

A CD25-targeted pyrrolobenzodiazepine antibody-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

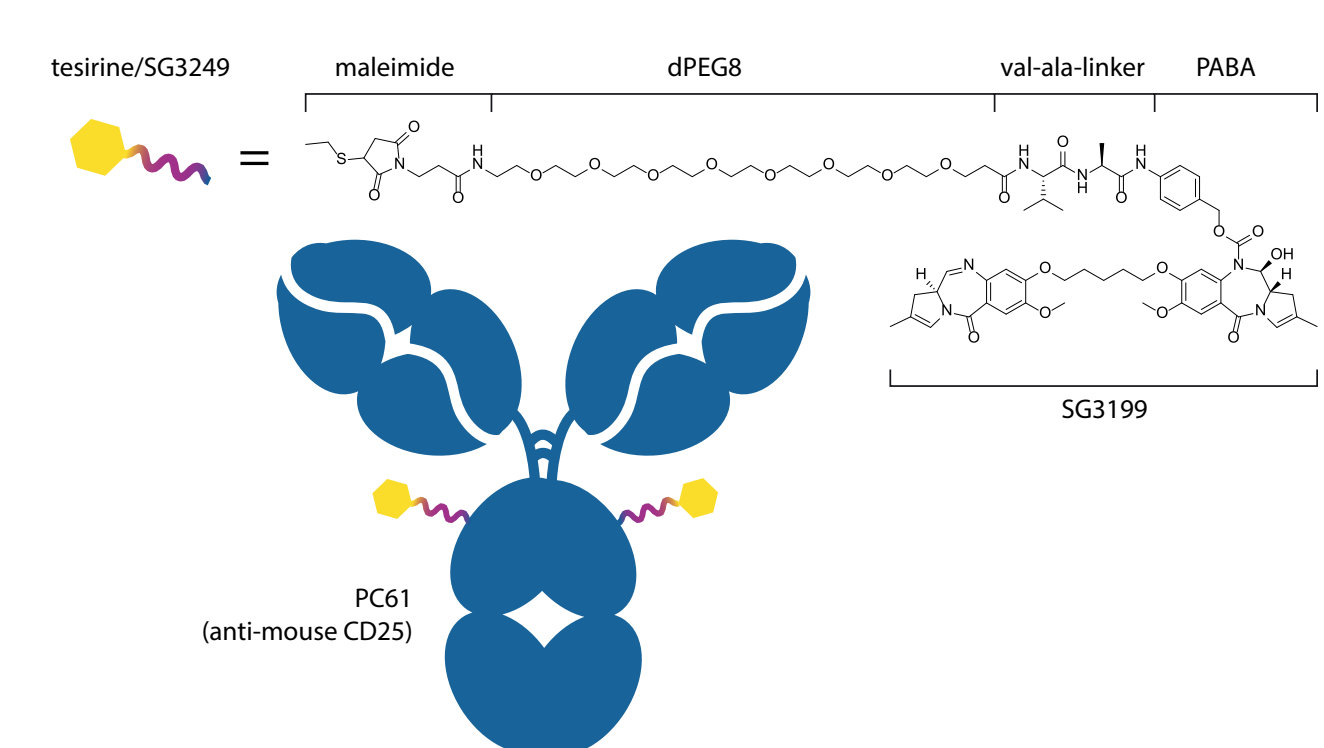
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Introduction

- 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].
- 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.

