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Antibody blockade of IL1RAP signaling reduces metastasis in a breast cancer model

David Liberg [1], Per-Ola Önnervik [2], Matteo Riva [2], Liselotte Larsson [1], Göran Forsberg [1], Karin von Wachenfeldt [2]

[1] Cantargia AB, Medicon Village, SE-223 81 Lund, Sweden; [2] Truly Labs AB, Medicon Village, SE-223 81 Lund, Sweden

ABSTRACT

Blockade of tumor inflammation has potential for cancer therapy, both as a primary mechanism to counter tumor growth but also in combination with other therapeutics. IL-1 signaling has been shown preclinically to be involved in tumor development and chemoresistance of pancreatic cancer, and blockade of IL-1 was recently shown to have a significant clinical impact on development of lung cancer. IL-1 receptor associated protein (IL1RAP) is a coreceptor for the IL-1 receptor (IL1R1) and is required for IL-1 signaling. IL1RAP is expressed in a number of tumor tissues, including lung and pancreatic cancer, both on tumor cells and on infiltrating immune cells. We have, using antibodies directed against IL1RAP, shown the ability to target and kill IL1RAP-expressing tumor cells by ADCC, to inhibit IL-1 signaling in those cells and to reduce growth of transplanted human tumors in vivo. To study effects of IL1RAP targeting on the tumor microenvironment and in an immune competent setting, an antibody towards mouse IL1RAP was generated. This antibody potently blocks mouse IL-1 (IC50 = 13 nM), binds to IL1RAP protein with high affinity (Kd = 4,2 nM), labels IL1RAP-expressing cells and can be administered to mice with good pharmacokinetics. In vivo imaging shows that the antibody is not generally distributed in tissues but localizes to tumor sites after injection. Treatment of mice with orthotopically implanted 4T1 breast cancer cells did not reduce primary tumor growth significantly but reduced both the number of (47% reduction, p=0.02) and size of lung metastases. Interestingly, 4T1 tumor cells express low levels of IL1RAP and are not responsive to IL1RAP blockade, but the effects instead relate to effects on the tumor microenvironment. We conclude that targeting of IL1RAP can, in addition to induce ADCC of tumor cells and block their response to IL-1, also inhibit metastasis by affecting the tumor microenvironment.

BACKGROUND

Interleukin-1 receptor associated protein (IL1RAP) is a co-receptor of the IL-1 receptor (IL1R1) and is required for IL-1 signaling. We have previously described IL1RAP as a target on solid tumors (e.g. pancreatic cancer, non-small cell lung cancer (NSCLC), triple-negative breast cancer and colorectal cancer) and on leukemic cells (chronic myeloid leukemia, acute myeloid leukemia, acute lymphoblastic leukemia) and shown that an anti-human IL1RAP antibody have anti-tumor effects in human xenograft models. A humanized and ADCC-enhanced form of this antibody (CAN04) is currently in phase I/IIa clinical development (CANFOUR [see ClinicalTrials.gov NCT03267316]) with primary focus on pancreatic cancer and NSCLC. In order to study effects not only on the tumor cells but in animals with an intact immune system, we developed a surrogate murine-specific antibody (3A9). This antibody recognizes murine IL1RAP and binds in a manner that blocks signal transduction (see figure 1), similar to CAN04. IL-1 has previously been shown to be important for growth and metastasis of the syngeneic 4T1 breast cancer model, an effect involving myeloid suppressor cell functions [2, 3]. Since 4T1 cells express little IL1RAP and IL1R1 and hence do not respond to IL-1 (figure 4 and [2]), we used this model to study effects of IL1RAP targeting of the immune system and of the tumor microenvironment.

