SOT201 is a novel targeted IL-15Rβγ agonist to alleviate PD-1-mediated immune cell suppression and potentiate anti-tumor efficacy

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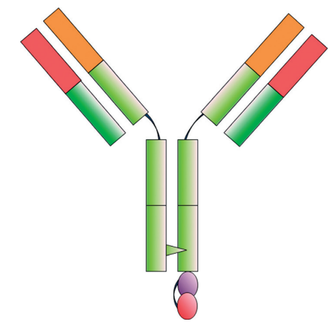
Introduction

SOT201 is a novel immunocytokine consisting of a monoclonal humanized, Fc silenced antibody against PD-1 fused to a covalent RLI-15 complex of a human IL-15 mutein linked to the high-affinity binding site of the IL-15Rα, the sushi+ domain. SOT201 is developed for immunotherapeutic treatment of various types of cancers. The activity of SOT201 is based on spatiotemporal reinvigorating of anti-tumor immune responses by disrupting co-inhibitory T-cell signaling by blocking PD-1 and synergistically activating adaptive as well as innate immunity by IL-15-mediated signaling via the IL-2/IL-15βγ receptor on T cells, NK, NKT, and γδ T cells. SOT201 showed a superior potentiation of T cell stimulation over pembrolizumab in mixed lymphocyte reaction *in vitro*. Studies in cynomolgus monkeys showed that decreased affinity of IL-15 mutein in SOT201 for its IL-15Rβγ is well optimized to ensure favorable pharmacokinetic properties while inducing strong CD8⁺ T cell and NK cell activation and expansion. Synergistic action on CD8⁺ T cell activation of both anti-PD-1 and RLI-15 moieties was confirmed using mouse surrogate SOT201 molecules *in vivo*. Strong anti-tumor efficacy after SOT201 treatment was achieved in a human PD-1 transgenic mouse model implanted with the MC38-hPD-L1 cell line. These data represent a promising therapeutic candidate molecule leveraging the synergistic concerted action of anti-PD-1 blockage and simultaneous immune cell activation directed preferentially to the high PD-1⁺ T cell tumor environment. The therapeutic potential of SOT201 is currently being prepared for evaluation in a Phase I clinical study in metastatic advanced cancer patients as well as for PD-1 resistant/refractory patients as our clinical stage IL-2/IL-15Rβγ agonist SOT101 already showed a clinical benefit in these patients in its ongoing Phase I study.

SOT201



Efficient PD-1 blocking by anti-PD-1 humanized IgG4 monoclonal antibody
- targeting specifically PD-1⁺ CD8⁺ T cells

Fc part with a minimal effector activity
- knob-in-hole substitutions for heavy chain heterodimerization

RLI-15 mutein
- IL-15Rα sushi+ domain-linker-IL-15 mutein
- reduced binding affinity to IL-2/15Rβγ (increased half-life)

Figure 1

anti-PD-1 antibody and RLI-15 mutein moieties in SOT201 synergize in activation of CD8⁺ T cells