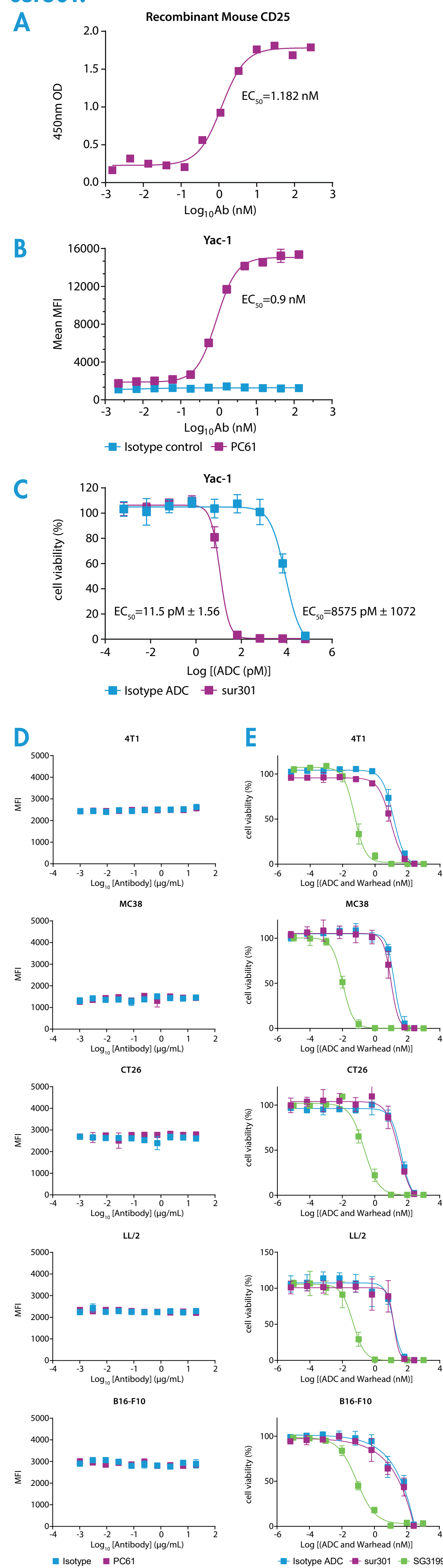
Material & methods

- 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

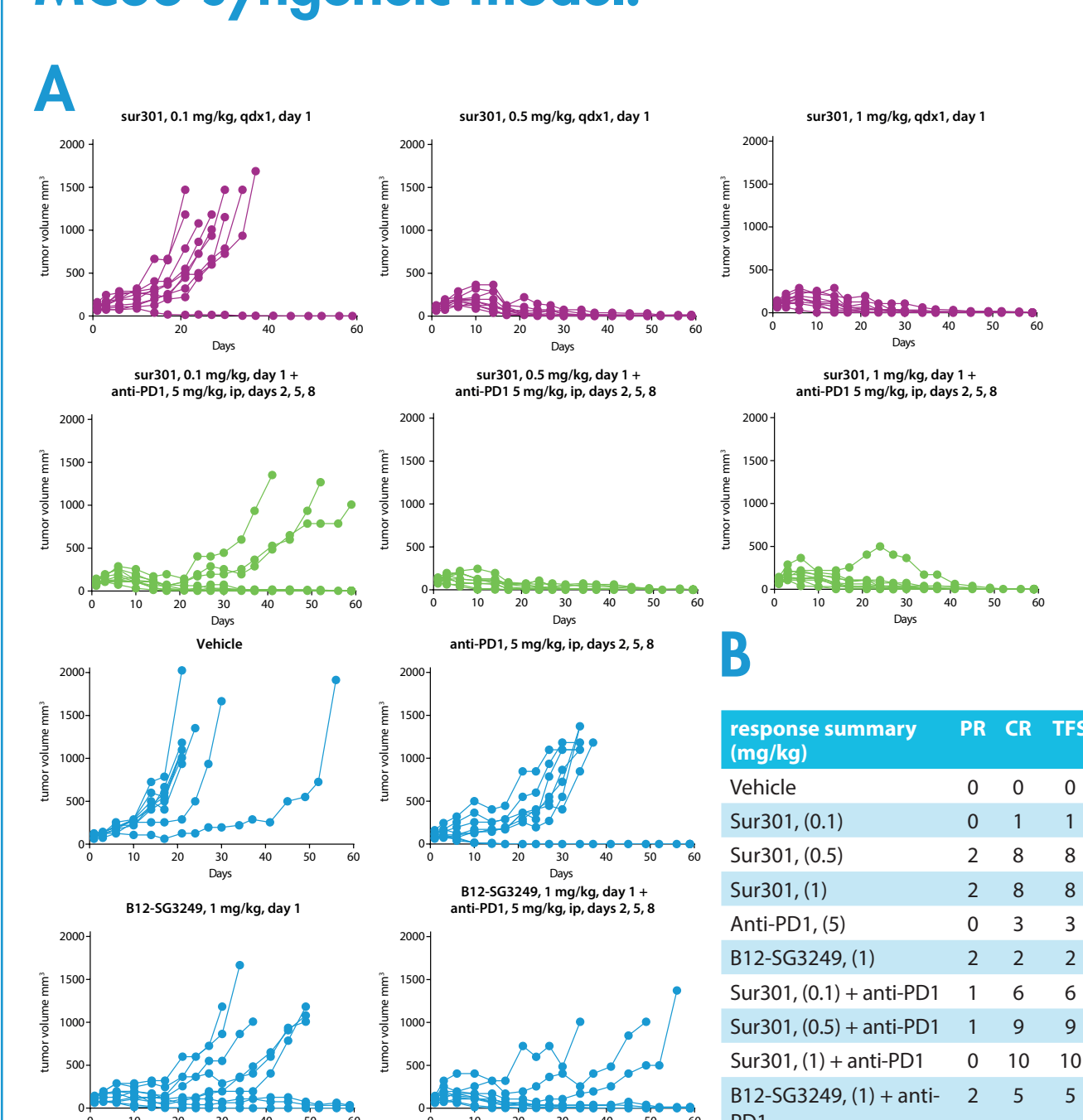
Figure 2. *In vitro* characterization of sur301.



