A CD25-targeted pyrrolobenzodiazepine dimer-based antibody-drug conjugate shows potent anti-tumor activity in pre-clinical models of solid tumors either alone or in combination with a PD-1 inhibitor

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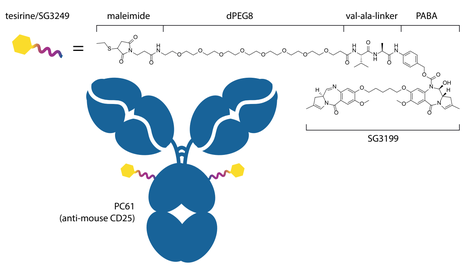
Introduction

1. Regulatory T (Treg) cells infiltrate into various types of human cancers and contribute to the immunosuppressive tumor microenvironment [1]. The intra-tumoral balance between Tregs versus Teffectors (Teffs) cells appears to impact the outcome of the immune system-mediated tumor eradication and numerous attempts are currently underway to reduce the CD25-expressing Treg cells [2].
2. Sur301 is an antibody-drug conjugate (ADC) composed of PC61, a rat monoclonal antibody directed against mouse CD25, stochastically conjugated to tesirine, a protease-cleavable, pyrrolobenzodiazepine (PBD) dimer-based payload [3], with a drug-to-antibody ratio of 2 (Figure 1).

Figure 1. Structure of sur301.



Aim

The purpose of this study was to characterize the *in vitro* and *in vivo* anti-tumor activity of sur301 in CD25-negative syngeneic colon cancer models with tumor infiltration of Tregs cells and to determine its pharmacokinetic in the mouse.

Material & methods

- Binding of PC61 to mouse recombinant CD25 (R&D Systems) was done by ELISA.
- Analysis of CD25 expression on mouse cell lines was performed by flow cytometry using PC61 and an isotype control antibody.
- Cytotoxicity of sur301, the free PBD dimer SG3199 and isotype-control ADC was determined by the CellTiterGlo® assay (Promega).
- *In vivo*, sur301 was administered intraperitoneally (i.p.) as single dose to C57BL/6 mice containing established MC38 tumors and to BALB/c mice containing established CT26 tumors (group mean tumor volumes 103-172 mm3) on Day 1. The other compounds used i.p. were B12-SG3249 (non-binding ADC), an isotype control PBD-ADC, anti-PD1 antibody (clone RMP1-14) and anti-CD8 antibody (clone 2.43).
- The Coefficient of Drug Interaction (CDI) was assessed for sub-additive, additive, or supra-additive (synergism) properties on the last day all evaluable animals remained on study, as previously described [4].
- Pharmacokinetic (PK) analysis of sur301 was performed in female C57BL/6 mice. Serum samples were collected for each time point after a single dose administration of sur301 (0.1, 0.5 or 1 mg/kg). Quantitation of total (unconjugated and conjugated) Ab was determined by ECLIA using recombinant mouse CD25 as capture and a biotin-labelled polyclonal Goat anti-Rat IgG (Mouse adsorbed) in combination with sulfoTAG streptavidin as detector.

