Glycoengineered Murine Antibodies as Surrogates to the Humanized and ADCC-enhanced Anti-IL1RAP Antibody CAN04 (nidanilimab)

Camilla Rydberg Millrud [1]; Pablo Peña [2]; Gabriel Svensson Birkedal [1]; Hanna Håkansson Falk [3]; Camilla Ekenstierna [3]; Kjell Sjöström [4]; Liselotte Larsson [1]; Marcus Järås [2]; Karin von Wachenfeldt [3]; Göran Forsberg [1]; David Liberg [1]

[1] Cantargia AB, Ideon Science Park, Lund, Sweden; [2] Division of Clinical Genetics, Department of Laboratory Medicine, Lund University, Sweden; [3] Truly Labs AB, Medicon Village, Lund, Sweden; [4] Innovagen AB, Ideon Science Park, Lund, Sweden.

INTRODUCTION

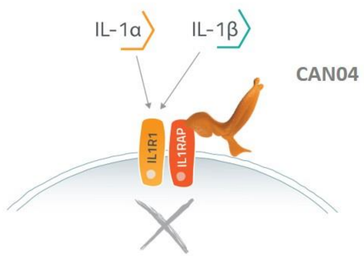
Antibodies targeting Interleukin-1 (IL-1) receptor accessory protein (IL1RAP) are investigated for treatment of malignant and inflammatory diseases. A fully humanized IgG1 antibody (CAN04, nidanilimab) is currently in phase II clinical development in non-small cell lung cancer (NSCLC) and pancreatic cancer. IL1RAP is a co-receptor of the IL-1 receptor (IL1R1) and is required for IL-1 signaling. CAN04 disrupts both IL-1α and IL-1β signaling and is engineered to mediate enhanced effector cell functions, such as antibody-dependent cellular cytotoxicity (ADCC). As several antibodies developed for clinical use, CAN04 is not cross-reactive to rodent IL1RAP resulting in obvious limitations in preclinical mouse studies.

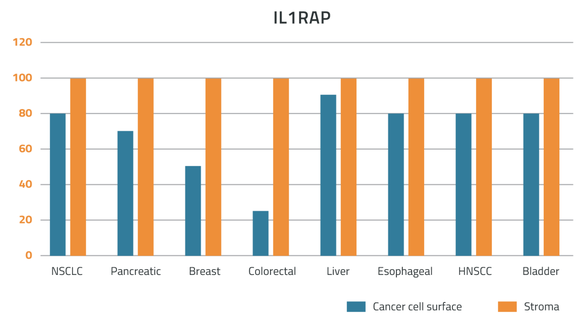
In order to study effects of IL1RAP-targeting in murine systems, mouse IgG2a anti-mouse IL1RAP antibodies were developed as surrogates to CAN04. These antibodies are functional in preclinical models, but they do not mimic the ADCC-enhancement of the clinical antibody. It is well established that afucosylated human antibodies have an increased affinity for Fc-receptors and an augmented ADCC and antibody-dependent cellular phagocytosis (ADCP), but less is described for mouse antibodies. In this study we analyzed the potential of murine afucosylated antibodies to act as surrogates for afucosylated human antibodies.

STUDY OBJECTIVES

- To express normal, afucosylated and LALA-PG mutated (non-Fc-binding) murine antibodies and study the binding of these antibody variants to different Fc-receptors.
- To study and compare the in vitro and in vivo activity of normal, afucosylated and LALA-PG mutated murine anti-IL1RAP antibodies.