PC61 binding to:

- mouse recombinant CD25 and
- mouse CD25 on YAC-1 cells.
- sur301 *in vitro* cytotoxicity in the CD25-expressing YAC-1 cell line.
- PC61 and an isotype control antibody did not bind to a panel of CD25-negative murine solid cancer cell lines.
- 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.

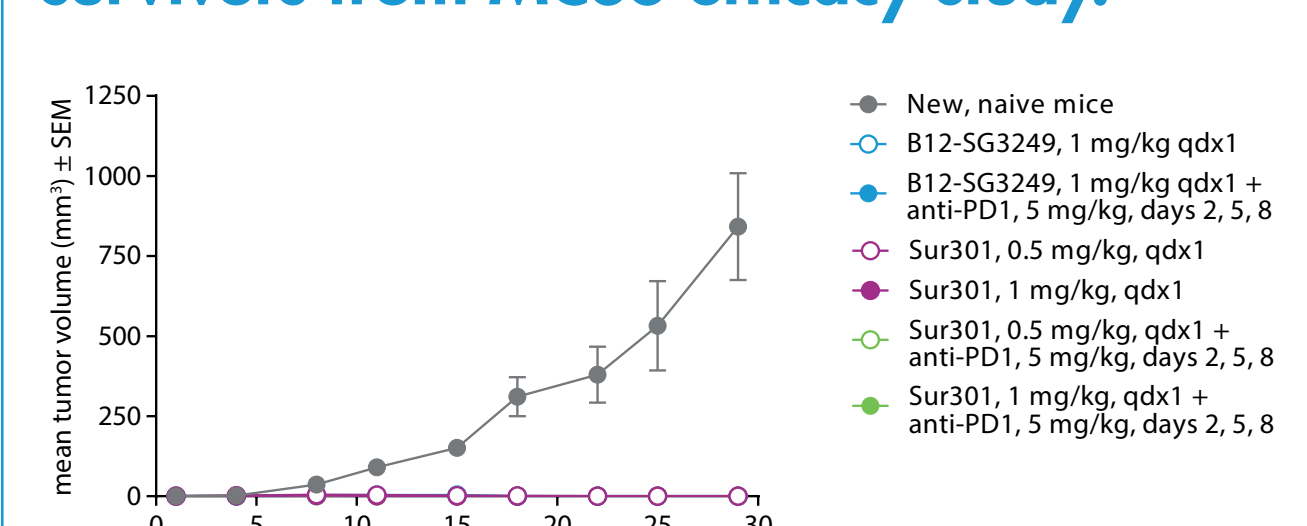


Each graph represents tumor volumes (TV) over time for each individual mouse (10 mice/group). Treatment and dosing are indicated in each graph's title.