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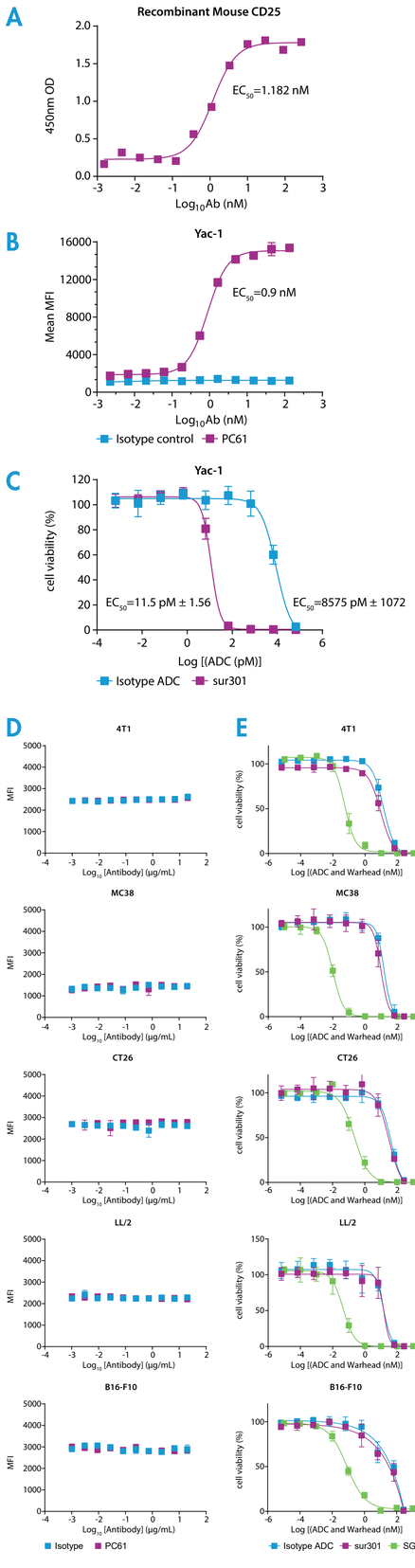
Results

Figure 2. *In vitro* characterization of sur301.



PC61 binding to:

A. mouse recombinant CD25 and

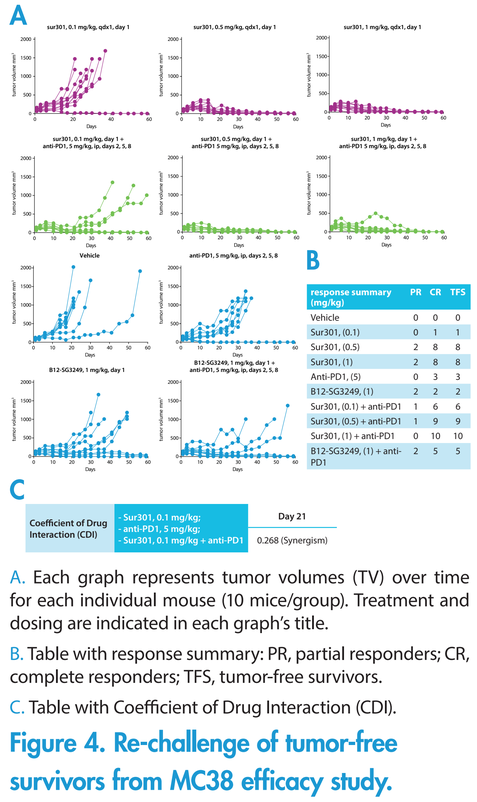
B. mouse CD25 on YAC-1 cells.

C. sur301 *in vitro* cytotoxicity in the CD25-expressing YAC-1 cell line.

D. PC61 and an isotype control antibody did not bind to a panel of CD25-negative murine solid cancer cell lines.

E. *In vitro* cytotoxicity of sur301, isotype-control ADC and the naked PBD-dimer SG3199 in a panel of CD25-negative murine solid cancer cell lines.

Figure 3. *In vivo* anti-tumor activity in the MC38 syngeneic model.



B

response summary (mg/kg)	PR	CR	TFS
Vehicle	0	0	0
Sur301, (0.1)	0	1	1
Sur301, (0.5)	2	8	8
Sur301, (1)	2	8	8
Anti-PD1, (5)	0	3	3
B12-SG3249, (1)	2	2	2
Sur301, (0.1) + anti-PD1	1	6	6
Sur301, (0.5) + anti-PD1	1	9	9
Sur301, (1) + anti-PD1	0	10	10
B12-SG3249, (1) + anti-PD1	2	5	5

C

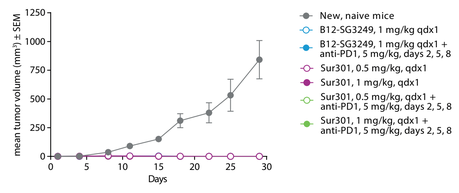
Coefficient of Drug Interaction (CDI)	- Sur301, 0.1 mg/kg; - anti-PD1, 5 mg/kg; - Sur301, 0.1 mg/kg + anti-PD1	Day 21
		0.268 (Synergism)

A. Each graph represents tumor volumes (TV) over time for each individual mouse (10 mice/group). Treatment and dosing are indicated in each graph's title.

