A bispecific antibody targeting CD40 and EpCAM induces superior anti-tumor effects compared to the combination of monospecific antibodies

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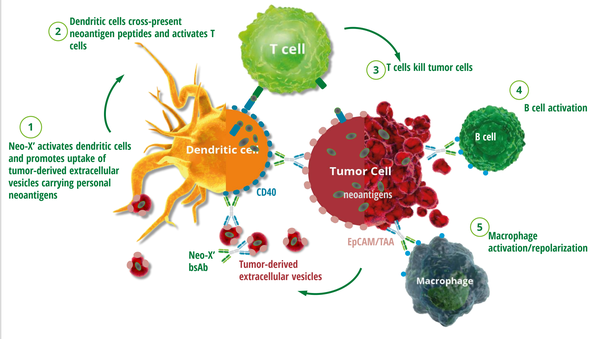
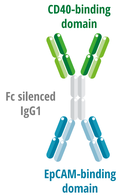
Proposed mode of action of Neo-X' antibodies





- Bispecific antibody within the Neo-X' concept targeting CD40 and EpCAM
- Designed to activate antigen presenting cells only in the presence of EpCAM
- Aims to increase the tumor neoantigen-specific T cell pool by targeting tumor extracellular vesicles to DCs and boosting cross-presentation of neoantigens

4224 induces superior anti-tumor effect compared to monotargeting antibodies

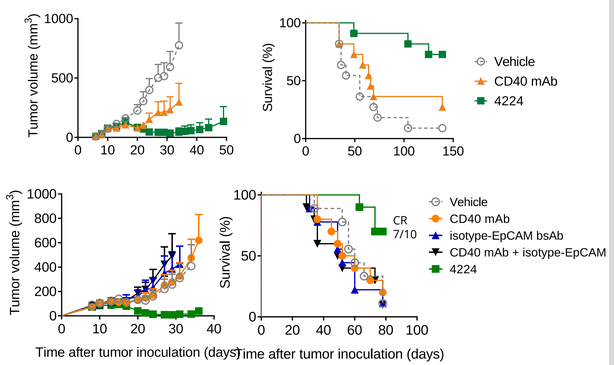


Figure 1. Human CD40 transgenic (hCD40tg) mice were inoculated with MB49-hEpCAM$^+$ cells s.c. and administered 100 µg anti-CD40 antibody (CD40 mAb) or an equivalent dose (167 µg) 4224 i.p. on days 10, 13 and 16 (top graphs). Alternatively, mice were inoculated with MB49-hEpCAM$^+$ cells s.c. and administered 100 µg CD40 mAb, 167 µg isotype-EpCAM bsAb, 100 µg CD40 mAb plus 167 µg isotype-EpCAM bsAb or 167 µg 4224 as previously (bottom graphs).

4224 induces tumor antigen dependent immune activation

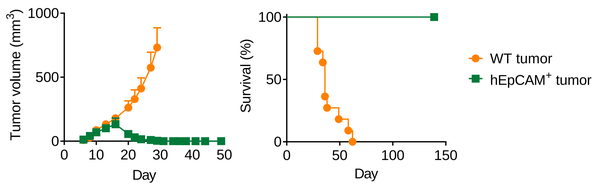


Figure 2. Human CD40 transgenic (hCD40tg) mice were inoculated with either MB49-WT or MB49-hEpCAM$^+$ cells s.c. and administered 417 µg 4224 i.p. on days 10, 13 and 16.

4224 induces broad tumor-specific immunity

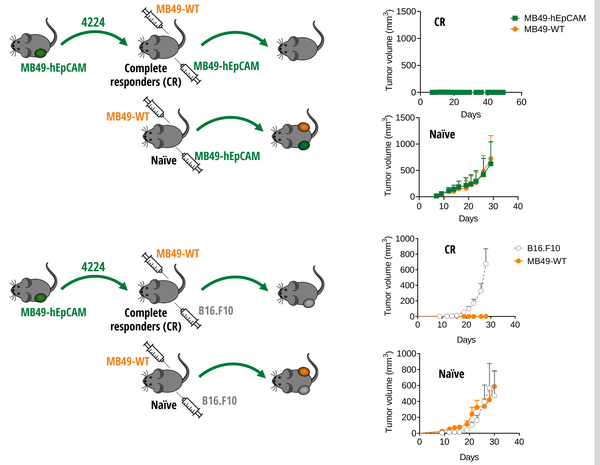


Figure 3. Naïve CD40tg mice or complete responders (CR) cured from MB49-hEpCAM$^+$ tumors by 4224 were rechallenged with the same tumor on one flank and an MB49-WT tumor on the other flank. Graphs show tumor volume of MB49-hEpCAM (green) and MB49-WT (orange) in CR (top) and naïve (second from top) mice. Alternatively, naïve or CR mice were rechallenged with MB49-WT tumor on one flank and an irrelevant tumor (B16.F10) on the other flank. Graphs show tumor volume for MB49-WT (orange) and B16.F10 (grey) in CR (second from bottom) and naïve (bottom) mice.

