Neo-X-Prime™ bispecific antibodies targeting CD40 and tumor antigens promote cross-presentation of tumor exosome-derived neoantigens and induce a superior anti-tumor response

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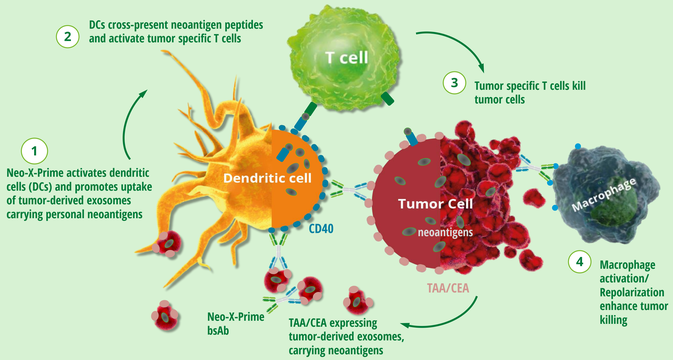
Unique mode of action of Neo-X-Prime antibodies



Neo-X-Prime Lead candidate: CD40-CEA bsAb

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CD40

- Activates dendritic cells (DCs) and promotes cross-priming of T cells
- Activates macrophages to promote anti-tumor functions

CEA

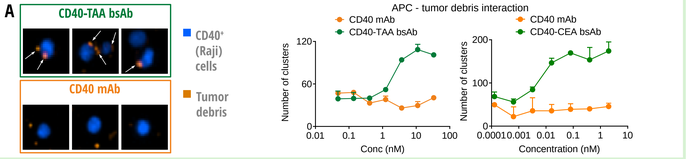
- Tumor associated antigen CEA – expressed in several solid tumors including colorectal, lung, gastric, breast and pancreatic cancer
- Limited CEA expression in healthy tissue

Neo-X-Prime concept:

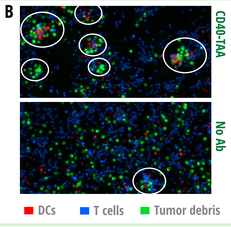
- Bispecific antibodies targeting CD40 and highly expressed tumor associated antigens (TAA)
- Activates antigen presenting cells only in the presence of TAA
- Aims to increase the tumor neoantigen-specific T cell pool by targeting TAA expressing tumor exosomes to DCs and boosting cross-presentation of neoantigens to T cells

Neo-X-Prime bsAbs promote tumor antigen uptake and cross-presentation

Neo-X-Prime bsAbs deliver TAA-expressing tumor debris to antigen presenting cells



Neo-X-Prime bsAb promotes clustering of DC, T cells and tumor debris



Neo-X-Prime bsAb promotes DC uptake of tumor exosomes and cross-presentation of neoantigen, enhancing expansion of neoantigen-specific T cells

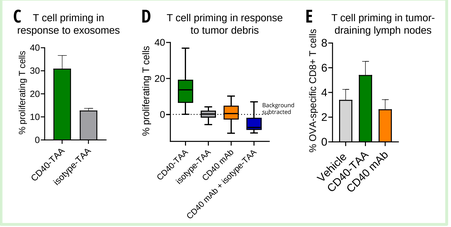
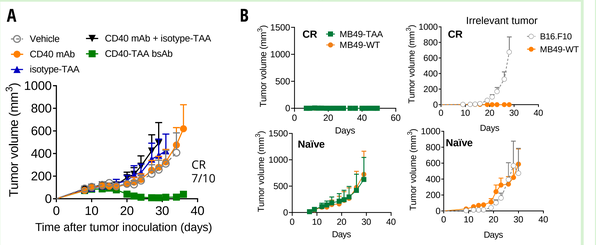