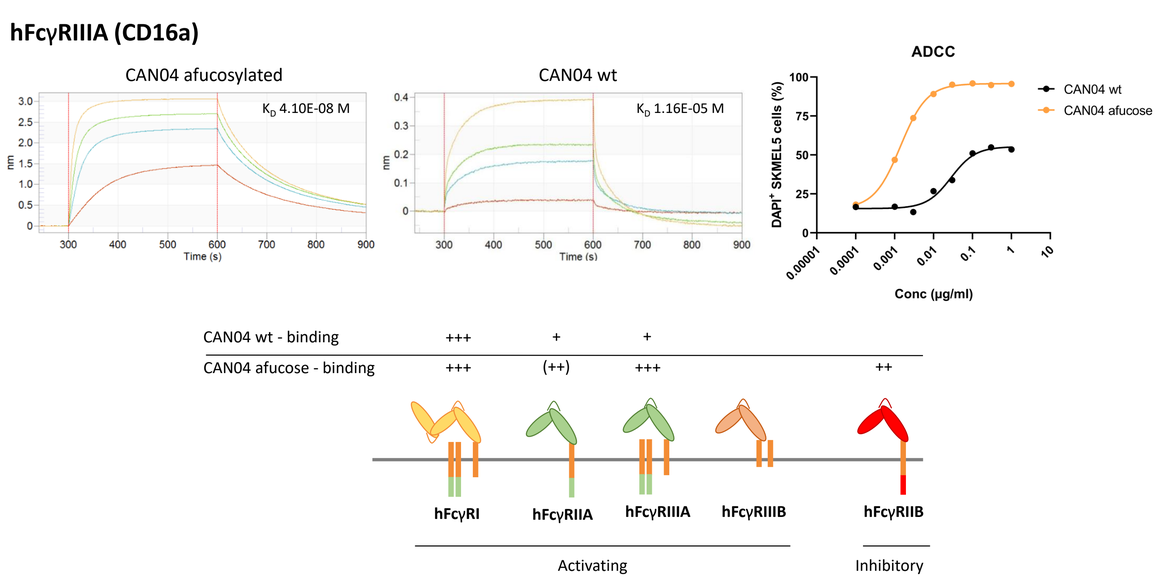
CAN04





CAN04 targets IL1RAP, blocks signaling from IL1α and IL1β and induces ADCC of IL1RAP-expressing cells. Left panel: IL1RAP associates with IL1R1 to allow IL-1α and IL-1β signaling, CAN04 binds to IL1RAP and blocks IL1RAP function. Right panel: IL1RAP is commonly expressed in solid and hematological cancers. Staining of frozen tissue sections from patient samples of the specified indications (n ranging from 14 to 46) with CAN04

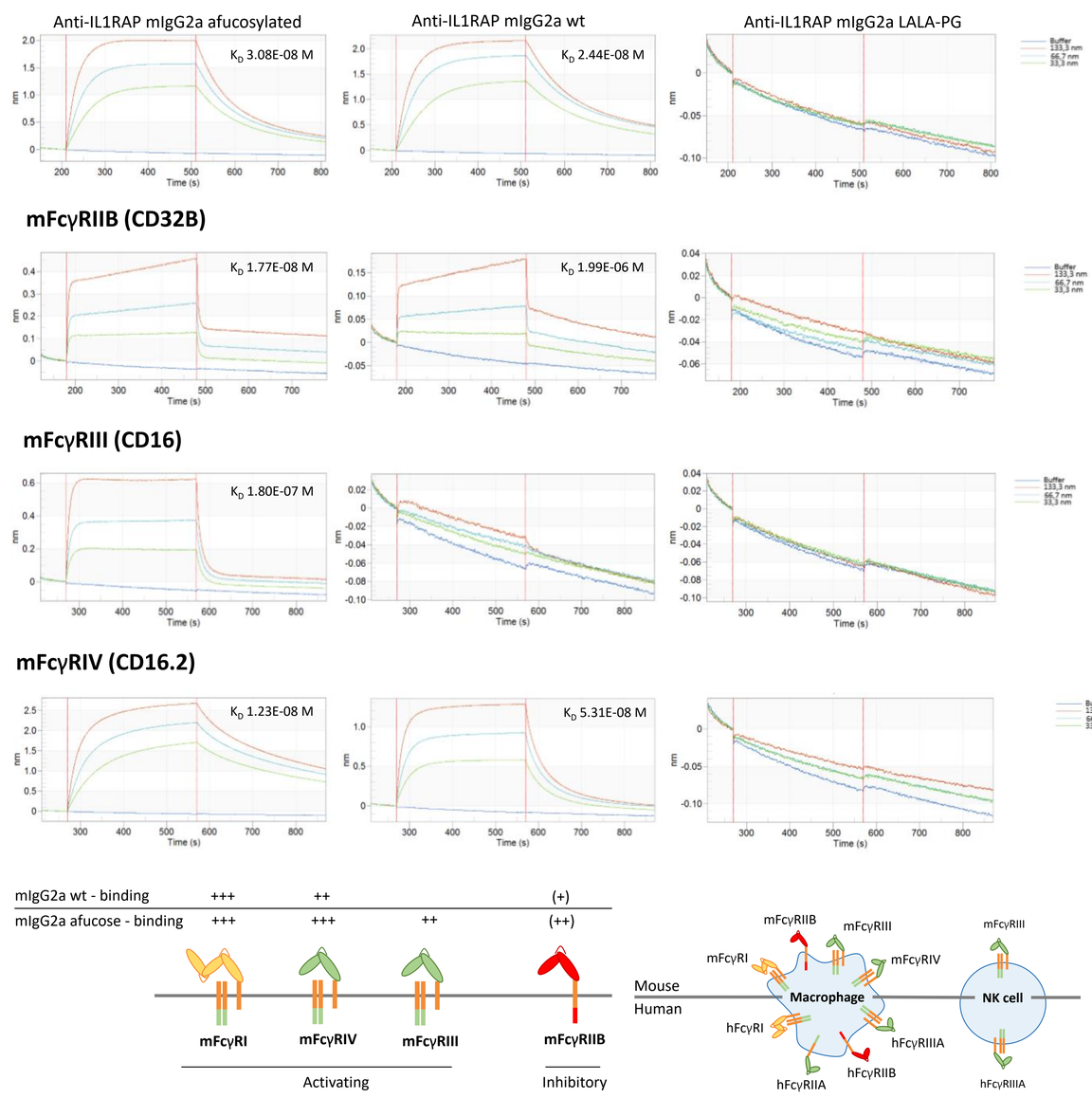
CAN04 (afucosylated human IgG1 anti-IL1RAP) has an increased affinity to FcγRIIIa and an enhanced ADCC



Afucosylated CAN04 binds FcγRIIIa with a higher affinity compared to CAN04 wild type, corresponding to the enhancement of ADCC by afucosylated CAN04. The two left panels: The binding of CAN04 afucosylated and CAN04 wild type was determined with optical biosensor technology analysis. Right panel: Primary NK cells was cultured with PE labeled IL1RAP expressing SKMEL5 tumor cells in presence of afucosylated or wild type CAN04. Lower panel: The binding of CAN04 and CAN04 wild type to the different human Fcγ-receptors.

