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The anti-IL1RAP antibody CAN04 increases tumor sensitivity to platinum-based chemotherapy.

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INTRODUCTION

Interleukin-1 (IL-1) receptor accessory protein (IL1RAP) is a co-receptor of the IL-1 receptor (IL1R1) and is required for IL-1 signaling. IL1RAP is overexpressed in various solid tumors (Figure 1), both on cancer cells and in the tumor microenvironment. CAN04 is a fully humanized antibody that binds IL1RAP with high affinity, disrupts IL-1α and IL-1β signaling (IC50 = 3.9 and 4.1 nM respectively) and is glycoengineered to mediate an enhanced antibody-dependent cellular cytotoxicity (ADCC, EC50 < 1 nM). Currently, CAN04 is in phase I/IIa clinical development (CANFOUR [see ClinicalTrials.gov NCT03267316]) with primary focus on pancreatic ductal adenocarcinoma (PDAC) and non-small cell lung cancer (NSCLC), two indications with high expression of IL1RAP and where IL-1 signaling has been proven important for tumor development. CANFOUR investigates CAN04 treatment in combination with gemcitabine/cisplatin in NSCLC and gemcitabine/nab-paclitaxel in PDAC. In an interim analysis, 4/7 patients with metastatic pancreatic cancer (PDAC) and 2/3 patients with metastatic non-small cell lung cancer (NSCLC) had objective responses, including 1 NSCLC patient with complete response (CR).

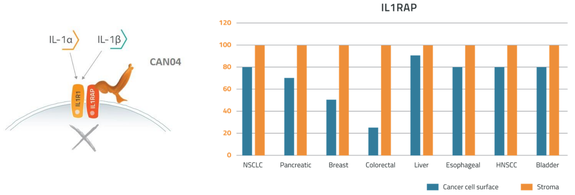

Figure 1. CAN04 targets IL1RAP, blocks IL-1 signaling and is overexpressed in various solid tumors. Left panel: IL1RAP associates with IL1R1 to allow IL-1α and IL-1β signaling, CAN04 binds to IL1RAP and blocks signaling. Right panel: IL1RAP is frequently expressed in cancer cells from solid tumors and always in the tumor stroma (n ranging from 14 to 46).

IL-1α and IL-1β has been implicated in the resistance to several therapeutic regimens, including gemcitabine and 5-FU chemotherapy. However, less is known about the relationship between IL-1α/β and platinum-based chemotherapy, which is commonly used in several cancer forms.

STUDY OBJECTIVES

- To generate data supporting development of CAN04, a humanized anti-IL1RAP antibody in phase I/IIa clinical development
- To analyze consequences of chemotherapy treatment on IL-1 expression in the tumor microenvironment
- To study the effect of IL1RAP-targeting in combination with platinum-based chemotherapy, both in a human tumor xenograft model and in a syngeneic model

RESULTS — NSCLC PDX

IL1RAP is expressed in human LU2503 NSCLC PDX tumors, both on cancer cells and in the tumor stroma, and CAN04 blocks tumor cell IL-1 receptor signaling

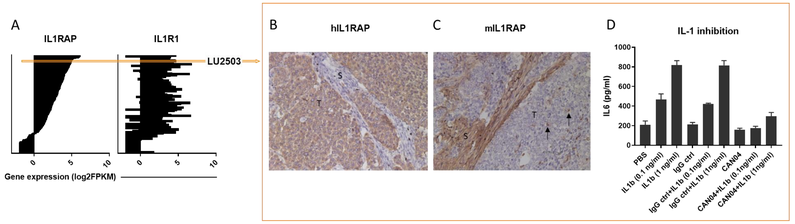

Figure 2. The NSCLC patient-derived xenograft (PDX) LU2503 express IL1RAP, IL1R1 and CAN04 blocks IL-1 receptor signaling in the tumor cells. A: RNA-seq gene expression data from 300 lung cancer PDX models were obtained from CrownBio huBase®, the PDX models were sorted for IL1RAP expression and the corresponding data on IL1R1 expression are also shown, the orange arrow indicate the LU2503 NSCLC PDX model. **B:** IHC-staining of human IL1RAP in tumors from LU2503 PDX mice display IL1RAP expression of the human tumor cells (T), whereas staining for mouse IL1RAP **(C)** show IL1RAP expression in the murine derived stromal regions (S) and infiltrating cells (arrows) into the tumor region. **D:** CAN04 (20 μg/ml) inhibits IL-1β induced IL-6 production from LU2503 cells *in vitro*.