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

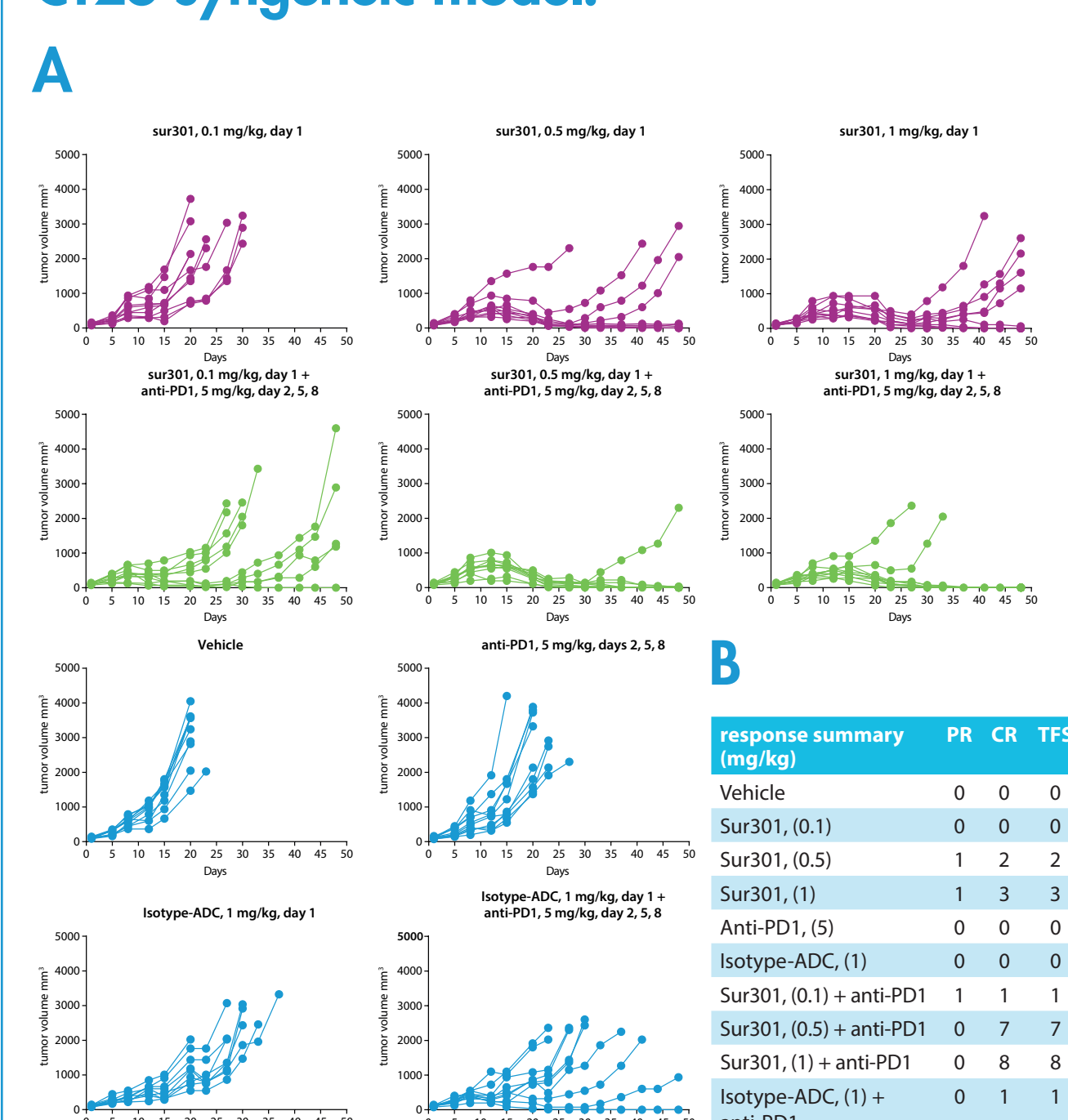
C. Table with Coefficient of Drug Interaction (CDI).