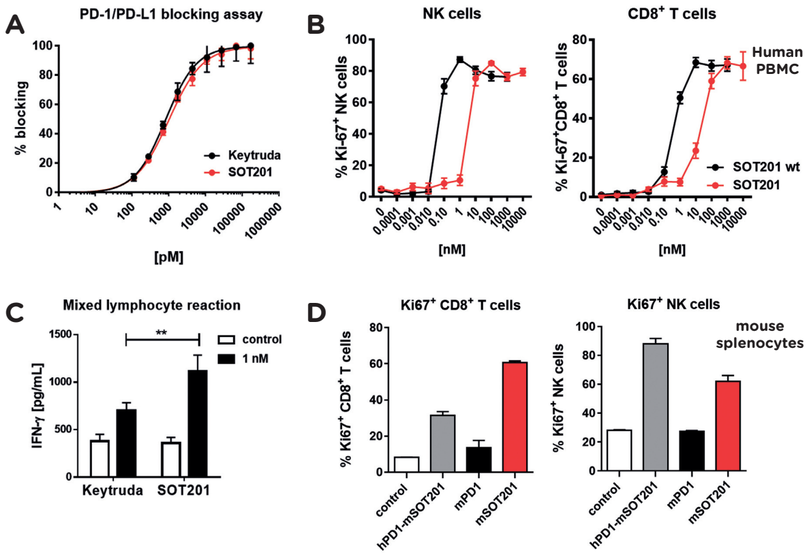


Figure 1. **(A)** SOT201 effectively blocks PD-1/PD-L1 interactions similarly to Keytruda **(B)** SOT201 activates proliferation of NK and CD8⁺ T cells at higher EC50 concentration in comparison to non-mutated wt immunocytokine molecule (SOT201 wt). Cell proliferation (Ki67) was detected by flow cytometry after 7 days *in vitro* **(C)** SOT201 potentiates IFN-γ production in a mixed lymphocyte reaction of human mismatched paired donor PBMCs after 5 days *in vitro*. **(D)** anti-PD-1 antibody and RLI-15 mutein moieties in mouse SOT201 surrogate (mSOT201) synergize in activation of CD8⁺ T cells. Murine monoclonal anti-PD-1 antibody (mPD1) and human targeted anti-PD1 mouse IgG1-RLI-15 mutein immunocytokine (hPD1-mSOT201) were used as single activity controls. Cell proliferation (Ki67) was detected in spleen by flow cytometry 5 days after IV injection of compounds at equimolar amount to 5 mg/kg of mSOT201 in healthy C57BL/6 mice (n=2/group).

Figure 2

SOT201 induces a strong anti-tumor efficacy in syngeneic MC38 mouse tumor model and hPD1-transgenic mouse bearing MC38-hPD-L1 tumors

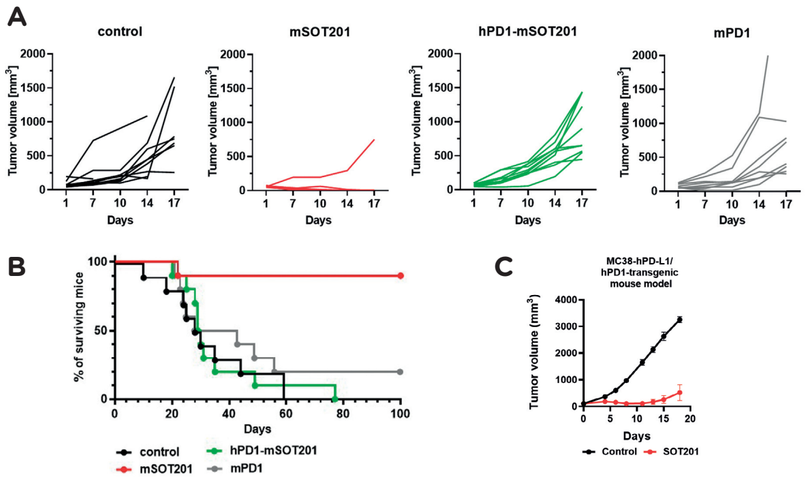


Figure 2. (A) mSOT201 (mouse SOT201 surrogate) induces tumor regression (9/10) in MC38 mouse model after a single IV administration in comparison to control, murine monoclonal anti-PD-1 antibody (mPD1) and human anti-PD1 mouse IgG1-RLI-15 mutein immunocytokine (hPD1-mSOT201). Compounds were injected at equimolar amount to mSOT201 (5 mg/kg) intravenously on day 1 (randomization day, tumor volumes 80-100 mm³) in syngeneic MC38 tumor bearing C57BL/6 mice. **(B)** Survival of MC38 tumor bearing mice up to 100 days post treatment **(C)** SOT201 induces a strong anti-tumor efficacy in comparison to control in hPD1-transgenic mouse implanted with MC38-hPD-L1 expressing cell line. SOT201 was injected IV at 20 mg/kg on day 1 (randomization day, tumor volumes 100 mm³) (n=10/group).

Figure 3

mSOT201 induces pathways and genes connected to anti-tumor immunity in MC38 tumors and activates immune cells in spleen and lymph nodes

