
Ki67$^+$ NK cells	4.1	2.7	0.6
Ki67$^+$ CD8$^+$ T cells	9.8	34.9	3.6
Memory Ki67$^+$ CD8$^+$ T cells	0.9	17.4	19.3

Figure 1. A) Cis-acting SOT201-induces proliferation of Kit225 cell line expressing PD-1$^+$ and IL-2/15Rβγ (kit225 PD-1$^+$) with ~ 3000x higher potency than of kit225 wt (expressing IL-2/15Rβγ only). The potency of naked mutein RLI-15$_m$ is similar on both Kit225 PD-1$^+$ and wt. **B)** SOT201-induced proliferation of PD-1$^+$ and PD-1$^-$ immune cells. Mean ± SEM from 4-8 donors. **C)** SOT201 blocks PD-1/PD-L1 interactions *in vitro* with IC50 of 1nM. **D)** SOT201 enhances IFN-γ production at 1 nM in MLR after 5 days *in vitro*. Mean ± SEM of 12 donor pairs. E) SOT201 reinvigorates partially exhausted human T cells *in vitro*. Means ± SEM of 3 donors (****P < 0.0001, ***P < 0.001 **P < 0.01). SEB – staphylococcus aureus enterotoxin B.

Figure 2: Single administration of SOT201 and mSOT201 induces anti-tumor efficacy in anti-PD-1 sensitive and resistant mouse tumor models

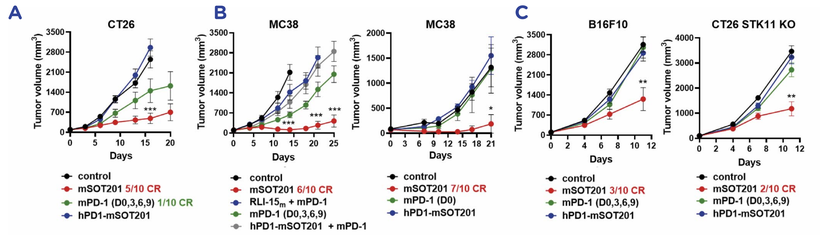


Figure 2: SOT201 induces anti-tumor efficacy in anti-PD-1 responsive models **A)** CT26 (10 mg/kg all compounds) and **B)** MC38 (5 mg/kg mSOT201 or equimolar to the other compounds including naked mutein RLI-15$_m$) and **C)** in anti-PD-1 resistant mouse tumor models B16F10 or CT26 STK11 KO (10 mg/kg all compounds). Representative experiments, n = 8-10 animals/group. If not stated otherwise, single dose on Day 0 (~100 mm^3).

Figure 4: mSOT201 elicits qualitatively superior anti-tumor efficacy and a better effector CD8$^+$ Tex than mPD1-IL2v

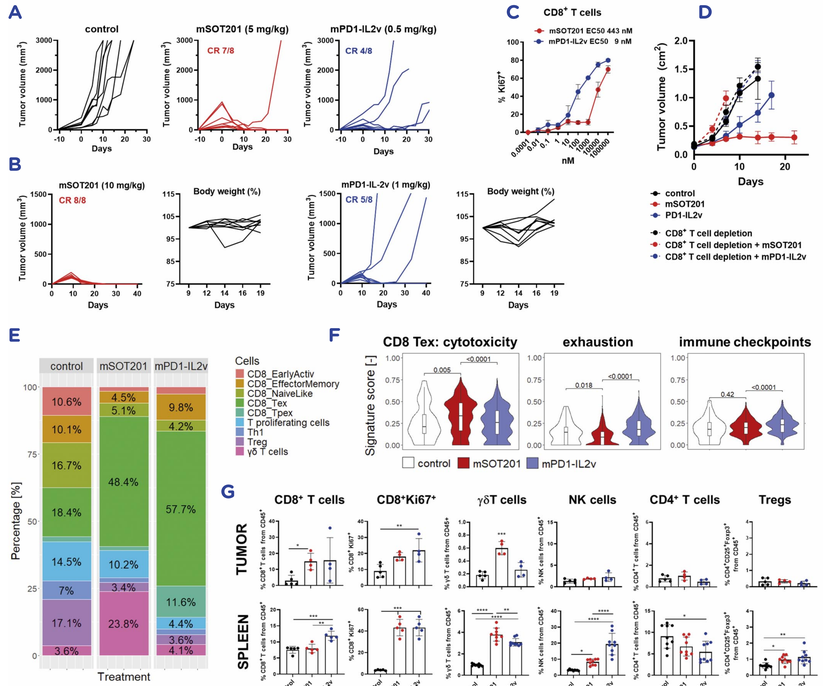


Figure 4: A) Anti-tumor efficacy of SOT201 (5 mg/kg i.v) and mPD1-IL2v (0.5 mg/kg i.v) administered as single doses on Day 0 (~100 mm^3) in MC38 mouse tumor model. **B)** Higher single dose administration of mPD1-IL2v does not compensate for efficacy due to an increasing toxicity in MC38. **C)** mSOT201 and mPD1-IL2v-induced splenic CD8$^+$ T cell proliferation after 5 days *in vitro* with EC50. **D)** Anti-tumor efficacy in MC38 mouse model ± CD8$^+$ T cell depletion. **E)** Proportion of T cell populations and γδ T cells in MC38 tumors 5 days after a single dose treatments. ProjecTILs was used for the cell identification and clustering *(Andreatta et al., 2021 Nat Commun.)*. **F)** Violin gene signatures in CD8$^+$ Tex: cytotoxicity (Gzma, Gzmc, Gzmf, Prf1, Klgr1, Fasl), exhaustion (Tox, Nfatc1, Nr4a2, Irf4, Tcf7, Batf) and immune checkpoints (Pdcd1, Havrc2, Lag3, Tigit, CD38, Cd101, CD39). **G)** Flow cytometry phenotyping of MC38 tumors and spleen day 5 post-treatments with mSOT201 (5 mg/kg, i.v) and mPD1-IL2v (0.5 mg/kg, i.v), both on Day 0 (~100 mm^3). Means ± SEM.

