2875

IL1RAP-targeting antibody-drug conjugate: A novel therapeutic targeting both tumor cells and the tumor microenvironment

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Introduction

Antibody-drug conjugates (ADCs) are a class of highly targeted therapies that have brought significant advancements in cancer treatment by combining the selective targeting power of monoclonal antibodies with the potent cell-killing effects of chemotherapy.

ADCs consist of a monoclonal antibody that targets specific cancer-associated antigens conjugated to a potent cytotoxic payload via a linker. By harnessing the specificity of monoclonal antibodies, ADCs are designed to precisely target tumor cells while minimizing off-target cytotoxicity.

Interleukin-1 receptor accessory protein (IL1RAP) is low in normal tissues but highly expressed in various solid tumors, serving as a prognostic marker correlated to poor clinical outcomes. Clinical studies with nadunolimab, an anti-IL1RAP antibody, show prolonged survival in pancreatic cancer patients with high tumor cells expression of IL1RAP when combined with chemotherapy (NCT03267316)[1].

Cantargia's IL1RAP ADCs exploit IL1RAP expression by tumor cells and other cells in the tumor microenvironment (TME) to deliver toxic drugs into the tumor.

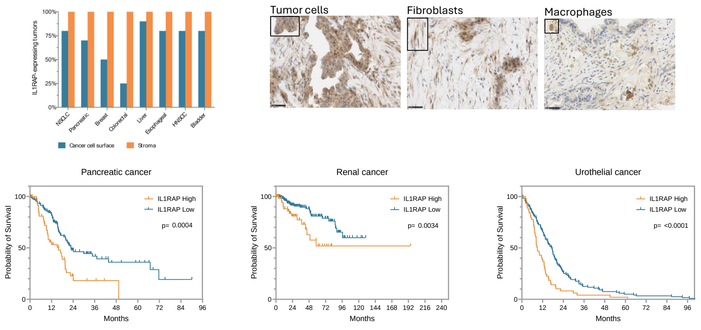


Figure 1. IL1RAP expression in the tumor and stroma of different cancer indications. High IL1RAP mRNA expression correlates with poor survival in several tumors, including pancreatic cancer, renal cancer and urothelial cancer (RNAseq data from TCGA).

Strategy and Aim

The Cantargia CANxx antibody library has over 200 anti-IL1RAP clones, which act as a source for new drug candidates and reagents. The antibody library includes antibodies with diverse binding patterns, domains, and epitopes of IL1RAP, as well as varying species cross-reactivity and functional properties such as internalization and inhibition patterns.

In the presented work an antibody, screened and selected from the CANxx library of IL1RAP-binding antibodies, was conjugated to the tubulin-targeting payload DM51 via a cleavable tri-peptide linker using ImmunoGen technology.

Using preclinical *in vitro* and *in vivo* models, the tolerability, and cytotoxic efficacy of the novel anti-IL1RAP ADC with the potential to target IL1RAP-expressing tumor cells and other tumor promoting cells within the TME, was assessed.

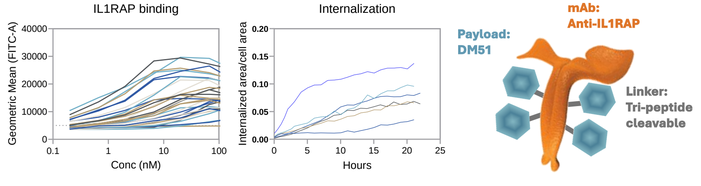
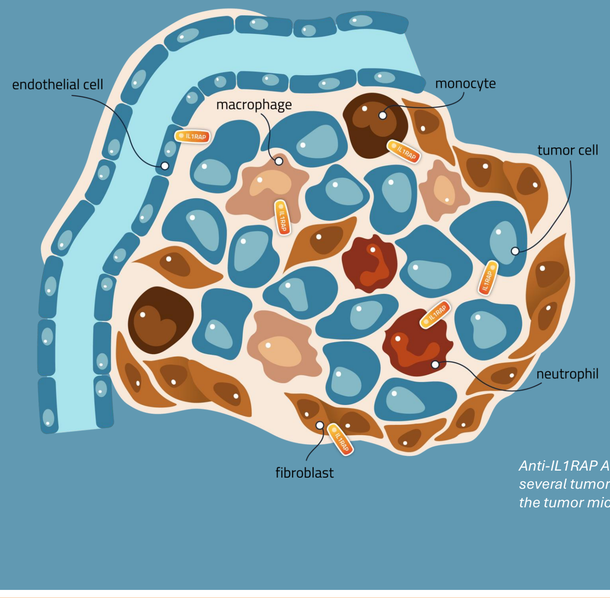