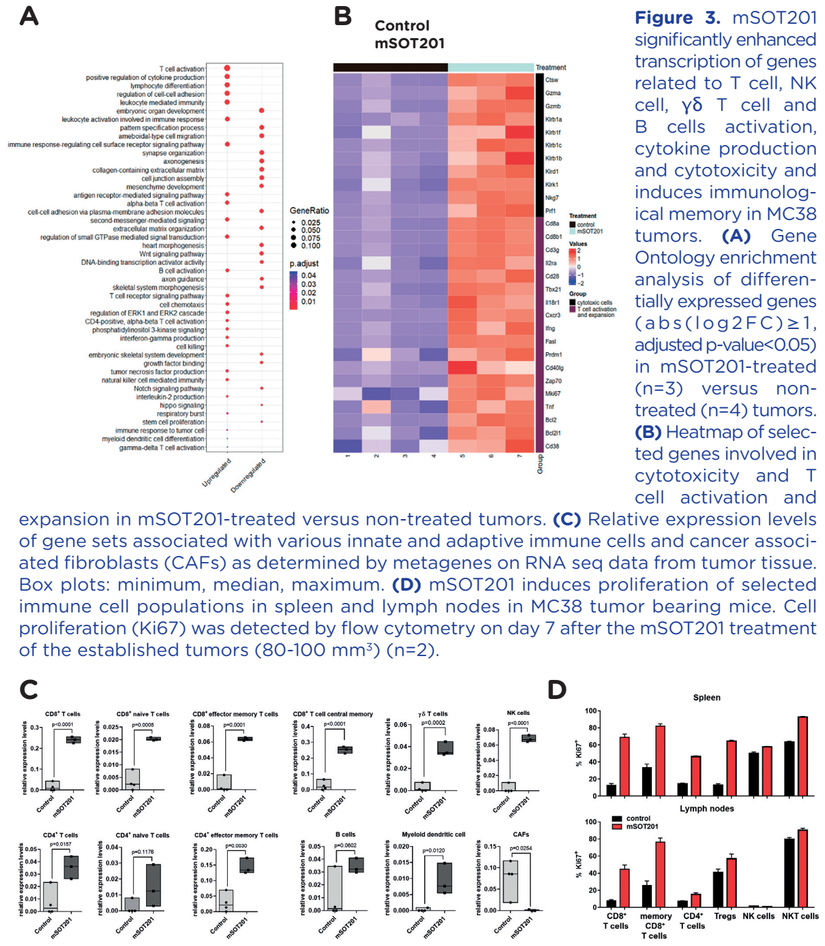


Figure 3. mSOT201 significantly enhanced transcription of genes related to T cell, NK cell, γδ T cell and B cells activation, cytokine production and cytotoxicity and induces immunological memory in MC38 tumors. **(A)** Gene Ontology enrichment analysis of differentially expressed genes (abs(log2FC)≥1, adjusted p-value<0.05) in mSOT201-treated (n=3) versus non-treated (n=4) tumors. **(B)** Heatmap of selected genes involved in cytotoxicity and T cell activation and expansion in mSOT201-treated versus non-treated tumors. **(C)** Relative expression levels of gene sets associated with various innate and adaptive immune cells and cancer associated fibroblasts (CAFs) as determined by metagenes on RNA seq data from tumor tissue. Box plots: minimum, median, maximum. **(D)** mSOT201 induces proliferation of selected immune cell populations in spleen and lymph nodes in MC38 tumor bearing mice. Cell proliferation (Ki67) was detected by flow cytometry on day 7 after the mSOT201 treatment of the established tumors (80-100 mm³) (n=2).

Figure 4

mSOT201 induces a higher tumor regression rate than mPD1-IL2Rβγ agonist in MC38 tumor mouse model

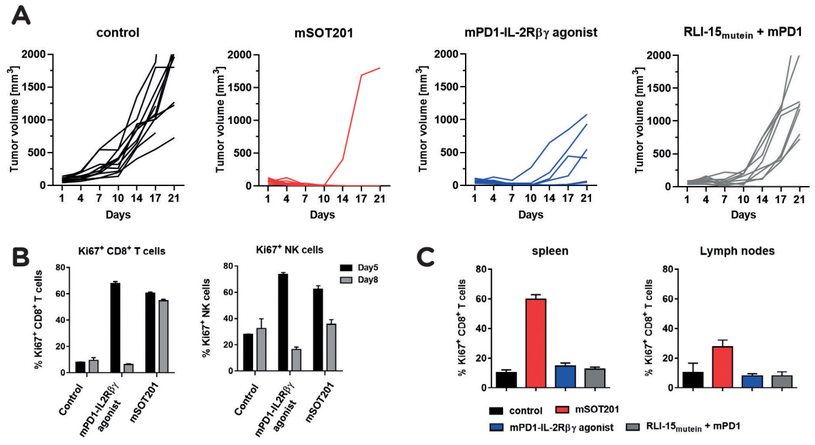
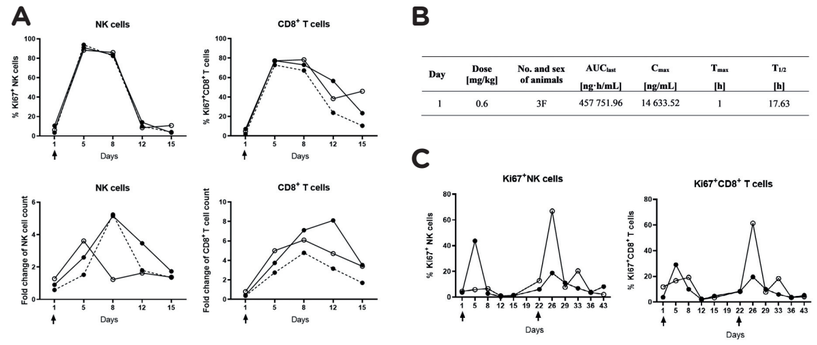


