An agonistic CD40 bispecific antibody designed for flexible synthetic peptide-cargo delivery through an affinity interaction, a way to ensure optimal T cell activation

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Background

CD40 agonistic antibodies targeting antigen-presenting cells rely on antigen presentation for optimal efficacy, as the co-stimulatory signal alone will not lead to T cell activation. Agonistic CD40 antibodies have a dose limited toxicity that can be avoided by low-dose subcutaneous administration combined with antigen presentation[1]. We have previously reported the development of a novel Adaptable Drug Affinity Conjugate (ADAC), based on an agonistic CD40 bispecific antibody carrying out a dual mode of action (immune stimulation and cargo delivery)[2]. The ADAC technology makes use of a high-affinity interaction between a short non-immunogenic peptide-tag (pTag) and a single-chain fragment (scFv) providing a modular peptide delivery platform enabling data-driven neoantigen-based drug production and delivery.

[1] L. Sandin et al, 2014 PMID: 24778163, DOI: 10.1158/2326-6066.CIR-13-0067
[2] M. Eltahir et al, 2022 DOI: 10.1002/adtp.202200008

Conserved agonistic activity with the bispecific format

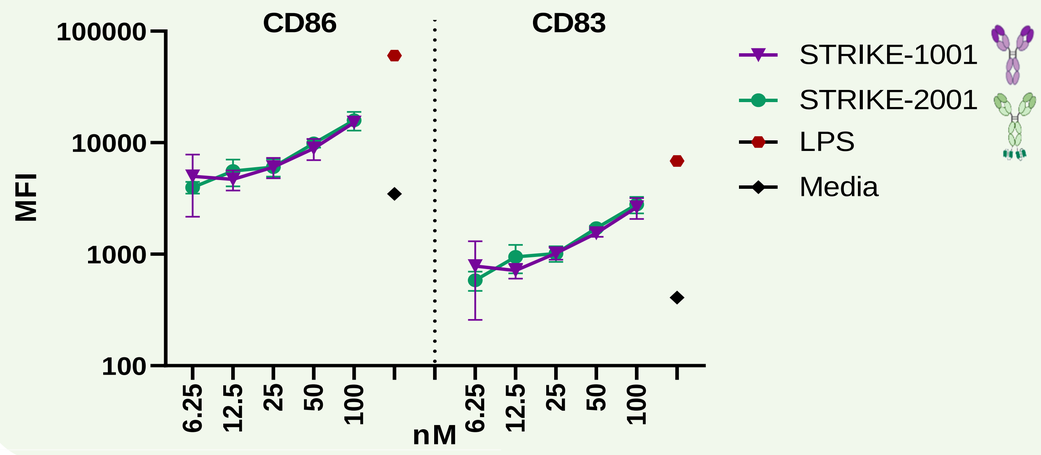
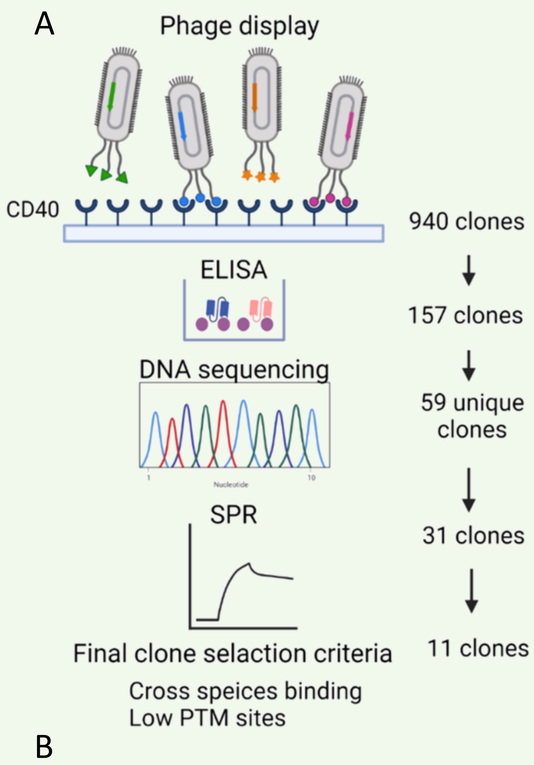
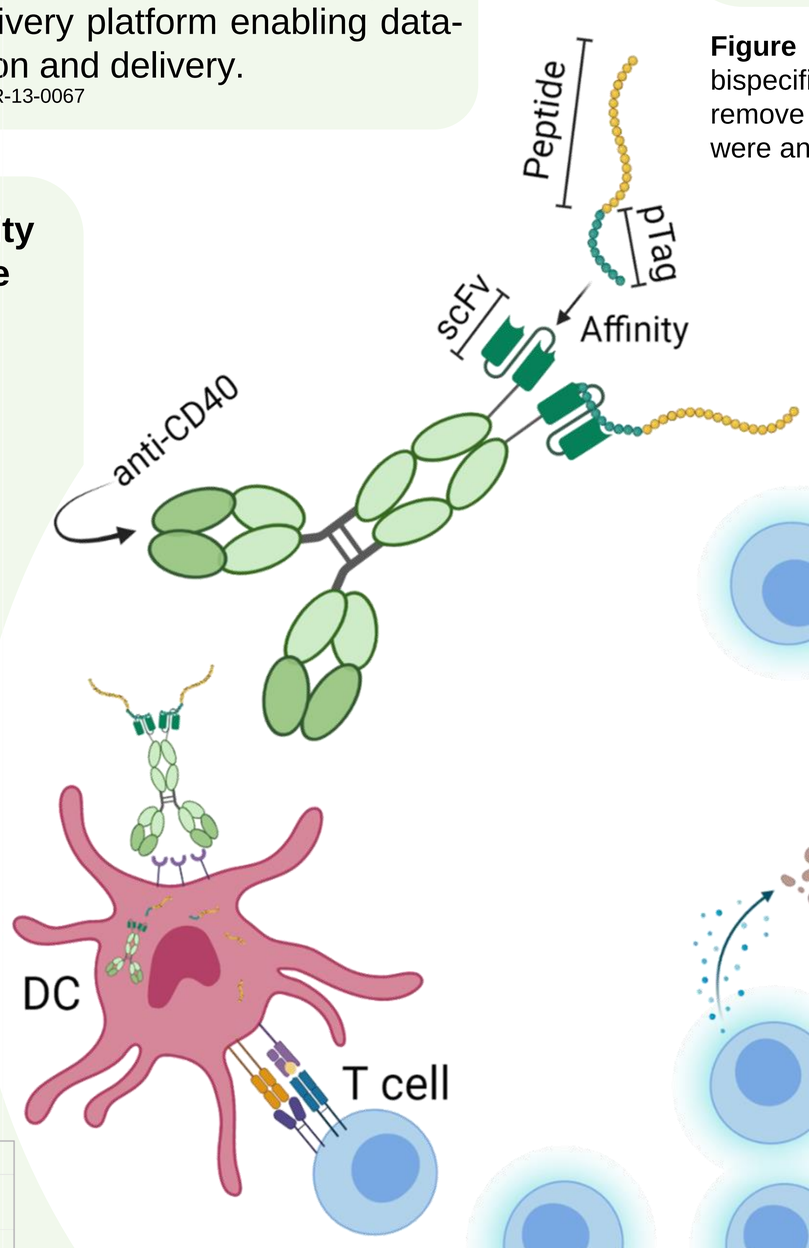
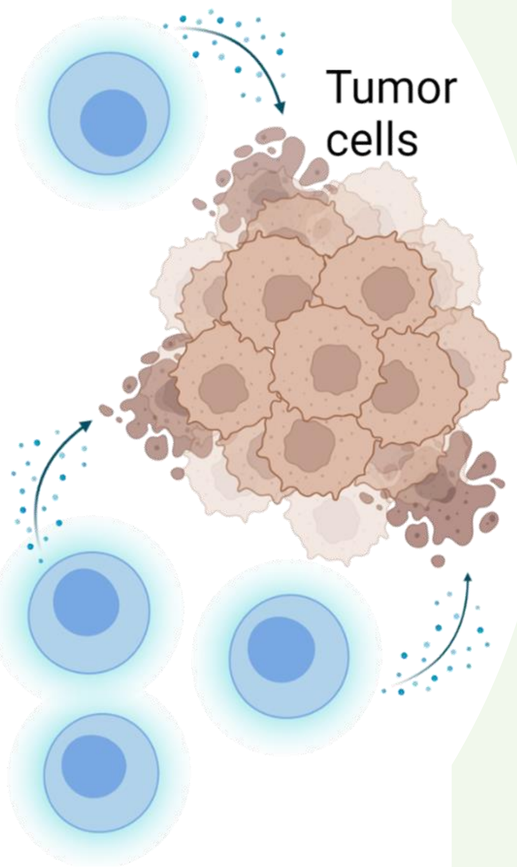


