The development of an Adaptable Drug Affinity Conjugate (ADAC) targeting CD40 for a flexible therapeutic peptide cargo delivery to dendritic cells

Ida Laurén [1,3], Mohamed Eltahir [1,3], Aman Mebrahtu [2,3], Rosanne Veerman[3], Juan Astorga [4], Aljona Saleh [1], Jimmy Ytterberg [1], Annika Lindqvist [1], Leif Dahllund [4], Anders Olsson [4], Oskar Andersson [4], Johan Rockberg [2,3], Helena Persson [2,4], Sara Mangsbo [1,3].

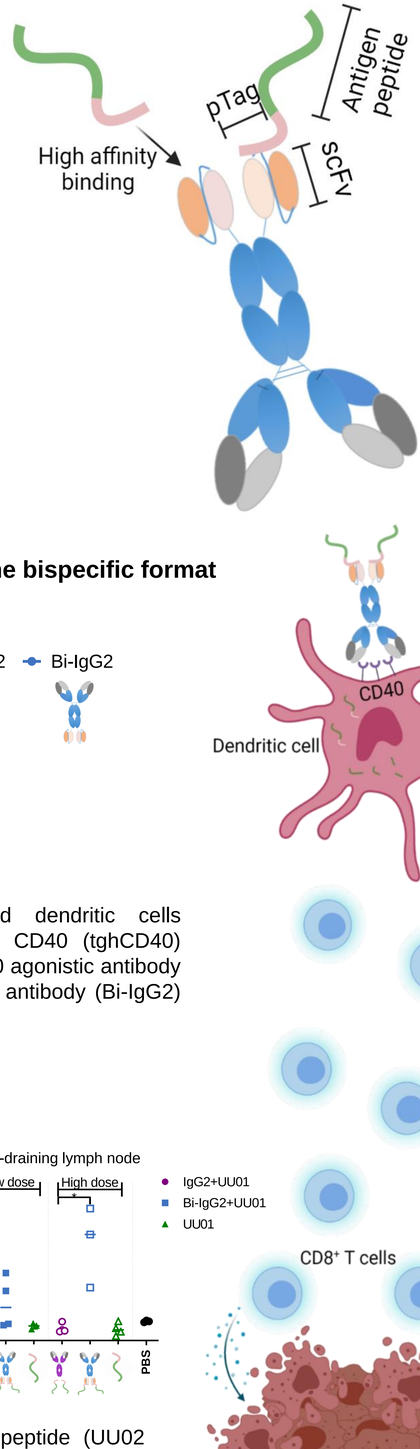
[1]Uppsala University, Uppsala, Sweden, [2]KTH Royal Institute of Technology, Stockholm, Sweden, [3]Strike Pharma AB, Uppsala, Sweden, [4]Karolinska University, Stockholm, Sweden

AACR abstract #2890

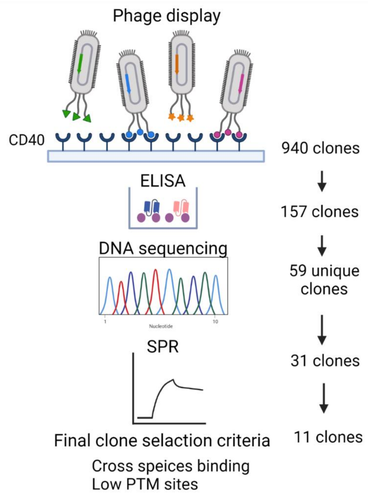
Proof of concept of the ADAC technology

Background

CD40 agonistic antibodies targeting antigen-presenting cells rely on simultaneous antigen presentation for optimal efficacy, as the co-stimulatory signal alone will not lead to T cell activation. Herein we have developed a novel Adaptable Drug Affinity Conjugate (ADAC), a refinement of a traditional Antibody Drug Conjugate (ADC) with a focus on tailored drug design. The ADAC technology relies on a high-affinity interaction between a short non-immunogenic peptide-tag (pTag) and a single-chain fragment (scFv).



Drug candidate screening



Agonistic activity screen of novel anti-CD40 antibodies

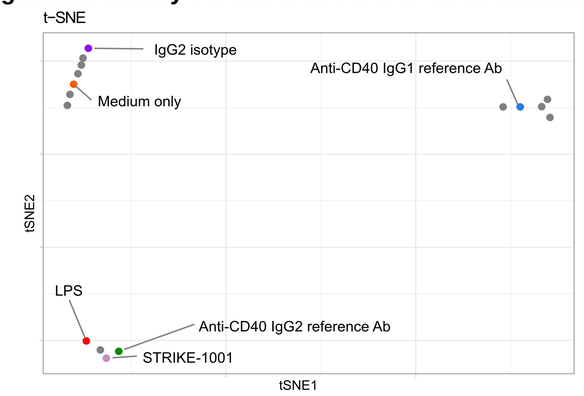


Figure 4. Human monocyte-derived dendritic cells (moDCs) were stimulated with a dose titration of eleven anti-CD40 antibody clones (IgG2 format) for 48h and were analysed for upregulation of MHC-II, CD86, CD83 and CD40 using flow cytometry. Data illustrated with a t-SNE plot. Herewith data from the 250 nM stimulation. In grey are all clones analysed but not pursued further. STRIKE-1001 cluster with the positive controls.