Figure 4. (A) mSOT201 (mouse SOT201 surrogate) induces tumor regression (9/10) in MC38 mouse model after a single IV administration in comparison to control, murine monoclonal anti-PD-1 antibody (mPD1) (0/10), mPD1-IL-2Rβγ agonist (mPD1-IL-2Rβγ agonist) with blocked CD25 binding (5/10) and naked RLI-15 mutein + murine monoclonal anti-PD1 antibody (mPD1) (0/10). **(B)** The dosing was selected to match NK and CD8⁺ T cell proliferation on day 5 between mSOT201 (5 mg/kg) and mPD1-IL-2Rβγ agonist (0.25 mg/kg) after IV administration in healthy C57BL/6 mice. Cell proliferation (Ki67) was detected by flow cytometry. mSOT201 induces activation of CD8⁺ T cells and NK cells which persisted up to day 8 in contrast to mPD1-IL-2Rβγ agonist. **(C)** mSOT201 induces proliferation of NK and CD8⁺ T cells in MC38 tumor bearing mice which persist 7 days after dosing in contrast to mPD1-IL-2Rβγ agonist and the equimolar amount of RLI-15 mutein + mPD1 combination. The treatment of MC38 tumors was at randomization day 1, tumor volumes 100 mm³ (n=10/group).

Figure 5

SOT201 induces strong NK and CD8⁺ T cell activation and displays a favorable PK profile in cynomolgus monkeys



Day	Dose [mg/kg]	No. and sex of animals	AUC_{last} [ng·h/mL]	C_{max} [ng/mL]	T_{max} [h]	$T_{1/2}$ [h]
1	0.6	3F	457 751.96	14 633.52	1	17.63

Figure 5. (A) SOT201 induces high proliferation and expansion of NK and CD8⁺ T cells in blood in cynomolgus monkeys after an IV administration. The animals were dosed at 0.6 mg/kg on day 1 and the cell proliferation (Ki67) and absolute cell numbers was evaluated at the indicated days by flow cytometry and hematology, respectively. **(B)** The table of pharmacokinetic parameters obtained in the study (A). **(C)** SOT201 induces activation of NK and CD8⁺ T cells after a repetitive IV administration in cynomolgus monkeys. SOT201 was administered at 0.3 mg/kg on day 1 and 21 and the cell proliferation (Ki67) was evaluated by flow cytometry at the indicated days. Each graph curve represents one animal.

Conclusions

- **Anti-PD-1 antibody and RLI-15 mutein moieties in SOT201 synergize in the activation of CD8⁺ T cells *in vivo* and IFN-γ production from human PBMC *in vitro*.**
- **SOT201 induces a strong anti-tumor efficacy in hPD1-transgenic mouse bearing MC38-hPD-L1 tumors and syngeneic MC38 mouse tumor model.**
- **mSOT201 induces pathways connected to anti-tumor immunity and increases expression of genes linked to effector and central memory CD8⁺ T cells in MC38 tumors. mSOT201 activates immune cell population in spleen and lymph nodes of MC38-tumor bearing mice.**
- **mSOT201 induces a higher tumor regression rate than mPD1-IL2Rβγ agonist in MC38 tumor mouse model at doses inducing similar PD activity.**
- **SOT201 induces strong NK and CD8⁺ T cell proliferation and expansion and displays a favorable PK profile in cynomolgus monkeys.**

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