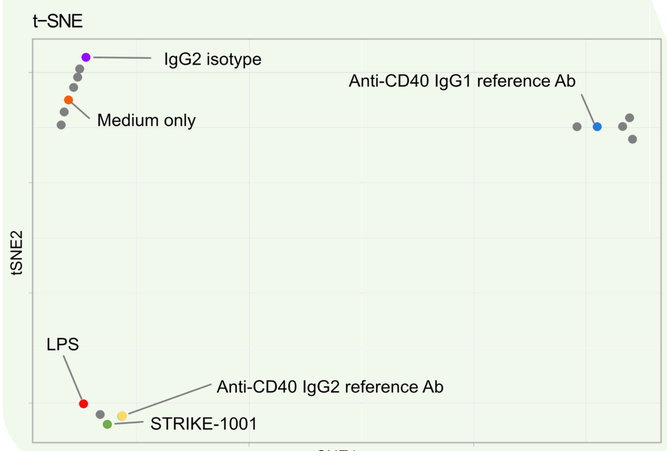
Figure 3. Human moDCs were stimulated with parental antibody (STRIKE-1001), bispecific antibody (STRIKE-2001) and LPS control for 6h prior 2x washing (to remove excess antibodies) and further incubated for a total of 48h and were analysed by flow cytometry for CD86 and CD83 expression.