B. Table with response summary: PR, partial responders; CR, complete responders; TFS, tumor-free survivors.

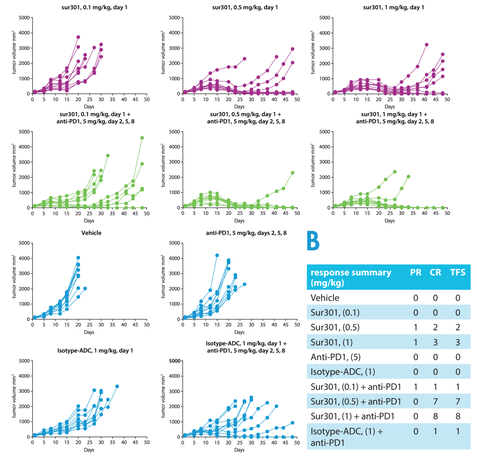
C. Table with Coefficient of Drug Interaction (CDI).

Figure 4. Re-challenge of tumor-free survivors from MC38 efficacy study.



Tumor-free survivors from the MC38 study (treated with 0.5 or 1 mg/kg, figure 3) were re-challenged with a subcutaneous (s.c.) implant of MC38 cells (contralateral to the original cell implant) and tumor formation was monitored over time. A group of naive mice (10/group) was implanted with MC38 cells and served as control.

Figure 5. *In vivo* anti-tumor activity in the CT26 syngeneic model.



B

response summary (mg/kg)	PR	CR	TFS
Vehicle	0	0	0
Sur301, (0.1)	0	0	0
Sur301, (0.5)	1	2	2
Sur301, (1)	1	3	3
Anti-PD1, (5)	0	0	0
Isotype-ADC, (1)	0	0	0
Sur301, (0.1) + anti-PD1	1	1	1
Sur301, (0.5) + anti-PD1	0	7	7
Sur301, (1) + anti-PD1	0	8	8
Isotype-ADC, (1) + anti-PD1	0	1	1

C

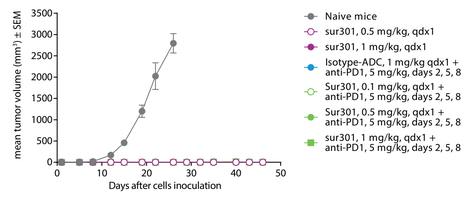
Coefficient of Drug Interaction (CDI)	- Sur301, 0.1 mg/kg; - anti-PD1, 5 mg/kg; - Sur301, 0.1 mg/kg + anti-PD1	Day 20
		0.285 (Synergism)

A. Each graph represents TV over time for each individual mouse (10 mice/group). Treatment and dosing are indicated in each graph's title. Table reports the response summary.

B. Table with response summary.

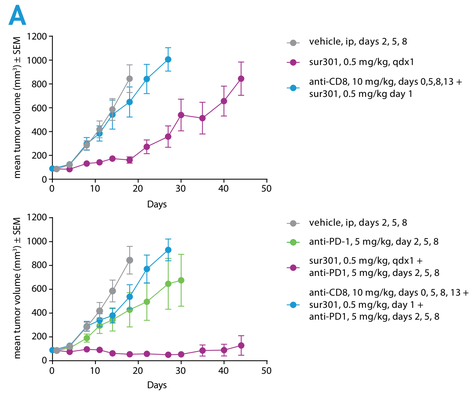
C. Table with CDI.

Figure 6. Re-challenge of tumor-free survivors from CT26 efficacy study.



Each tumor-free survivor from the CT26 study (figure 5) was re-challenged with a s.c. implant of CT26 cells (contralateral to the original cell implant) and tumor formation was monitored over time. A group of naive mice (10/group) was implanted with CT26 cells and served as control.

Figure 7. Sur301 anti-tumor activity is dependent on CD8+ T cells.