Figure 2: Examples of binding efficacy and internalization to the IL1RAP high expressing cell line SK-MEL5 of anti-IL1RAP antibodies in the CANxx library. An anti-human IL1RAP monoclonal antibody was conjugated to the tubulin-targeting payload DM51 via a cleavable tri-peptide linker using ImmunoGen technology. DAR ~ 4.

Anti-IL1RAP ADCs demonstrated safety, tolerability, and significant anti-tumor efficacy suggesting promising therapeutic potential across a broad spectrum of cancers



Results

Anti-IL1RAP ADC maintains IL1RAP binding and exhibits target specific tumor cytotoxicity

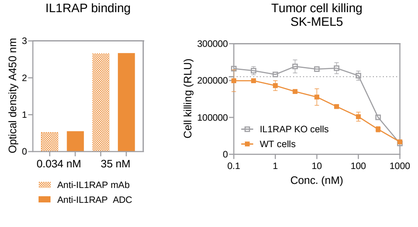


Figure 3: Binding affinity of the anti-human IL1RAP antibody to IL1RAP *in vitro* before and after ADC conjugation. IL1RAP specific, dose dependent tumor cell killing of the anti-IL1RAP ADC in SK-MEL5 WT and SK-MEL 5 KO cells.

Anti-IL1RAP ADC is well tolerated

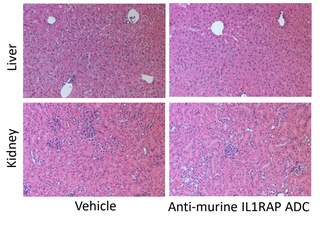


Figure 4. No effect on liver and kidney was detected with an anti-murine IL1RAP ADC. A surrogate anti-murine IL1RAP ADC with the same payload and linker was developed to evaluate the safety and tolerability of the anti-IL1RAP ADC. The anti-murine IL1RAP ADC was tested in concentrations 1, 3, 5 and 10 mg/kg in C57BL/6 mice and 20 mg/kg in BALB/c mice. Body weight and liver and kidney enzymes was measured together with histology assessment.

Results

Anti-IL1RAP ADC shows dose dependent anti-tumor effects and treatment tolerability *in vivo* in an IL1RAP high expressing tumor model

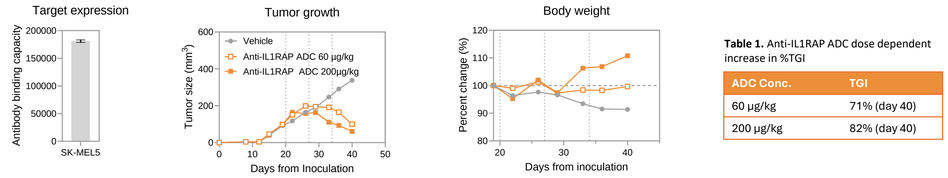


Table 1. Anti-IL1RAP ADC dose dependent increase in %TGI

ADC Conc.	TGI
60 µg/kg	71% (day 40)
200 µg/kg	82% (day 40)

Figure 5: Mice were inoculated with the melanoma cell line SK-MEL5 and treated with or without 60 µg/kg or 200 µg/mg anti-IL1RAP ADC (n=7 per group). At day 40, the percentage of tumor growth inhibition (TGI) was calculated compared to vehicle. Payload doses of 60 µg/kg and 200 µg/kg correspond to approximately 3mg/kg and 10 mg/kg of antibody, respectively. Vertical lines represent day of dosing.