Improved peptide stability

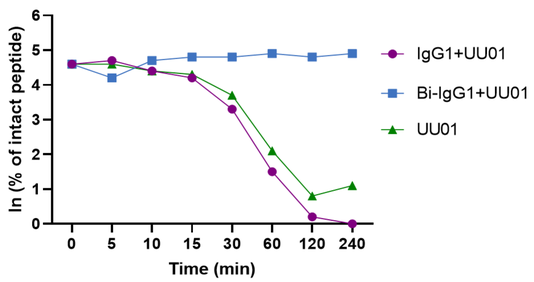


Figure 1. The synthetic long peptide (SLP) UU01 (pTag+gp100-derived MHC class I epitope) alone or mixed with an anti-CD40 agonistic antibody (IgG1) or the designed bispecific antibody (Bi-IgG1) and incubated in mouse plasma before analysed by mass spectrometry set to detect intact peptides to measure peptide stability over time.

Retained agonistic activity in the bispecific format

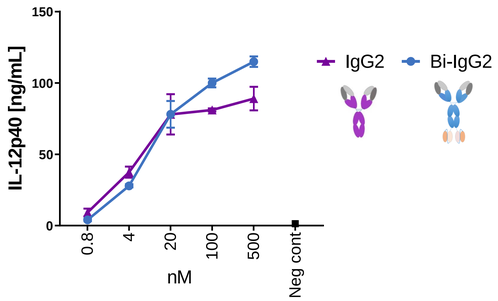


Figure 2. Bone marrow-derived dendritic cells (BMDCs) from transgenic human CD40 (tghCD40) mice were incubated with anti-CD40 agonistic antibody (IgG2) and the designed bispecific antibody (Bi-IgG2) for 48h.

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

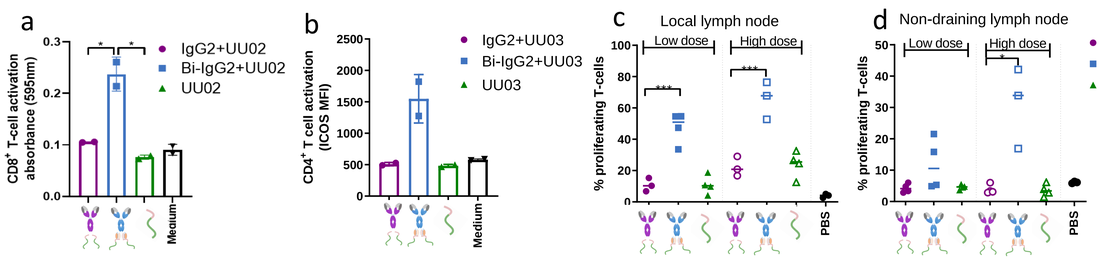


Figure 3. (a) B3Z CD8⁺ T cell co-culture with tghCD40 BMDCs stimulated with 125nM peptide (UU02 SLP=pTag+OVA-derived MHC class I epitope) and 10nM of anti-CD40 agonistic antibody (IgG2 or Bi-IgG2). (b) OT-II CD4⁺ T cells in co-culture with tghCD40 BMDCs stimulated with 10nM antibody (IgG2 or Bi-IgG2) and 20nM SLP (UU03=pTag+OVA-derived class II epitope). (c) Antibodies (IgG2 or Bi-IgG2) were administrated in a low (7.5pmol) and higher (22.5pmol) concentration with 2.5 molar ratio of SLP (UU01=pTag+gp100-derived MHC class I epitope). Proliferation of PMEL CD8⁺ T cells (CFSElow) was analysed 4 days after cell transfer.

Epitope mapping of STRIKE-1001

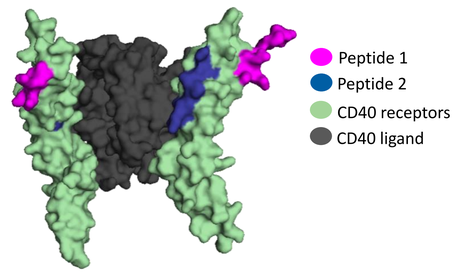


Figure 5. Epitope mapping through HDX-MS with no overlap with CD40 ligand.

Off-target analysis of STRIKE-1001

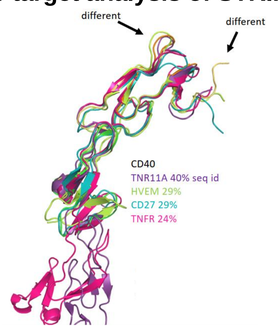


Figure 6. Off-target screening of other receptors in the tumor necrosis factor receptor superfamily (TNFRSF) with the similar folded structure as CD40. TNR11A (RANK) was identified as a risk. SPR analysis showed that STRIKE-1001 did not bind the TNR11A protein (not shown).

Agonistic activity of the selected clone in an IgG2 and the bispecific format

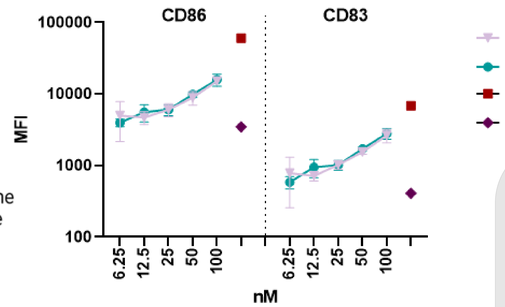


Figure 7. Human MoDCs were stimulated with parental antibody (STRIKE-1001) and bispecific antibody (STRIKE-2001) for 48h and were analysed by flow cytometry for CD86 and CD83 upregulation. The bispecific format maintains full agonistic activity.

Conclusion

We have developed a first-in-class drug delivery strategy using the ADAC technology. ADAC is designed to meet the demands of a flexible peptide cargo drug delivery strategy, ensuring *in vivo* T cell priming via a CD40-targeted approach. Simultaneous immune activation and peptide presentation ensures optimal drug efficacy and safety.