Drug candidate and agonistic activity screening to identify final candidate

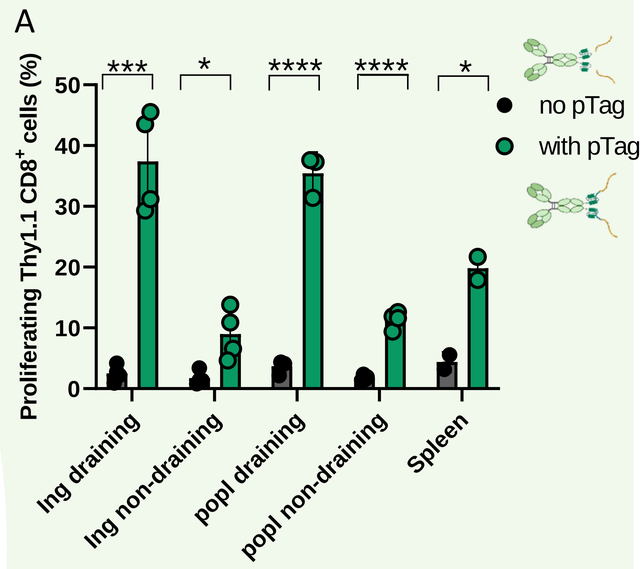








Superior T-cell activation/expansion induced with the bispecific antibody



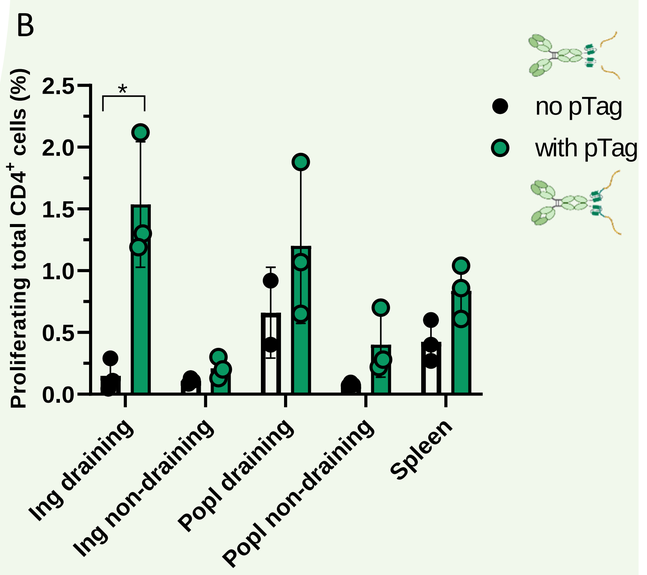


Figure 1. A) Phage display selection workflow **B)** Human monocyte derived DCs (moDC) were stimulated with 250nM of each antibody for 48h and were analysed by flow cytometry for MHC-II, CD86, CD83 and CD40 upregulation illustrated with a t-SNE plot illustrating the clustering based on agonistic activity.

Figure 4. T cells derived from transgenic mice that carries a T cell receptors specific for A) a class I epitope of hgp100 (PMEL) or **B)** a class II epitope from ovalbumin (OTII) were injected into tgCD40 mice prior vaccination with 15pmol STRIKE-2001 antibody with 2.5X mol ratio of respectively peptide with or without pTag. T cell proliferation was tracked by CSFE labelling measured post 96h. Ing = inguinal, popl = popliteal

Epitope and off-target binding of STRIKE-1001

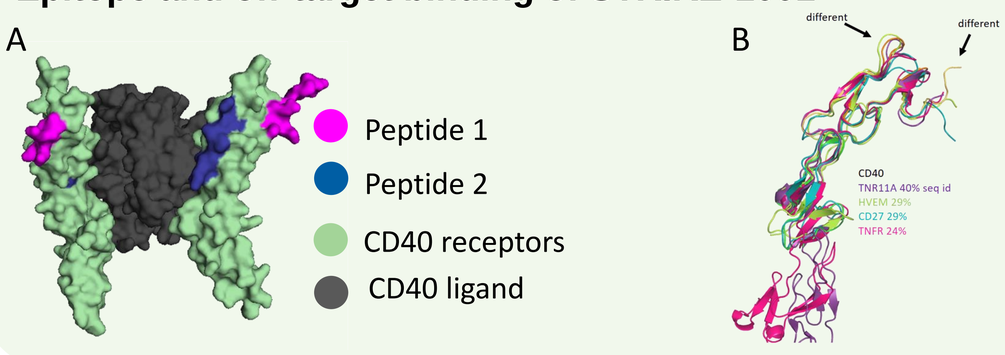


Figure 2. **A)** Epitope mapping through HDX-MS, with no overlap of the CD40 ligand binding sites. **B)** Off-target screening of receptors with homologous folded structure. TNR11A (RANK) was identified as an off-target risk candidate. SPR analysis showed no binding by STRIKE-1001 to TNR11A protein (data not shown).

Conclusion

We have developed a first-in-class drug delivery strategy using the ADAC technology. ADAC facilitates a flexible peptide cargo delivery strategy ensuring antigen-specific *in vivo* T cell priming via a CD40-targeting approach. The flexibility of cargo delivery provided by the affinity interaction enables the transformation of the drug development pipeline into omics-driven immunotherapy approaches. The dual effector functions of the bispecific format, together with local administration, allows for T cell activation at a lower dose ensuring optimal drug efficacy and safety.

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