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.

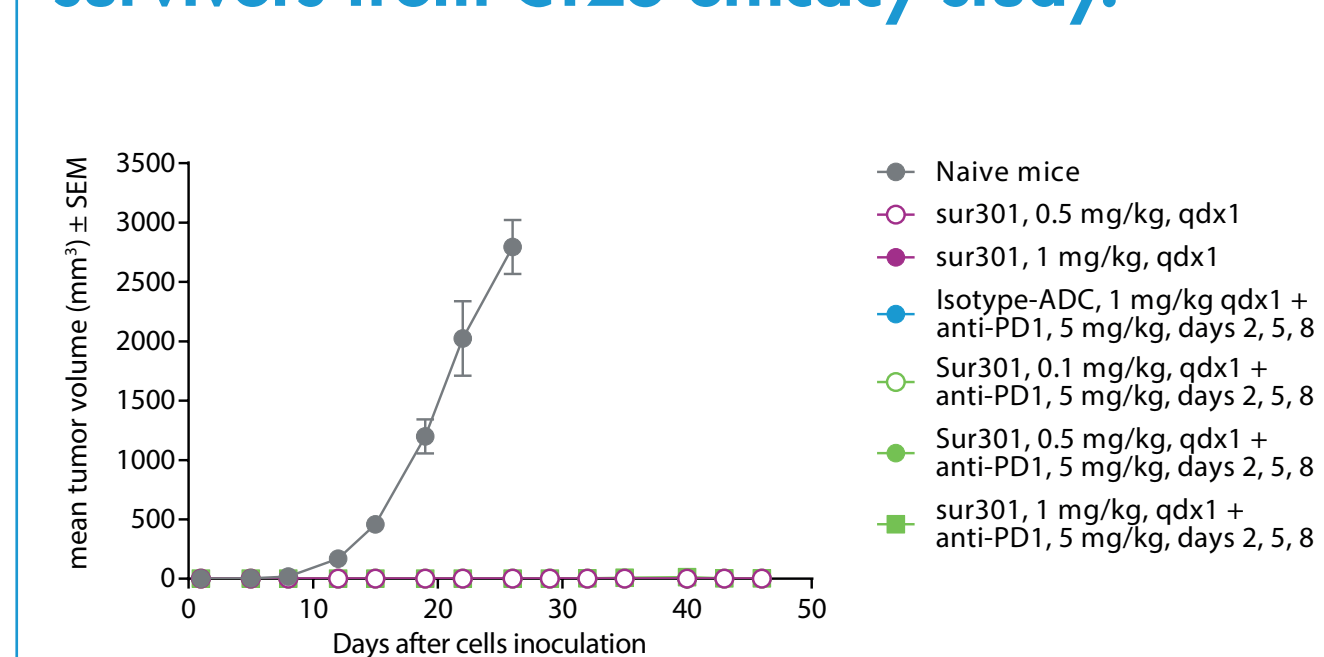
Figure 5. *In vivo* anti-tumor activity in the CT26 syngeneic model.



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.