Cisplatin/gemcitabine induces tumor cell death, tumor cell upregulation of IL-1α and stromal activation of Interleukin-1β Converting Enzyme (ICE, Caspase-1)

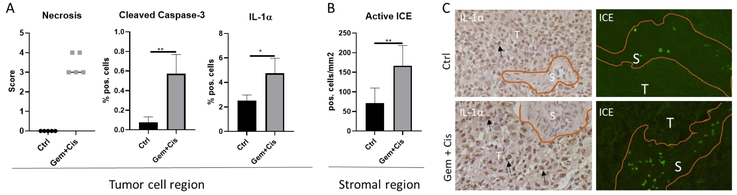

Figure 3. Cisplatin/gemcitabine treatment induces cell death and activates IL-1 signaling in the NSCLC PDX model LU2503. Mice were treated with Cisplatin (Cis, 2.5 mg/kg) and Gemcitabine (Gem 30 mg/kg). Tumors were excised at day 18 and investigated by IHC or IF. **A:** In the tumor cell region, Cis/gem induced tumor cell death as indicated by an increased necrosis (defined by H&E stain) and an increased fraction of cells with cleaved Caspase-3. LU2503 tumor cells also express IL1α, which was upregulated after cis/gem treatment and displayed a translocation with stronger cytoplasmic/cell membrane staining compared to control. **B:** Cis/gem treated tumors had activated ICE (Caspase-1) in the stromal compartment. **C**: Representative IHC or IF images of IL1α and ICE upregulation as described above. (T=tumor cell, S=stroma). N=5, *p<0.05, **p<0.01.

CAN04 increases the efficacy of cisplatin/gemcitabine and carboplatin/gemcitabine, two commonly used platinum-based chemotherapies

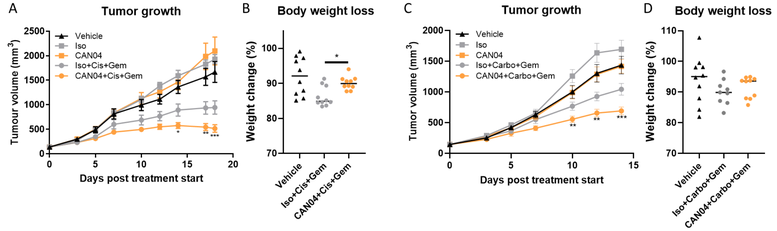

Figure 4. CAN04 increases the efficacy of cisplatin/gemcitabine and carboplatin/gemcitabine in the LU2503 NSCLC PDX model. A: Combination treatment with cisplatin (Cis, 2,5 mg/kg), gemcitabine (Gem, 30 mg/kg) and CAN04 (3F8, 10 mg/kg) displayed a significantly stronger anti-tumor effect compared to isotype control with chemotherapy. **B:** The CAN04 and chemotherapy combination showed a reduced body weight loss compared to the chemotherapy control. Body weight loss was determined as the maximum differences (%) compared to weight at inoculation,. **C:** CAN04 also increased the activity of the carboplatin (Carbo, 30 mg/kg) and gemcitabine (Gem, 30 mg/kg) combination, with a trend to reduced body weight loss with inclusion of CAN04 **(D)**. *p<0.05, **p<0.01, n=10 nude BALB c mice/group.