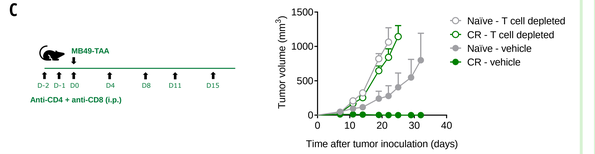
Figure 1. (A) Fluorescently labeled CD40+ Raji cells were cultured with fluorescently labeled hEpCAM+ tumor debris in the presence of titrated CD40 mAb or CD40-EpCAM. Alternatively, Raji cells were cultured with CEA+ tumor debris and CD40 mAb or CD40-CEA. Images were captured using a live cell imaging system and clusters of CD40+ cells co-localized with tumor debris were quantified after 2-8 hrs of culture. (B) Fluorescently labeled DCs from CD40tg mice were cultured with fluorescently labeled necrotic EpCAM+ OVA+ MB49 tumor cells and TCR transgenic OVA-specific T cells (OT1 cells) in the presence or absence of CD40-EpCAM. Images were captured after 12 h of coculture. (C) Proliferation of Celltrace Violet-labeled OT1 cells cultured with hCD40tg DCs and MB49-EpCAM-OVA tumor exosomes or (D) debris in the presence of CD40-EpCAM or control antibodies was assessed by flow cytometry after 72h. (E) OT1 cells were adoptively transferred to CD40tg mice bearing MB49-EpCAM-OVA tumors, followed by i.p. administration of 417 µg CD40-EpCAM or 250 µg CD40 mAb (at equivalent molar concentrations). Mice were subsequently treated with FTY720 to prevent T cell egress from lymph nodes, and the frequency of OVA-specific CD8+ T cells in tumor draining lymph nodes was assessed 3 days after antibody administration. The graphs show mean ± SEM in one representative experiment of three (A, E), pooled data from 3 experiments (C) or median ± min and max as pooled data from 4 experiments (D). TAA = EpCAM in this figure.

Neo-X-Prime bsAb induces superior anti-tumor effect vs combination of monotherapies and a broad tumor-specific immunological memory that is T cell dependent

Neo-X-Prime bsAb cures tumor-bearing mice in a TAA dependent manner and induces tumor-specific immunological memory to antigens other than TAA



Neo-X-Prime bsAb-induced immunological memory is T cell-dependent



Neo-X-Prime bsAb does not induce increased levels of systemic cytokines

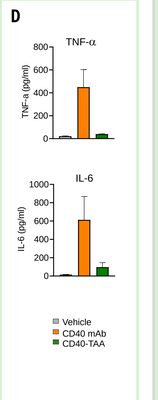
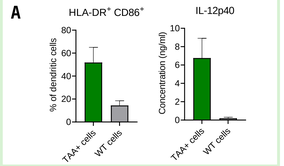


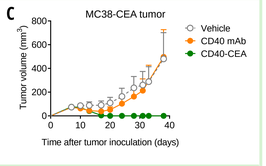
Figure 2. Human CD40 transgenic (hCD40tg) mice were inoculated with MB49-hEpCAM+ cells s.c. and administered (A) 100 µg anti-CD40 antibody, a molar equivalent dose (167 µg) of CD40-EpCAM, 167 µg isotype-EpCAM bsAb, or 100 µg CD40 mAb plus 167 µg isotype-EpCAM bsAb i.p. on days 10, 13 and 16. (B) Cured mice (complete responders, CR) were rechallenged with the same tumor on one flank and an MB49-WT tumor on the other flank, or with MB49-WT tumor on one flank and an irrelevant tumor (B16.F10) on the other flank. (C) Cured mice and naïve controls were treated with T cell depleting antibodies or vehicle control and re-challenged with MB49-EpCAM tumor cells s.c. Tumor volume over time is shown as mean ± SEM. (D) hCD40tg mice were inoculated with MB49-hEpCAM+ cells s.c. and administered molar equivalent doses of a CD40 mAb or CD40-EpCAM bsAb (250 and 417 µg, respectively) i.p. on days 10, 13 and 16. Systemic immune activation was evaluated by measuring plasma cytokine levels 4 hrs after the second therapy dose and shown as mean ± SEM. TAA = EpCAM in this figure.

