The Adaptable Affinity Drug Conjugate (ADAC™) targeting CD40 facilitates modular neoantigen peptide delivery through affinity-based drug cargo delivery

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Background

Agonistic anti-CD40 therapy has shown promising results in pre-clinical models but the narrow therapeutic window upon intravenous (i.v) administration limits its use. Additionally, monoclonal agonistic CD40 therapies depend on antigen presentation, in addition to its inherent immune stimulation capacity, for efficient tumor-specific T cell activation. We have developed a format named the **Adaptable Drug Affinity Conjugate (ADAC)** that facilitates co-delivery of CD40 stimulation and antigen delivery to enable subcutaneous drug administration and efficient local immune activation. ADAC is based on a novel agonistic CD40 antibody equipped with unique scFvs, enabling rapid cargo loading by an high affinity interaction to a peptide tag (pTag), eliminating the need for complex chemistry or linkers for antigen release.

1. The agonistic activity remains when converted to the bispecific format

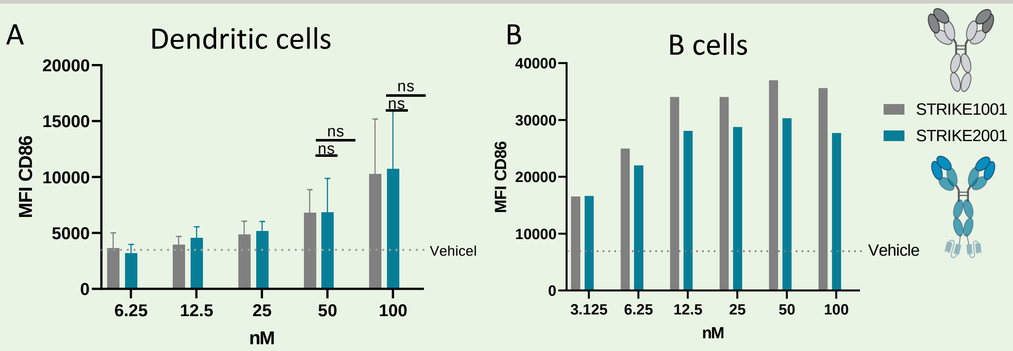
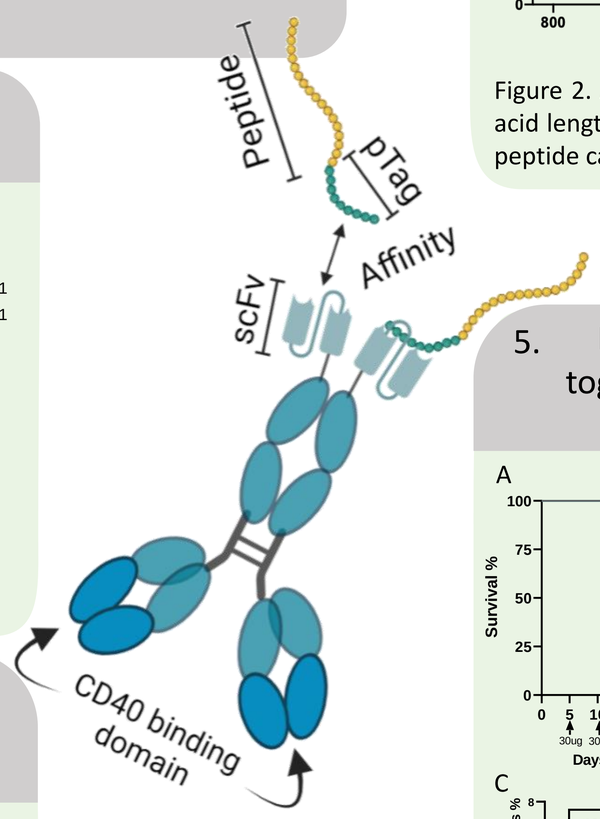


Figure 1. Agonistic activity of the monoclonal Ab (STRIKE1001) and bispecific Ab (STRIKE2001) was determined with (A) human moDCs and (B) B cells by upregulation of CD86 analysed by flow cytometry post 48h stimulation for moDCs and 24h for B cells.



2. Trimming of pTag or cargo variation does not impact the loading capacity on STRIKE2001

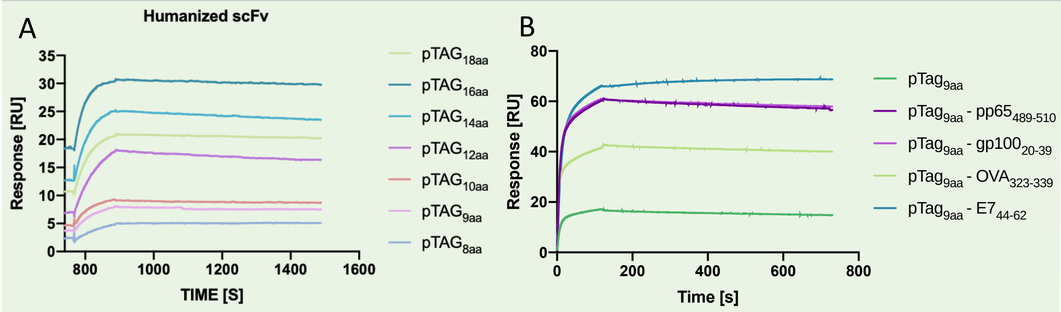


Figure 2. A) SPR sensogram of STRIKE2001 binding to pTag sequence with indicated amino acid length. B) SPR sensogram of STRIKE2001 binding to the pTag synthesized with different peptide cargo.