STUDY OBJECTIVES

- To generate data supporting translational research around CAN04, a fully humanized antibody against IL1RAP in phase I/IIa clinical development
- To develop a surrogate antibody to the human-specific clinical candidate CAN04 for studies in mice
- To study contributions of IL1RAP-targeting of non-tumor cells on tumor development and metastasis

RESULTS

The 3A9 antibody binds to murine IL1RAP and inhibits IL-1 signaling

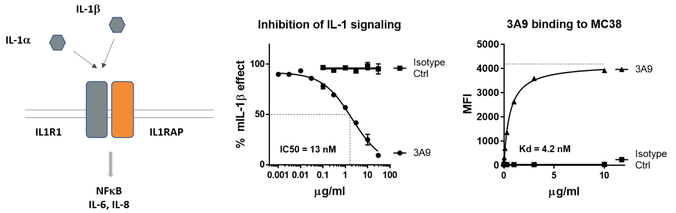
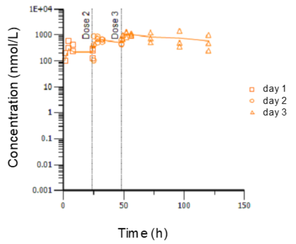


Figure 1. ***In vitro*** **activity of the 3A9 anti-murine IL1RAP antibody**. **Left Panel**: IL-1α and IL-1β binds to IL1R1 which in turn heterodimerize with IL1RAP. The complex transduces a signal to activate e.g. NFκB driven transcription. **Middle panel**: Inhibition of IL-1β induced activation of a SEAP reporter in HEK-Blue™ IL-1 Beta cells (Invivogen). Mouse mAb 3A9 generated an IC50 of 1.95 µg/ml corresponding to 13 nM. **Right panel**: FACS analysis of 3A9 binding to the IL1RAP+ MC38 cell line, Kd was calculated using the GraphPad Prizm software, one site specific binding model (y=Bmax*x/(Kd+x)).

3A9 can be administered in vivo with a favorable pharmacokinetic profile



Group	Tmax (h)	Cmax (SE) (nmol/L)	AUClast (SE) (h*nmol/L)	AUCINF_obs (h*nmol/L)	AUC_% Extrap_obs (%)	T 1/2 (h)	RSQ_ adjusted
10 mg/kg, day 1	4	298 (170)	5250 (2000)	NA	99	NA	1
10 mg/kg, day 2	4	698 (110)	13800 (1300)	50300	72	NA	1
10 mg/kg, day 3	4	1120 (160)	58500 (15000)	104000	44	54.4	1

Figure 2. Pharmacokinetic profile of murine anti-IL1RAP 3A9 after repeated i.p. administration in C57BL/6 mice. Plasma concentrations following repeated intreaperitoneal administration for three consecutive days of 3A9 to C57BL/6. The levels of 3A9 were quantified by ELISA and the data elaborated using the WinNonlin software. At a dose of 10 mg/kg, good exposure levels enabling interpretation of the pharmacological effects were achieved, for details see table.

3A9 accumulates in 4T1 tumors after intraperitoneal administration

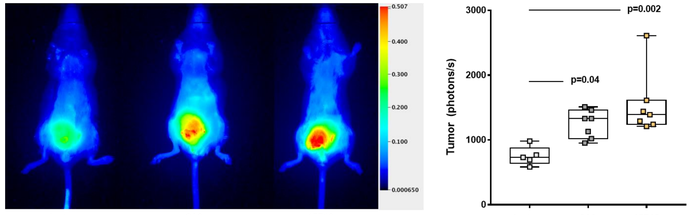


Figure 3. Near infrared imaging of 4T1 tumor-bearing mice using anti-IL1RAP probes. Isotype control, 3A9 and a second anti-IL1RAP antibody (15F12) were labeled with IRDye 800CW (LI-COR Biosciences, Germany). 40 µg of the labeled antibodies were administered intraperitoneally to mice 17 days after orthotopic inoculation of 10^5 4T1 cells in the mammary fat pad. Live imaging was performed on anesthetized animals using a Pearl Trilogy system (LI-COR Biosciences, Germany). Tumor, spleen and heart were dissected out and imaged after termination of the experiment. **Left panel**: Representative images 72h post injection of the indicated probes. **Right panel**: Total signal from the indicated probes for individual dissected tumors, the signal was adjusted for background by subtracting the signal from the heart (signal = total intensity for the tumor – (background mean Intensity of the heart x area of the tumor)).