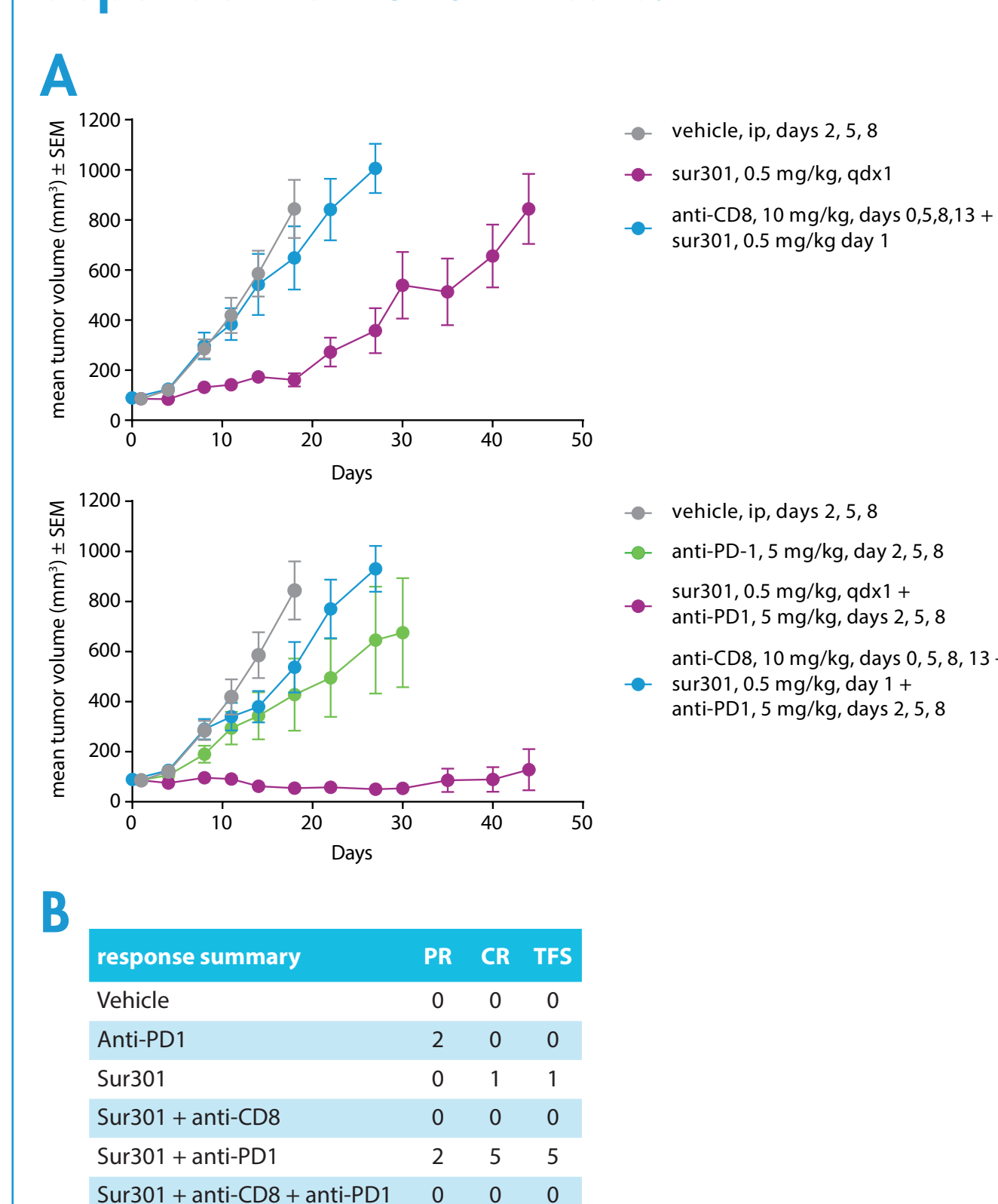
- Each graph represents TV over time for each individual mouse (10 mice/group). Treatment and dosing are indicated in each graph's title. Table reports the response summary.
- Table with response summary.
- Table with CDI.

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.

