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

Francesca Zammarchi, Karin Havenith, Francois Bertelli, Balakumar Vijayakrishnan, Patrick H. van Berkel

1ADC Therapeutics SA, London, United Kingdom, 2Spirogen, Medimmune, London, United Kingdom

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 cell population.

2Sur301 is an antibody-drug conjugate (ADC) composed of PC61, a rat monoclonal antibody directed against mouse CD25, stochastically conjugated to tesirine, a protein-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 volume 101-172 mm3) on Day 1. The other compounds used i.p. were BI2-SG3249 (non-binding ADC), an isotype control PBD-ADC, anti-PD1 antibody (clone 1H16-1A4) 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-labeled polyclonal Goat Anti-Mouse IgG (Mouse adsorbed) in combination with sulfoTAG streptavidin as detector.

Results

Figure 2. In vitro characterization of sur301.

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

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

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

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

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

Figure 8. sur301 PK in mice.

Figure 9. Proposed ADCT-301 mode of action.

Conclusions

1In 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.

2In 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).

3Combination of a sub-optimal dose of sur301 with an anti-PD1 antibody resulted in synergistic anti-tumor activity in both MC38 and CT26 models.

4Re-challenged animals from both efficacy studies did not develop new tumors indicating sur301 was able to induce tumor-specific protective immunity.

5Sur301 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.

6PK 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.

7Together, 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].

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2In vivo studies: Charles River Discovery Research Services USA

3Mouse PK assay: ADC Therapeutics PK team, London, UK.

References


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