Poster nr. 1087

SOT201 is a novel cis-acting immunocytokine targeting IL-15Rβγ and PD-1 to reinvigorate PD-1⁺ tumor infiltrating lymphocytes and potentiate anti-tumor efficacy

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Introduction

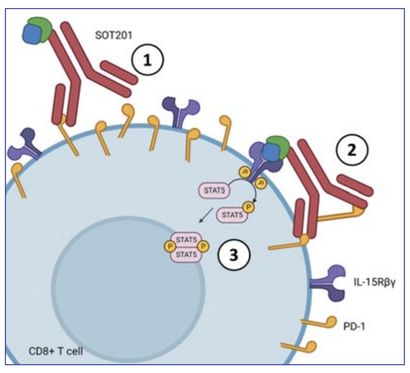
Background: SOT201 is a novel cis-acting immunocytokine consisting of a humanized, Fc-silenced monoclonal antibody against PD-1 fused to a covalent RLI-15 complex of a human attenuated IL-15 mutein linked to the high-affinity binding site of the IL-15Rα, the sushi+ domain. The activity of SOT201 is based on spatiotemporal reinvigoration of PD-1⁺ CD8⁺ tumor infiltrating lymphocytes (TILs) via cis activation and concomitant activation of innate immunity by IL-15-mediated signaling via the IL-2/IL-15βγ receptor.

Methods: Human PBMC, wt or PD-1-transfected Kit225 or Raji cells and *in vitro* exhausted human T cells were used to evaluate cis/trans activity of SOT201. Mouse surrogate SOT201-induced expansion and activation of ovalbumin-primed adoptively transferred OT-I CD8⁺ T cells *in vivo* was detected by flow cytometry. PD-1 responsive (MC38, CT26) and resistant mouse models (B16F10, CT26 STK11 KO) were used to determine the anti-tumor efficacy. The pharmacodynamics and pharmacokinetics of SOT201 were evaluated in cynomolgus monkeys.

Results: SOT201 delivers attenuated RLI-15 mutein to PD1⁺ TILs via cis presentation, stimulates *in vitro* exhausted T cells and expands antigen-specific PD-1⁺ CD8⁺ T cells *in vivo*. SOT201 treatment showed strong anti-tumor efficacy in PD-1 responsive and resistant tumor models *in vivo* and was shown to be superior to mouse PD1-IL-2Rβγ agonist. Studies in cynomolgus monkeys showed that decreased affinity of the novel IL-15 mutein in SOT201 for reduced IL-15Rβγ binding is well optimized to ensure favorable pharmacokinetic properties while potently stimulating PD-1⁺ CD8⁺ T cells and NK cells.

Conclusions: This data confirms SOT201 to be a promising therapeutic candidate molecule directed preferentially to the PD-1⁺ T cell tumor microenvironment. 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.