B

response summary	PR	CR	TFS
Vehicle	0	0	0
Anti-PD1	2	0	0
Sur301	0	1	1
Sur301 + anti-CD8	0	0	0
Sur301 + anti-PD1	2	5	5
Sur301 + anti-CD8 + anti-PD1	0	0	0

C

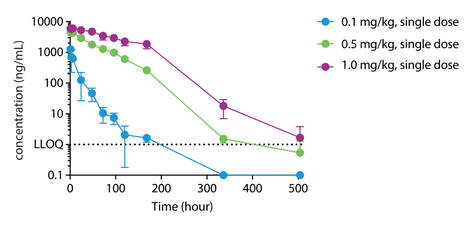
Coefficient of Drug Interaction (CDI)	- Sur301, 0.5 mg/kg; - anti-PD1, 5 mg/kg; - Sur301, 0.5 mg/kg + anti-PD1	Day 22
		0.471 (Synergism)

A. Each graph represents TV over time for each individual mouse (10 mice/group). Treatment and dosing are indicated in each graph's title.

B. Table with response summary.

C. Table with CDI.

Figure 8. sur301 PK in mice.



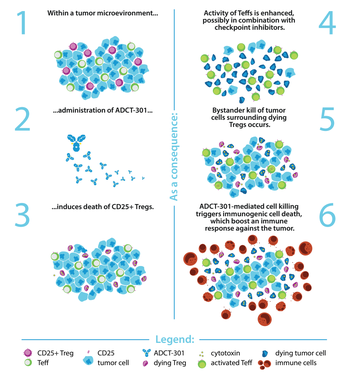
B

group	dose (mg/kg)	Tmax (h)	Cmax (ng/mL)	TAU (h)	AUC_TAU (ng*h/mL)
1	0.1	1	1254	504	14572
2	0.5	1	4495	504	271334
3	1.0	6	6044	504	751771

A. Quantification of total (conjugated and unconjugated) Ab. The graph shows the mean ± SD for the whole duration of the study (504 hours). For each dose group, 6 mice were i.v. injected and serum collected from 3 animals/group at 1, 6, 48, 96, 168 and 504 hours, and from the other 3 animals at 3, 24, 72, 120 and 336 hours.

B. Table with total Ab PK parameters according to a non-compartmental PK analysis (NCA).

Figure 9. Proposed ADCT-301 mode of action.



Conclusions

1. *In vitro*, sur301 demonstrated potent and specific cytotoxicity in a CD25-expressing mouse lymphoma cell line, while no specific cytotoxicity was observed in a panel of CD25-negative murine solid tumor derived cell lines.
2. *In vivo*, a single dose of sur301 at 0.5 or 1 mg/kg induced strong and durable anti-tumor activity against established CD25-negative solid tumors with infiltrating Treg cells (MC38 and CT26 syngeneic models).
3. Combination of a sub-optimal dose of sur301 with an anti-PD1 antibody resulted in synergistic anti-tumor activity in both MC38 and CT26 models.
4. Re-challenged animals from both efficacy studies did not develop new tumors indicating sur301 was able to induce tumor-specific protective immunity.
5. Sur301 anti-tumor activity, either alone or combined with an anti-PD1 antibody, was significantly reduced in the absence of CD8+ T cells, indicating that sur301 activity is CD8+ T cell-dependent and that overall effector T cell responses were not negatively impacted by sur301.
6. PK analysis in non-tumor bearing mice showed that sur301 has a dose dependent, target mediated drug disposition with nonlinear PK at the low dose and linear PK at higher dose levels.
7. Together, these data warrant further investigation of ADCT-301, a PBD-based ADC targeting human CD25 [5, 6], in patients with solid tumors, either alone or in combination with checkpoint inhibitors (clinical trial NCT03621982)[7].

Acknowledgements

- *In vitro* assays: Kathleen Santos (Spirogen/Medimmune; London, UK).
- *In vivo* studies: Charles River Discovery Research Services (USA).
- Mouse PK assay: ADC Therapeutics PK team (London, UK).

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