Durable anti-tumor effect with anti-IL1RAP ADC in the IL1RAP high expressing tumor model

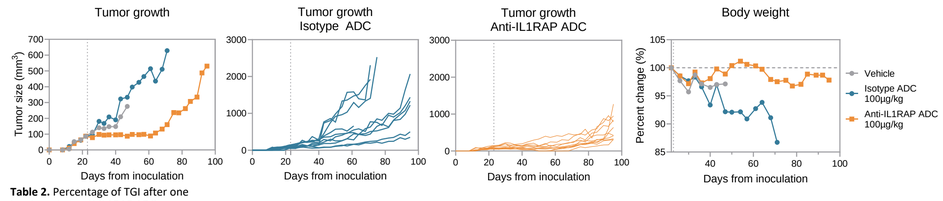


Table 2. Percentage of TGI after one treatment of anti-IL1RAP ADC

ADC Conc.	TGI
100 µg/kg	68% (day 47)

Figure 6: Mice were inoculated with the melanoma cell line SK-MEL5 and treated with or without 100 µg/mg anti-IL1RAP ADC or isotype ADC and monitored up to 95 days (n=10 per group). Vertical lines represent day of dosing. At day 47, the percentage of TGI was calculated compared to vehicle. A 100 ug/kg payload dose corresponds to approximately 5 mg/kg antibody. At day 95, 10 out of 10 mice were alive in the anti-IL1RAP ADC group compared to 6 out of 10 in the isotype ADC group.

Anti-IL1RAP ADC reduces tumor growth *in vivo* in an IL1RAP low expressing tumor model with pancreatic tumor cells and cancer-associated fibroblasts

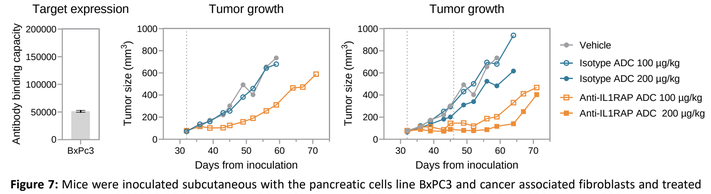


Table 3. Percentage of TGI after one treatment of anti-IL1RAP ADC

ADC Conc.	TGI
100 µg/kg	68% (day 49)
100 µg/kg	58% (day 59)

Table 4. Percentage of TGI after two treatments of anti-IL1RAP ADC

ADC Conc.	TGI
100 µg/kg	70% (day 49)
200 µg/kg	84% (day 49)
100 µg/kg	72% (day 59)
200 µg/kg	84% (day 59)

Figure 7: Mice were inoculated subcutaneous with the pancreatic cells line BxPC3 and cancer associated fibroblasts and treated with or without anti-IL1RAP ADC or isotype ADC (n=9 per group). Mice were dosed with either 100 µg/kg or 200 µg/kg once or twice. Vertical lines represent day of dosing. At day 49 and 59, the percentage of TGI was calculated compared to vehicle treatment at respective days. Payload doses 100ug/kg and 200ug/kg corresponds to approximately 5mg/kg and 10 mg/kg antibody, respectively.

Conclusions

- Anti-IL1RAP ADCs maintained the binding affinity to IL1RAP after conjugation to cytotoxic payloads
- Treatment with anti-IL1RAP ADCs were well tolerated
- The anti-IL1RAP ADC demonstrated potent anti-tumor efficacy in both mouse models evaluated

These preclinical results suggest that ADCs targeting IL1RAP may have promising therapeutic potential use across a broad spectrum of cancers.

References

1. Efficacy and safety of the anti-IL1RAP antibody nadunolimab (CAN04) in combination with gemcitabine and nab-paclitaxel in patients with advanced/metastatic pancreatic cancer. Clinical cancer research.2024; doi:10.1158/1078-0432.CCR-24-0645.