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

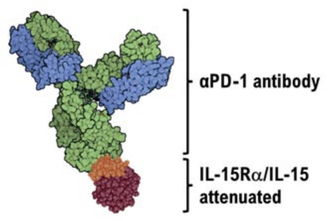
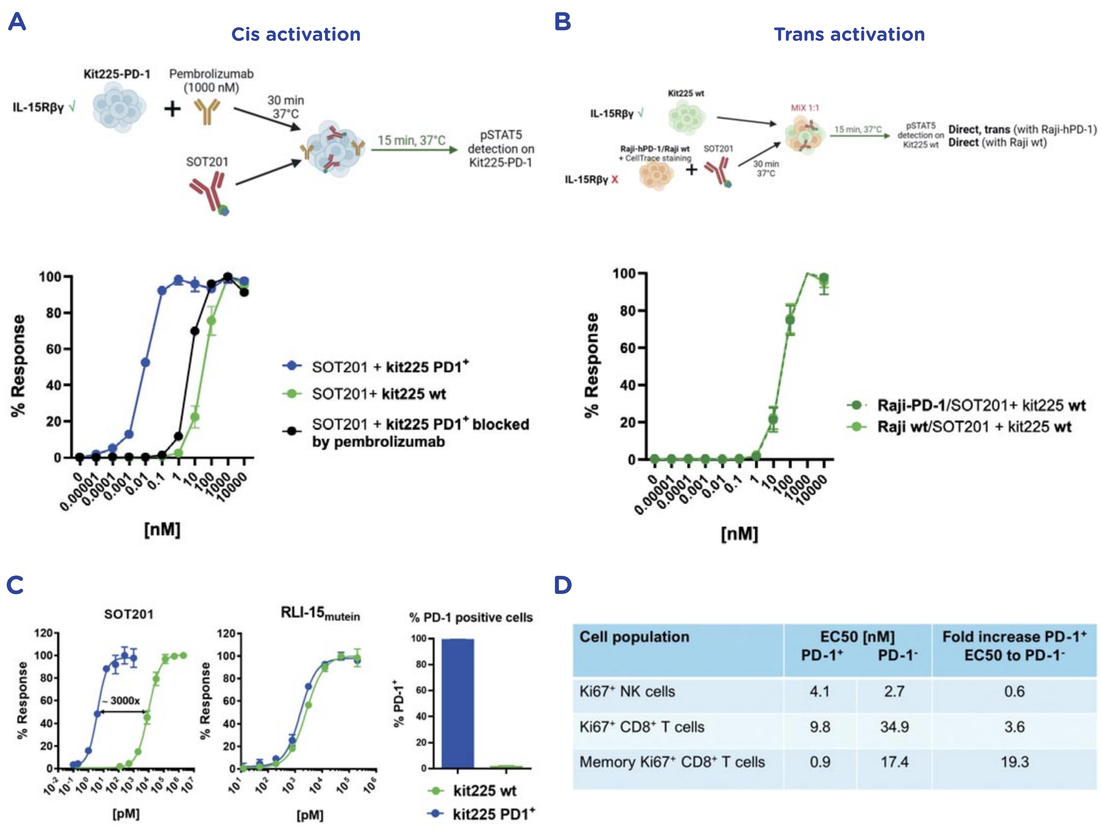


Figure 1: Cis activation is the predominant mechanism of SOT201 action



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) Kit225 cells transfected with human PD-1 (kit225 PD-1⁺) were incubated with or without pembrolizumab for 30 min. Then SOT201 was added for 15 min. Kit225 PD-1⁺ activation was detected via phosphorylation of STAT5 (pSTAT5) using flow cytometry. **B)** Kit225 wt cells were mixed with Raji-hPD-1 or Raji wt (no human PD-1 expression) and incubated with SOT201 for 15 min. Kit225 wt activation was detected via pSTAT5. Raji cells were excluded by CellTracer. The results are means ± SEM of n=2. **C)** Proliferation of kit225 PD-1⁺ or kit225 wt after 3 days with SOT201 or RLI-15 mutein only. **D)** Table of EC50 of proliferating (Ki67⁺) cell population stimulated with SOT201 for 7 days *in vitro*. The proliferation (Ki67⁺) of PD-1⁺ and PD-1⁻ CD8⁺ T cells, memory CD8⁺ T cells and NK cells was determined by flow cytometry and EC50 was calculated.

Figure 2: SOT201 blocks PD-1/PD-L1 interactions, enhances IFN-γ production and reinvigorates exhausted T cells in vitro