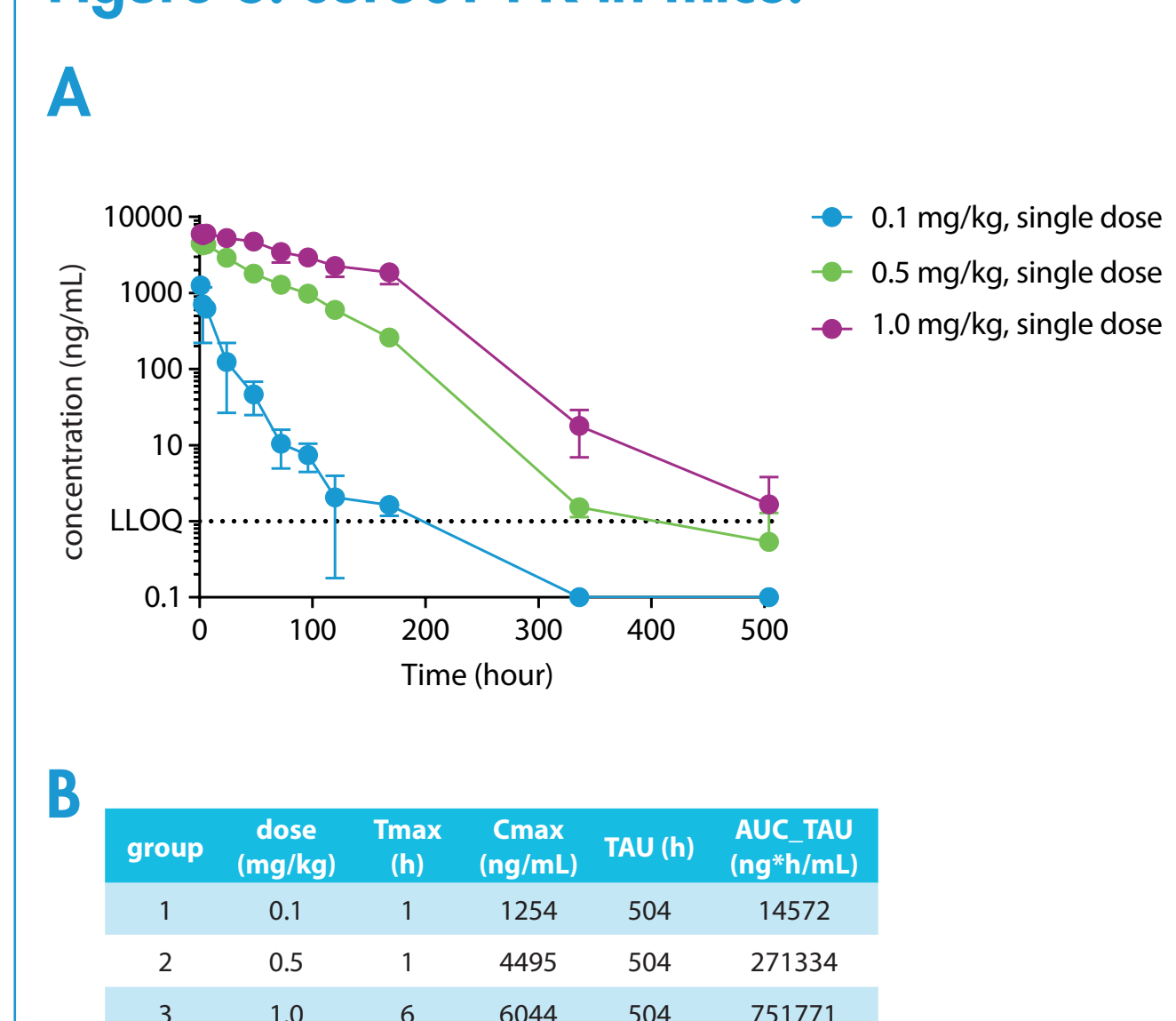


Each graph represents TV over time for each individual mouse (10 mice/group). Treatment and dosing are indicated in each graph's title.