MC38 SYNGENEIC

Syngeneic MC38 colon cancer tumor cells express IL1RAP and a CAN04 mouse surrogate antibody (mCAN04) blocks murine IL-1 receptor signaling *in vitro*

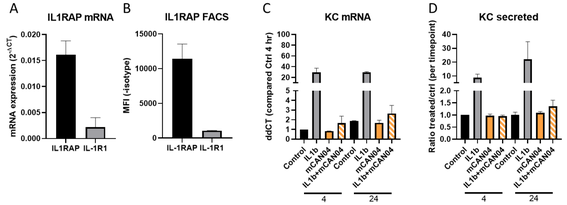

Figure 5. Murine MC38 colon cancer cells express IL1RAP and respond to IL-1 signaling. Baseline IL1RAP and IL1R1 expression at **A:** gene transcription level and **B:** cell surface expression. **C and D:** IL-1β induces upregulation of KC (GRO-alpha, mouse analogue of human IL-8) mRNA and protein in MC38 cells and mCAN04 (20 μg/ml), inhibits activation at gene transcription level (C) and as secreted protein (D). n=3 independent experiments.

mCAN04 increases activity of oxaliplatin and 5-FU in the MC38 colon cancer model

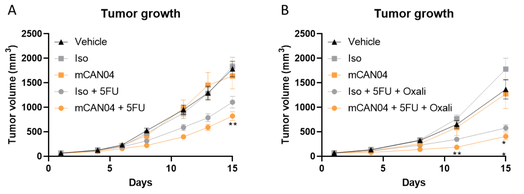

Figure 6. mCAN04 increases activity of 5-FU and oxaliplatin/5-FU in the syngeneic MC38 colon cancer model. 5-FU and oxaliplatin are part of standard of care chemotherapy regimens in several cancers including colorectal cancer and pancreatic cancer. MC38 cells were injected subcutaneously and mice were treated with **(A)** mCAN04, or isotype, (10 mg/kg) in combination with 5-FU (40 mg/kg) for 15 days resulting in a significant reduction in tumor growth in the group receiving mCAN04 combined with 5-FU, compared to the iso and 5-FU group. **B**: mCAN04, or isotype, (10mg/kg) in combination with 5FU (30 mg/kg) and oxaliplatin (Oxali, 6 mg/kg) for 15 days resulting in a strong reduction in tumor growth compared to vehicle treatment. Inclusion of mCAN04 led to a further attenuated tumor growth compared. Significances describe the differences between mCAN04 5-FU oxaliplatin compared to Iso 5-FU oxaliplatin. n=10 C57Bl/6 mice /group, *p<0.05, **<p.0.01.

mCAN04 in combination with platinum compounds are superior to platinum chemotherapy alone

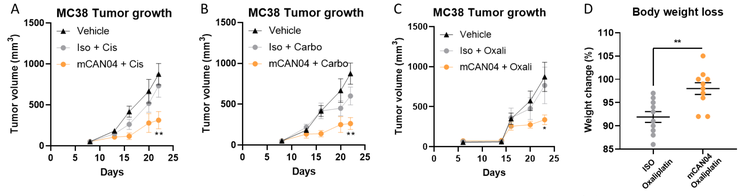

Figure 7. mCAN04 increases platinum-based chemotherapy activity in the MC38 tumor model. A-C: mCAN04 (10 mg/kg) in combination with cisplatin (2.5 mg/kg), carboplatin (20 mg/kg) or oxaliplatin (10 mg/kg) had a strong effect on tumor growth compared to the corresponding isotype/chemotherapy controls. **D:** Cisplatin and carboplatin at the tested doses did not induce body weight loss while the oxaliplatin treatment did, mCAN04 however reduced the drop in body weight in this model. Significances corresponds to mCAN04 in combination with platinum-based compound, compared to isotype in combination with platinum-based compound, n=10 C57Bl/6 mice /group, *p<0.05, **p<0.01.

CONCLUSIONS

- Cisplatin/gemcitabine chemotherapy induces cell death and IL-1α upregulation in tumor cells while Interleukin-1β Converting Enzyme is upregulated in the tumor stroma
- IL1RAP targeting by CAN04 increases the efficacy of platinum-based therapies
- CAN04 can counteract weight loss induced by chemotherapy
- The effects mediated by CAN04 are consistent with an effect on chemotherapy induced IL-1α and IL-1β
- These results support ongoing and future clinical development of CAN04