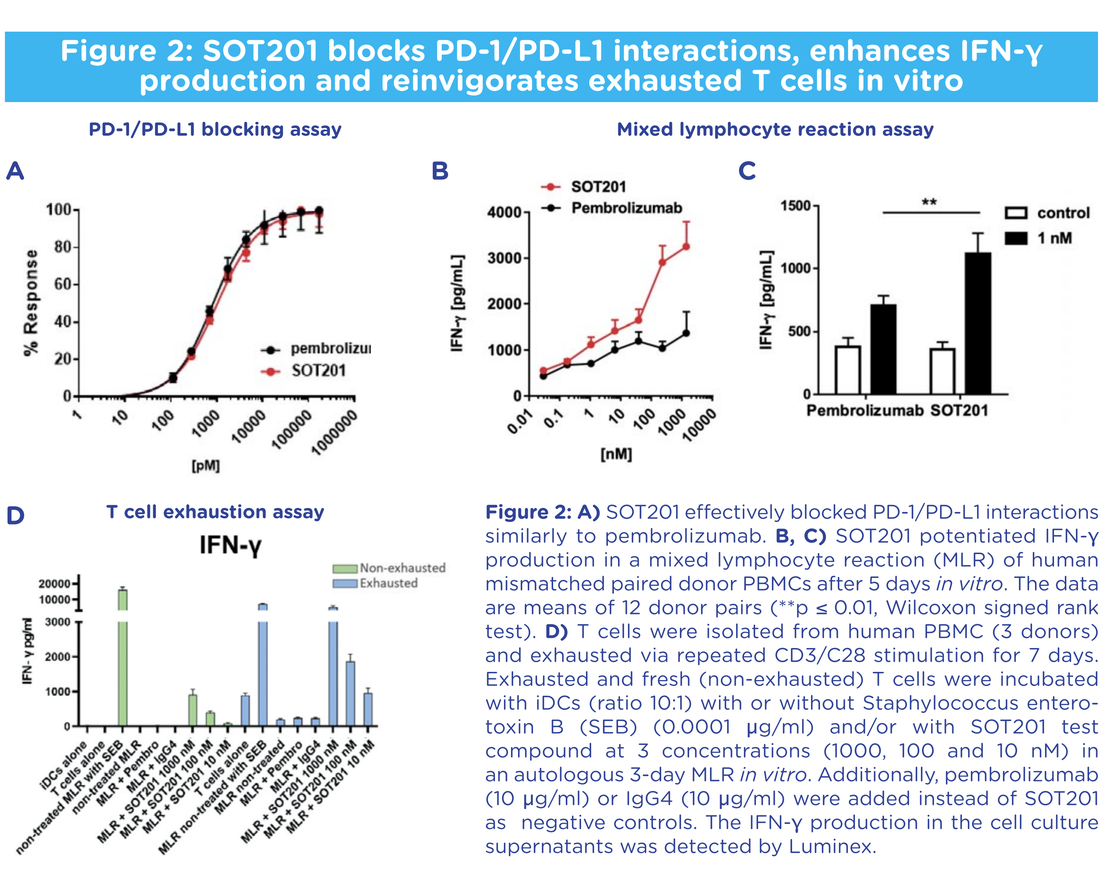


Figure 2: A) SOT201 effectively blocked PD-1/PD-L1 interactions similarly to pembrolizumab. **B, C)** SOT201 potentiated IFN-γ production in a mixed lymphocyte reaction (MLR) of human mismatched paired donor PBMCs after 5 days *in vitro*. The data are means of 12 donor pairs (**p ≤ 0.01, Wilcoxon signed rank test). **D)** T cells were isolated from human PBMC (3 donors) and exhausted via repeated CD3/C28 stimulation for 7 days. Exhausted and fresh (non-exhausted) T cells were incubated with iDCs (ratio 10:1) with or without Staphylococcus enterotoxin B (SEB) (0.0001 µg/ml) and/or with SOT201 test compound at 3 concentrations (1000, 100 and 10 nM) in an autologous 3-day MLR *in vitro*. Additionally, pembrolizumab (10 µg/ml) or IgG4 (10 µg/ml) were added instead of SOT201 as negative controls. The IFN-γ production in the cell culture supernatants was detected by Luminex.

Figure 3: Single dose of mSOT201 shows anti-tumor efficacy in PD-1 sensitive and resistant mouse models and expands antigen-specific CD8⁺ T cells in vivo