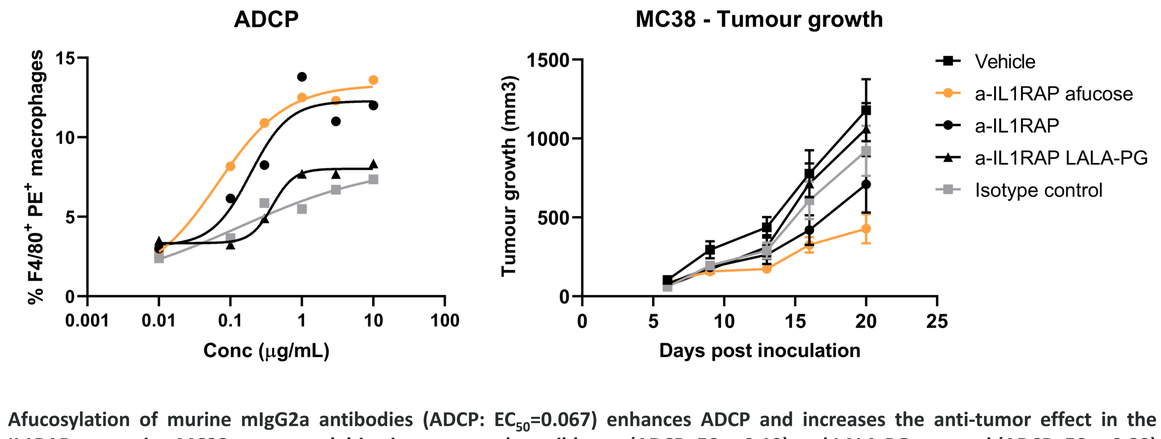
RESULTS

A murine afucosylated anti-IL1RAP IgG2a antibody binds FcγRIII and with a higher affinity to FcγRI compared to wild type antibody



The afucosylated mIgG2a anti-IL1RAP antibody has a different Fcγ-receptor binding pattern than normal mIgG2a. The wild type murine IgG2a antibody bound to murine FcγRI (K_D 2.44E-08) and FcγRIV (K_D 5.31E-08), but not to FcγRIII. The corresponding afucosylated antibody bound to FcγRI (K_D 3.08E-08), FcγRIII (K_D 1.80E-07) and with an enhanced affinity to FcγRIV (K_D 1.23E-08). As expected, the LALA-PG mutated antibody did not bind to any of the murine Fcγ-receptors. The binding of mIgG2a wild type, mIgG2a afucosylated and mIgG2a LALA-PG mutated antibodies to Fcγ-receptors was determined with optical biosensor technology analysis. Lower left panel: The binding of afucosylated mIgG2a and mIgG2a wild type to the different murine Fcγ-receptors. Lower right panel: The Fcγ-receptor expression by the ADCP and ADCC effector cells, macrophages and NK cells respectively, in mouse and human.

Afucosylation of a murine anti-IL1RAP antibody enhances ADCP almost 3-fold compared to the wild type antibody and increases the anti-tumor effect in the MC38 tumor model



Afucosylation of murine mIgG2a antibodies (ADCP: EC_{50}=0.067) enhances ADCP and increases the anti-tumor effect in the IL1RAP expressing MC38 tumor model *in vivo* compared to wild type (ADCP: EC_{50}=0.19) and LALA-PG mutated (ADCP: EC_{50}=0.39) variants of the antibody. Left panel: Primary murine macrophages was cultures with PE labeled IL1RAP-positive MC38 tumor cells for 6h in presence of anti-IL1RAP, anti-IL1RAP afucosylated, anti-IL1RAP LALA-PG or isotype control antibodies. Right panels: C57BL/6 mice were inoculated with MC38 cells and treated with or without anti-IL1RAP, anti-IL1RAP afucosylated, anti-IL1RAP LALA-PG or isotype control antibodies (n=10 mice/group). *** Anti-IL1RAP afucosylated vs Vehicle; * Anti-IL1RAP afucosylated vs Isotype control; ** Anti-IL1RAP afucsoylated vs Anti-IL1RAP.

CONCLUSIONS

- Afucosylation of CAN04 surrogate antibodies leads to enhanced immune cell interactions and increased anti-tumor effects.
  - Afucosylation of murine IgG2a CAN04 surrogate antibodies increases binding to mFcγRIII and mFcgRIV, enhances ADCP by macrophages and improves the anti-tumor effect.
  - Afucosylated murine surrogate antibodies can be used to illustrate the benefit of afucosylation in experimental models.