B. Table with response summary.

C. Table with CDI.

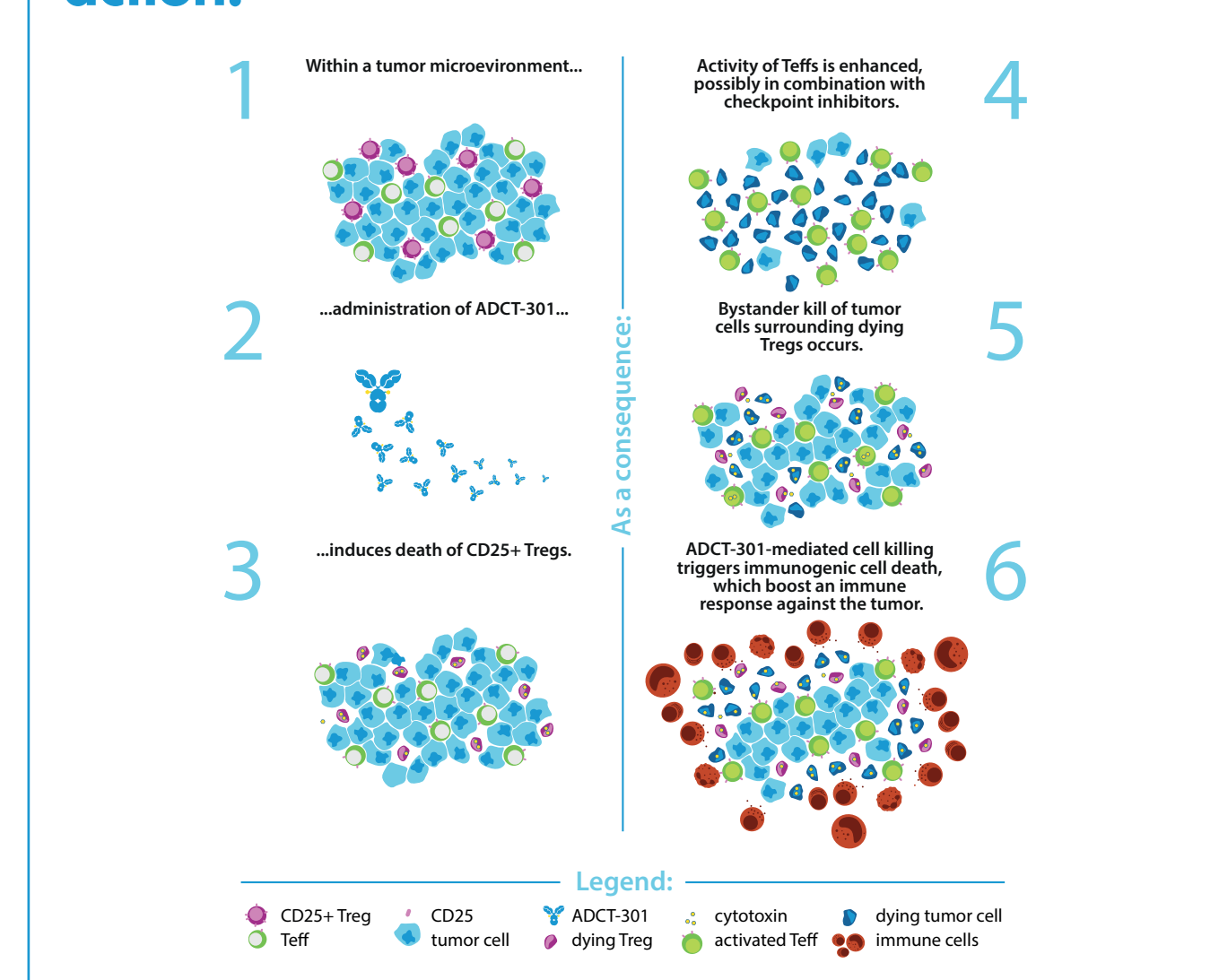
Figure 8. sur301 PK in mice.



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

- 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.
- 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).
- 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.
- Re-challenged animals from both efficacy studies did not develop new tumors indicating sur301 was able to induce tumor-specific protective immunity.
- 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.
- 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.
- 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

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- In vivo* studies: Charles River Discovery Research Services (USA).
- Mouse PK assay: ADC Therapeutics PK team (London, UK).

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