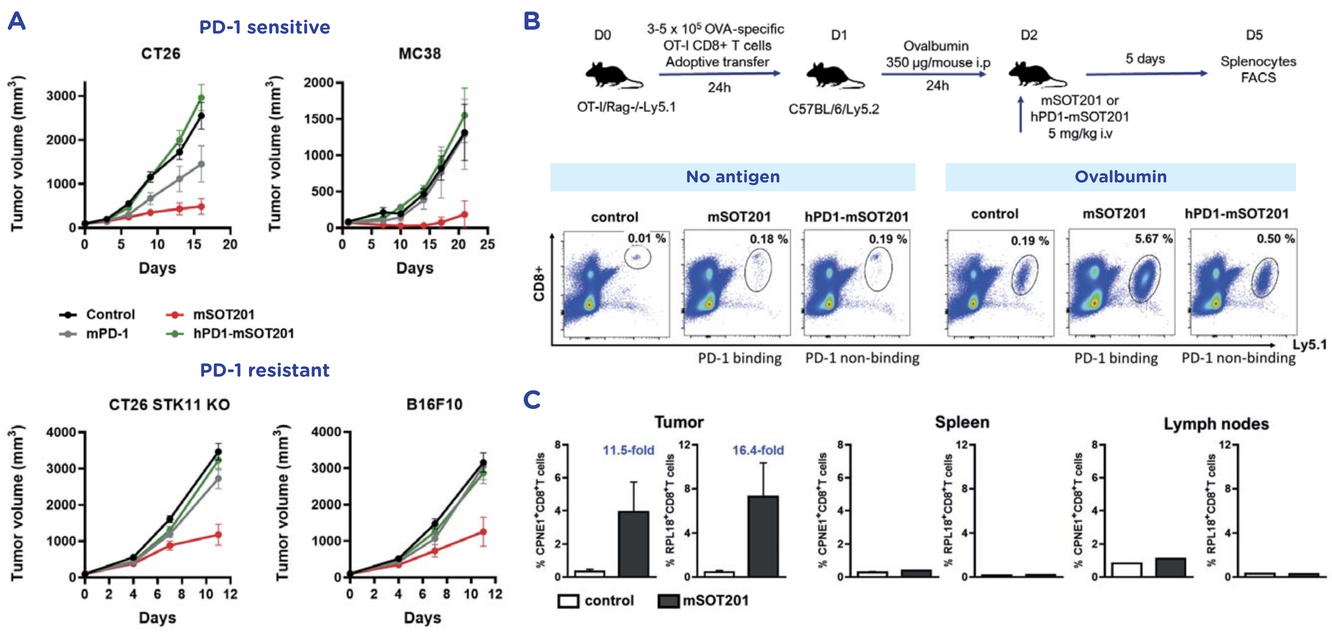


Figure 3: A) A single dose of mSOT201 induced anti-tumor efficacy in anti-PD-1 antibody treatment (PD1) sensitive models CT26 and MC38 when administered i.v. at 5 mg/kg on Day 0 (~100 mm³), mPD-1 i.p. (except CT26 Day 0,3,6,9) and hPD1-mSOT201 (human PD-1, mouse non-binding) i.v. dosed at 5 mg/kg on Day 0 (n=10/group). A single dose of mSOT201 induced anti-tumor efficacy in PD-1 resistant models CT26 STK11 KO and B16F10 when administered i.v. at 10 mg/kg on Day 0 (~100 mm³), mPD-1 i.p. dosed at 10 mg/kg, on Day 0,3,6,9 and hPD1-mSOT201 i.v. dosed at 10 mg/kg on Day 0 (n=10/group). **B)** mSOT201 expanded adoptively transferred ovalbumin-specific OT-I CD8⁺ T cells in the presence of ovalbumin in mice *in vivo*. **C)** mSOT201 expanded tumor antigen-specific CD8⁺ T cells in tumors but not in the spleen and lymph nodes in MC38 mouse model as detected by flow cytometry using dextramer staining for CPNE1⁺ and RPL18⁺CD8⁺ T cells. mSOT201 was injected i.v. at 5 mg/kg on Day 0 (~100 mm³), tissues were collected 5 days later.