4T1 cells express low levels of IL1RAP and IL1R1 on the cell surface

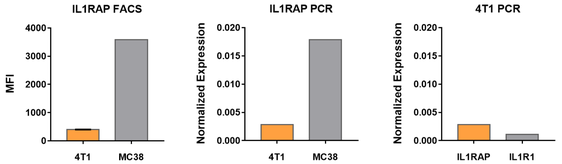


Figure 4. IL1RAP expression varies between different syngeneic mouse tumor cell-lines. Left panel: FACS analysis of IL1RAP expression using 3A9-PE for staining of two mouse tumor cell-lines. The colorectal cell-line MC38 has a clear expression (see also figure 1), whereas the 4T1 breast cancer line express only low levels of IL1RAP. This is replicated on the mRNA level as illustrated in the **middle panel.** **The right panel** compare the 4T1 mRNA expression of IL1RAP and IL1R1, the two components of the signaling IL-1 receptor .

3A9 recognizes IL1RAP on myeloid cell populations in the spleen and tumor of 4T1 tumor-bearing mice

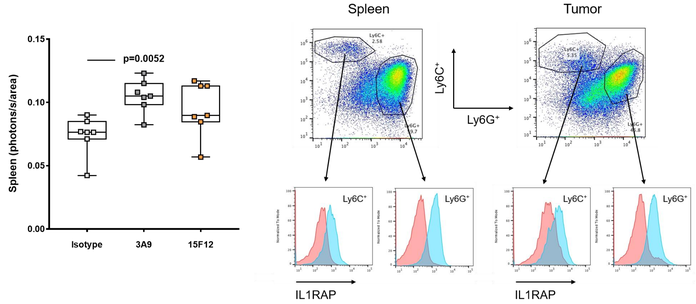


Figure 5. Near infrared imaging of spleens from 4T1 tumor bearing mice and FACS analysis of myeloid cell populations using anti-IL1RAP probes. **Left panel:** Spleens were dissected out and subjected to near infrared imaging 72h after intraperitoneal administration of the indicated probes (as described in figure 3). The signal represents total signal from the spleen divided by the area of the spleen in pixels. **Right panel:** FACS analysis of splenic and tumor infiltrating CD11b+ cells 17 days post-inoculation of 10^5 4T1 cells in the mammary fat pad. IL1RAP expression on $CD11b^+Ly6C^{hi}$ monocytic cells and on $CD11b^+Ly6G^+$ granulocytic cells is indicated.

3A9 blocks metastasis of orthotopically inoculated 4T1 tumors

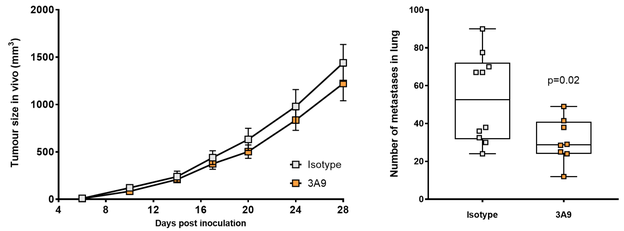


Figure 6. In vivo effects of 3A9 in the 4T1 orthotopic model. 10^5 4T1 cells were inoculated in the mammary fat pad of BALB/C mice. Bi-weekly treatment with 10 mg/kg 3A9 or isotype control started when tumors had reached 80-120 mm^3. **Left panel**: Tumor growth as measured by calipers on the indicated days. **Right panel**: Lung metastases at termination measured by coloring with India Ink and counting the number of metastases.

CONCLUSIONS

- A surrogate antibody to the clinical candidate CAN04 was developed, the antibody binds to murine IL1RAP and inhibits IL-1 signaling
- This surrogate antibody, 3A9, accumulates in tumors and spleen of 4T1 tumor-bearing mice where it recognizes myeloid cell populations
- Treatment of 4T1 tumor-bearing mice with 3A9 inhibits metastasis of tumor cells to the lungs
- The effects mediated by 3A9 are consistent with an effect on tumor-promoting myeloid cells to counter tumor metastasis

REFERENCES

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2. Bunt, SK., et al. Reduced Inflammation in the Tumor Microenvironment Delays the Accumulation of Myeloid-Derived Suppressor Cells and Limits Tumor Progression, Cancer Res 2007;67(20)

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