Figure 3: mSOT201 but not mPD-1 or hPD1-mSOT201 expands effector CD8$^+$ Tex and γδ T cells in MC38 tumors

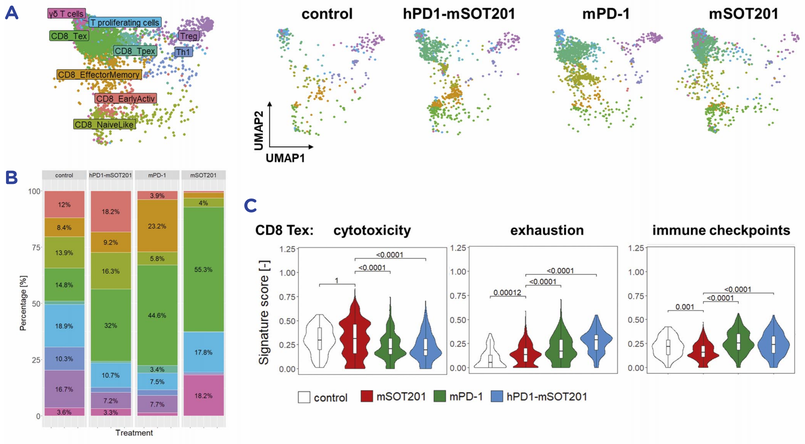


Figure 3: A) UMAP plots of various T cell populations. **B)** Proportion of T cell populations and γδ T cells in MC38 tumors 5 days after a single dose treatment with mSOT201, mPD-1 or hPD1-mSOT201 (5 mg/kg, i.v). ProjecTILs was used for the cell identification and clustering (*Andreatta et al., 2021 Nat Commun.*). **C)** Violin gene signatures in CD8$^+$ Tex: cytotoxicity (Gzma, Gzmc, Gzmf, Prf1, Klgr1, Fasl), exhaustion (Tox, Nfatc1, Nr4a2, Irf4, Tcf7, Batf) and immune checkpoints (Pdcd1, Havrc2, Lag3, Tigit, CD38, Cd101, CD39).

Figure 5: mSOT201 expanded OVA-primed CD8$^+$ T cells to become memory T cells which was limited upon mPD1-IL2v treatment due to the high level of peripheral sink on OVA-irrelevant CD8$^+$ T cells

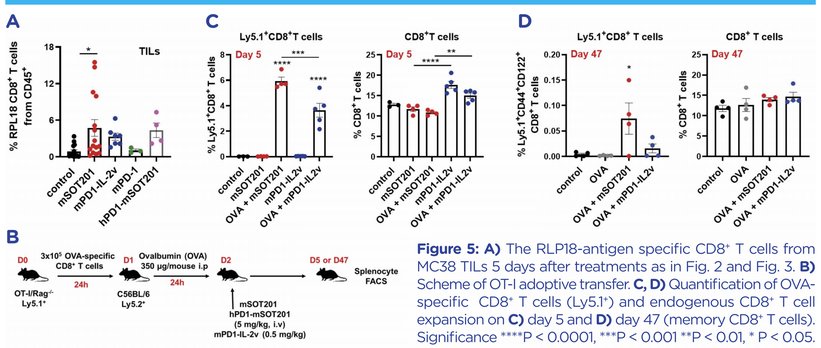


Figure 5: A) The RLP18-antigen specific CD8$^+$ T cells from MC38 TILs 5 days after treatments as in Fig. 2 and Fig. 3. **B)** Scheme of OT-I adoptive transfer. **C, D)** Quantification of OVA-specific CD8$^+$ T cells (Ly5.1$^+$) and endogenous CD8$^+$ T cell expansion on **C)** day 5 and **D)** day 47 (memory CD8$^+$ T cells). Significance ****P < 0.0001, ***P < 0.001 **P < 0.01, * P < 0.05.

Conclusion

- SOT201 is a PD-1-targeted and cis-acting attenuated IL-15 agonist that preferentially activates PD-1$^+$CD8$^+$ T cells thereby inducing a superior anti-tumor efficacy and reinvigorating exhausted CD8$^+$ T cells in PD-1 sensitive and resistant tumor models.
- mSOT201 induces a superior reinvigoration of tumoral CD8$^+$ Tex cells with a high cytotoxicity and low exhaustion/ immune checkpoint transcriptional signature compared to mPD1-IL2v, which correlated with a lower peripheral sink and more durable outcome of the treatment efficacy.
- SOT201 is currently being evaluated in a Phase I clinical study in metastatic advanced cancer patients that are considered responsive to checkpoint inhibition blockade as well as patients resistant/refractory to PD-1/PD-L1 therapies.

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