Figure 4: mSOT201 shows higher anti-tumor efficacy, activation of cytotoxicity and innate immunity than mPD1-IL-2Rβγ agonist in vivo

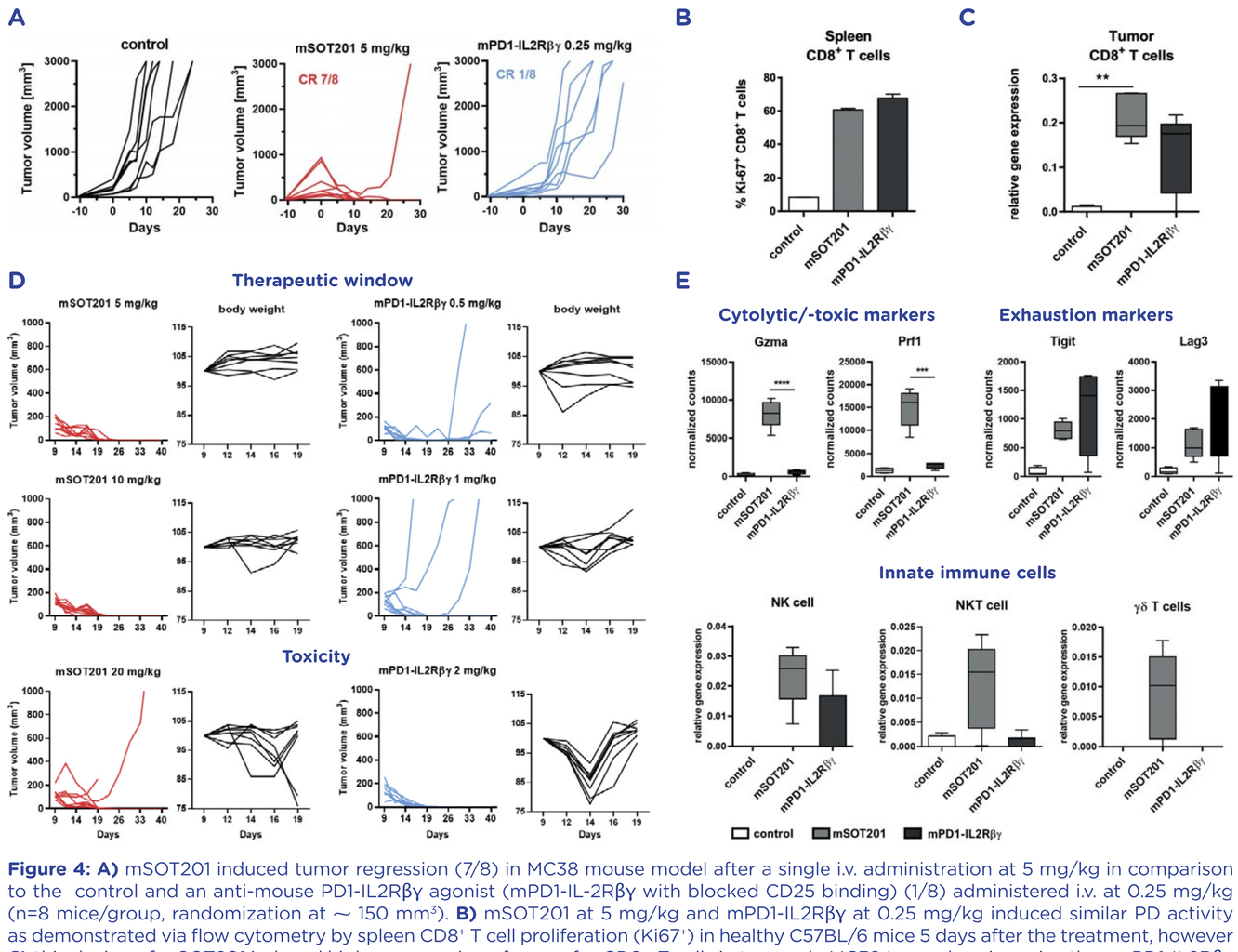


Figure 4: A) mSOT201 induced tumor regression (7/8) in MC38 mouse model after a single i.v. administration at 5 mg/kg in comparison to the control and an anti-mouse PD1-IL2Rβγ agonist (mPD1-IL-2Rβγ with blocked CD25 binding) (1/8) administered i.v. at 0.25 mg/kg (n=8 mice/group, randomization at ~ 150 mm³). **B)** mSOT201 at 5 mg/kg and mPD1-IL2Rβγ at 0.25 mg/kg induced similar PD activity as demonstrated via flow cytometry by spleen CD8⁺ T cell proliferation (Ki67⁺) in healthy C57BL/6 mice 5 days after the treatment, however **C)** this dosing of mSOT201 induced higher expression of genes for CD8+ T cells in tumors in MC38 tumor-bearing mice than mPD1-IL2Rβγ at day 7 after treatment as detected by RNAseq. **D)** Determination of the therapeutic window for mSOT201 and mPD1-IL2Rβγ agonist in MC38 mouse tumor model. **E)** RNAseq analyses of cytolytic/-toxic and exhaustion markers and genes representing the innate cell immune populations was conducted at day 7 after treatment from tumors (n = 5).

Figure 5: SOT201-mediated cis-acting mode of action confirmed in cynomolgus monkeys together with favorable pharmacokinetic profile

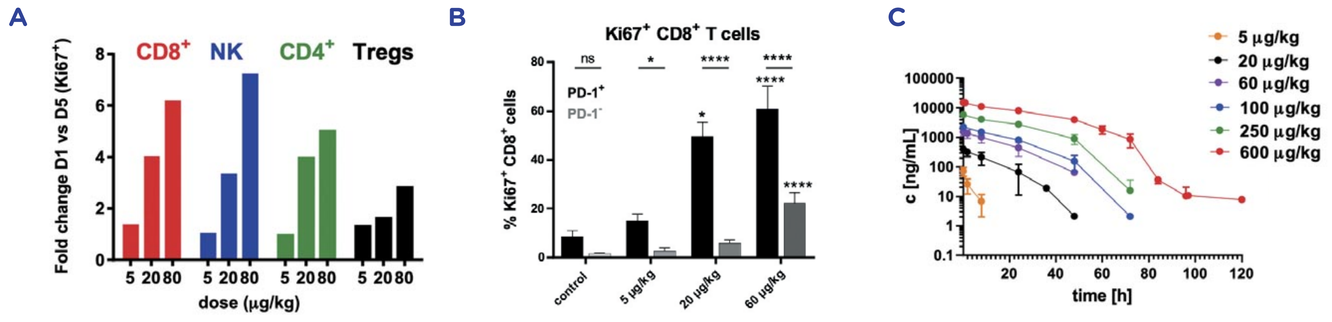