4224 displays low systemic immune activation

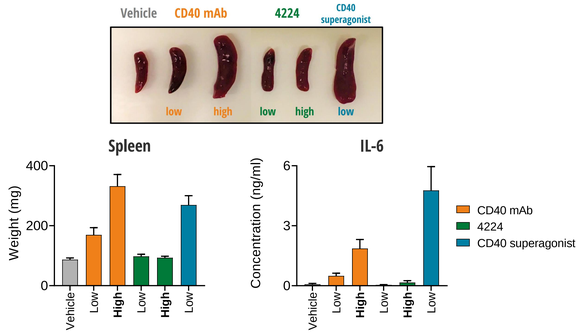


Figure 4. hCD40tg mice were inoculated with MB49-hEpCAM$^+$ cells s.c. and administered 100 (low) or 250 µg (high) CD40 mAb, or equivalent doses (167 or 417 µg) 4224, or 100 µg of a CD40 superagonist i.p. on days 10, 13 and 16. Systemic immune activation was evaluated by weighing spleens on day 19 (left) or by measuring plasma IL-6 levels 4 hrs after the second therapy dose (right).

4224 mediates EpCAM-dependent activation of dendritic cells

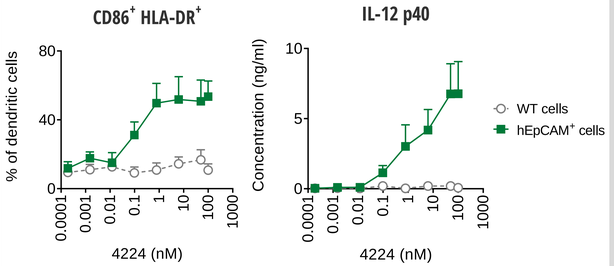


Figure 5. Human monocyte-derived dendritic cells were cultured with titrated 4224 in the presence or absence of hEpCAM expressed on CHO cells. After 2 days, expression of CD86 and HLA-DR on CD14$^-$ CD1a$^+$ dendritic cells was analyzed by FACS (left). Alternatively, supernatants were collected and IL-12 p40 content was analyzed by ELISA (right).

4224 mediates clustering of tumor debris and antigen presenting cells

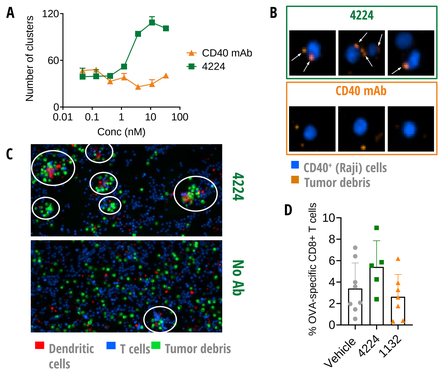


Figure 6. Fluorescently labeled CD40$^+$ Raji cells were cultured with fluorescently labeled hEpCAM$^+$ tumor debris in the presence of titrated CD40 mAb or 4224. Images were captured using a live cell imaging system (B) and clusters of CD40$^+$ cells co-localized with tumor debris were quantified after 8 hrs of culture (A). Fluorescently labeled dendritic cells from CD40tg mice were cultured with fluorescently labeled necrotic MB49 tumor cells expressing human EpCAM and membrane bound ovalbumin (OVA) and TCR transgenic OVA-specific T cells (OT1 cells) in the presence or absence of 4224. Images were captured after 12 h of coculture (C). OT1 cells were adoptively transferred to CD40tg mice bearing MB49-EpCAM-OVA tumors, followed by i.p. administration of 167 µg 4224 or 100 µg CD40 mAb. Mice were subsequentally 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 (D). The graph shows one representative experiment of three.

Summary

A Neo-X' bispecific antibody targeting CD40 and EpCAM

- Aims to turn tumor extracellular vesicles into autologous immune-activating neoantigen vaccination vehicles
- Mediates activation of dendritic cells and clustering of tumor debris and CD40$^+$ antigen presenting cells
- Induces a superior EpCAM-dependent anti-tumor effect compared to a combination of monotargeting compounds
- Induces a broad immunological memory against tumor antigen

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