Lead compound: CD40-CEA bsAb induces TAA-dependent immune activation, anti-tumor efficacy and immunological memory

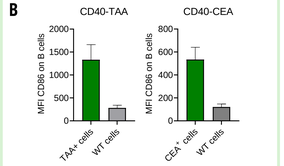
TAA-dependent DC activation with Neo-X-Prime



Anti-tumor efficacy of CD40-CEA bsAb



TAA-dependent B cell activation with Neo-X-Prime



Tumor-specific immunological memory

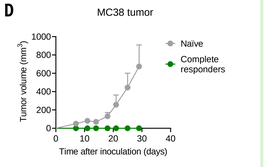
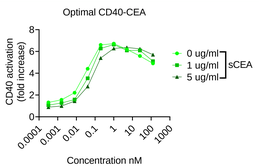


Figure 3. (A) Human monocyte-derived DCs were cultured with CD40-EpCAM in the presence EpCAM+ or EpCAM- cells. After 2 days, expression of CD86 and HLA-DR on CD14+ CD1a+ DCs was analyzed by FACS and IL-12p40 in supernatants was analyzed by ELISA. (B) Human primary B cells were cultured with CD40-EpCAM or CD40-CEA bsAbs in the presence of EpCAM+, CEA+ or WT cells. CD86 expression on CD19+ cells was analyzed by FACS after 2 days. (C) hCD40tg mice were inoculated with MC38-CEA+ cells s.c. and administered 100 µg anti-CD40 antibody or a molar equivalent dose (167 µg) CD40-CEA bsAb i.p. on days 7, 10 and 13. (D) Naïve hCD40tg mice or complete responders cured from MC38-CEA+ tumors by CD40-CEA bsAb treatment were rechallenged with MC38 WT tumors. Preliminary data from one initial in vivo experiment is shown as mean ± SEM. TAA = EpCAM in this figure.

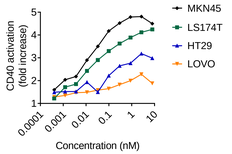
CD40-CEA binders designed for optimal performance

Optimal CD40-CEA bsAb efficiently activates CD40-expressing cells in the presence of soluble CEA



- Soluble CEA is present in the blood of patients and could act as a sink for CEA-targeting antibodies
- Our functional screening identified bsAbs that are not impaired by the presence of physiological levels of soluble CEA

CD40-CEA bsAbs efficiently activate CD40-expressing cells in the presence of tumor cells expressing high or intermediate levels of CEA, but have limited activity in the presence of cells with low CEA expression



Cell line	CEA receptor density
MKN45	353 000
LS174T	51 500
HT29	11 300
LOVO	5 500

Physiological levels in healthy tissue estimated to be <50% of LOVO cell line (Bacac et al, 2016)

Figure 4. (A) CD40 reporter cells were cultured with CD40-CEA bsAbs in the presence of CEA+ cells and 0, 1 or 5 µg/ml of soluble CEA (sCEA). CD40 activation was assessed after 6h. (B) CD40 reporter cells were cultured with CD40-CEA bsAbs and cell lines expressing different levels of CEA.

CD40-CEA Neo-X-Prime bsAb status and future plans

CD40-CEA bsAb displays good tolerability in non-human primates (NHP)

Exploratory toxicity study in cynomolgus NHP model

NHP treated with up to 37.5 mg/kg CD40-cCEA show:

- ✓ No clinical observations
- ✓ No histopathological changes
- ✓ No treatment-related changes in serum cytokines

CD40-CEA lead program development

Status:

Lead molecules identified - preclinical characterization ongoing

Cell line development initiated

Opportunities:

Potential for combination with standard of care in cold, macrophage dense tumors, e.g. pancreatic and colorectal cancer

Potential for combination with PD-1 in hot tumors, e.g. gastric and lung cancer

Summary

- Neo-X-Prime is a novel concept for priming tumor neoantigen-specific T cells
- Neo-X-Prime bispecific antibodies targeting CD40 and TAA induce:
  - Activation of dendritic cells
  - Stronger anti-tumor effects compared to combination of monospecific Abs
  - Engagement of DC/exosome interactions
  - Enhanced cross-priming and proliferation of tumor neoantigen-specific T cells
  - Strong anti-tumor T cell responses and immunological memory
- CD40-CEA lead program:
  - Excellent performance in target-tailored assays
  - Good tolerability of target combination in NHP model
  - Lead molecules identified
  - Cell line development initiated
  - Opportunities to meet key needs in multiple solid cancer indications, including colorectal, lung, gastric, breast and pancreatic cancer

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