Figure 5: A) Differential activity of SOT201 on NK cells, CD8⁺ and CD4⁺ T cells, and regulatory T cells (Treg) in the cynomolgus monkeys. SOT201 was administered i.v. on Day 1 (8 animals/group). Blood was drawn 5 days later and the proliferation (Ki67⁺) of immune cell populations was detected by flow cytometry. **B)** The proliferation (Ki67⁺) of PD-1⁺ and PD-1⁻ CD8⁺ T cells after SOT201 administration in cynomolgus monkeys was determined by flow cytometry on Day 5. The data are means of 8 animals (*p ≤ 0.05 ****p ≤ 0.0001, one-way ANOVA test). **C)** Pharmacokinetic profile of SOT201 administered i.v. at the indicated doses in cynomolgus monkeys, cycle 1.

Conclusions

- SOT201 is a PD-1-targeted and cis-acting IL-15 agonist that preferentially activates PD-1⁺CD8⁺ T cells and thereby enhance the production of IFN-γ and reinvigorates exhausted T cells in tumors
- A single dose of mSOT201 shows potent anti-tumor efficacy in PD-1 sensitive and resistant mouse models and expands tumor antigen-specific CD8⁺ Tumor Infiltrated Lymphocytes *in vivo*
- mSOT201 shows superior anti-tumor efficacy, activation of cytotoxicity and superior innate immunity as compared to a mPD1-IL-2Rβγ agonist *in vivo*
- In the Cynomolgus monkey the SOT201-mediated cis-acting mode of action was confirmed and a favorable pharmacokinetic profile was observed
- SOT201 represents a well-balanced candidate molecule for preferential and selective activation of memory-type and antigen-specific PD-1-expressing T cell populations and may provide superior response rates in patients
- SOT201 is currently being prepared for evaluation 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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