3. The ADAC technology induces both CD8⁺ and CD4⁺ antigen-specific T cell expansion in vivo

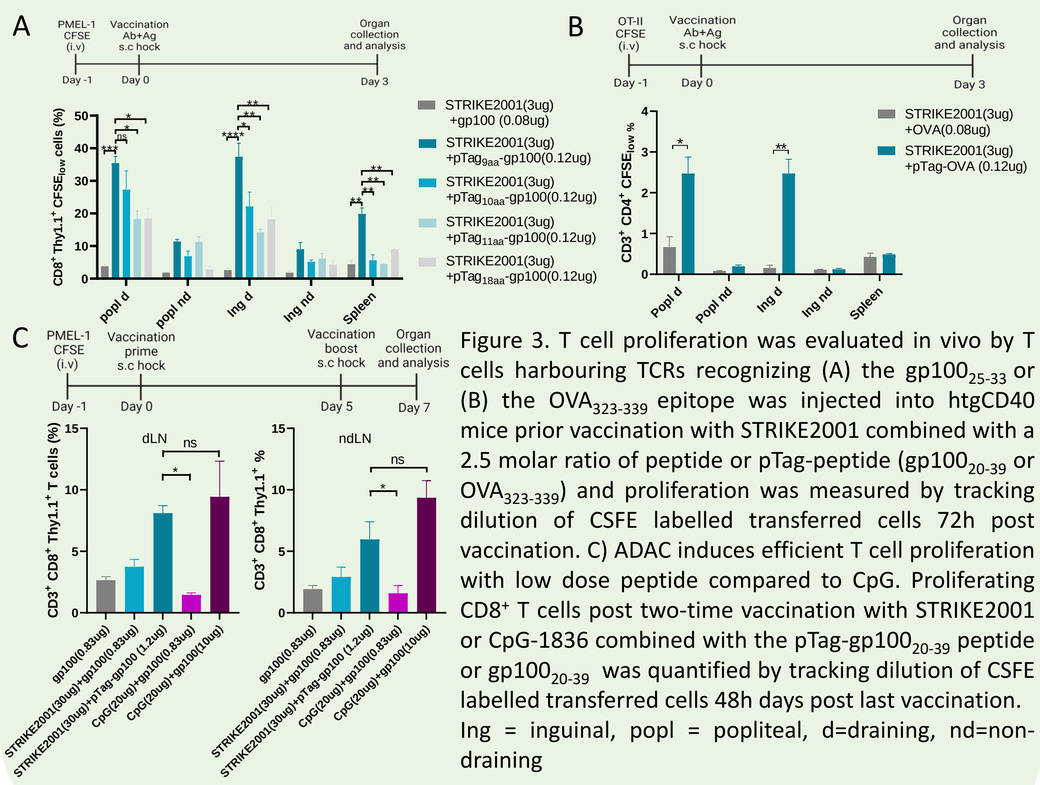
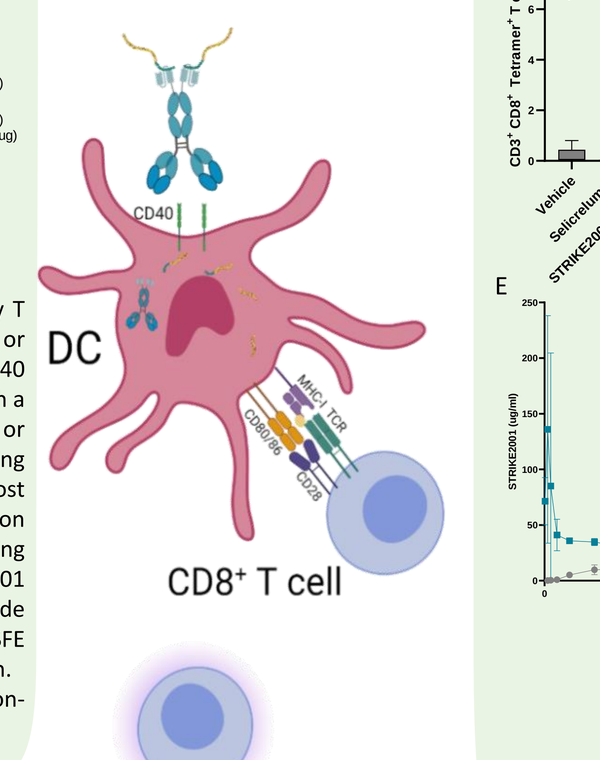


Figure 3. T cell proliferation was evaluated in vivo by T cells harbouring TCRs recognizing (A) the $gp100_{25-33}$ or (B) the $OVA_{323-339}$ epitope was injected into htgCD40 mice prior vaccination with STRIKE2001 combined with a 2.5 molar ratio of peptide or pTag-peptide ($gp100_{20-39}$ or $OVA_{323-339}$) and proliferation was measured by tracking dilution of CSFE labelled transferred cells 72h post vaccination. C) ADAC induces efficient T cell proliferation with low dose peptide compared to CpG. Proliferating CD8⁺ T cells post two-time vaccination with STRIKE2001 or CpG-1836 combined with the pTag-$gp100_{20-39}$ peptide or $gp100_{20-39}$ was quantified by tracking dilution of CSFE labelled transferred cells 48h days post last vaccination. Ing = inguinal, popl = popliteal, d=draining, nd=non-draining



4. Co-delivery of CD8 and CD4 T cell epitopes by ADAC generates synergistic T cell proliferation and activation

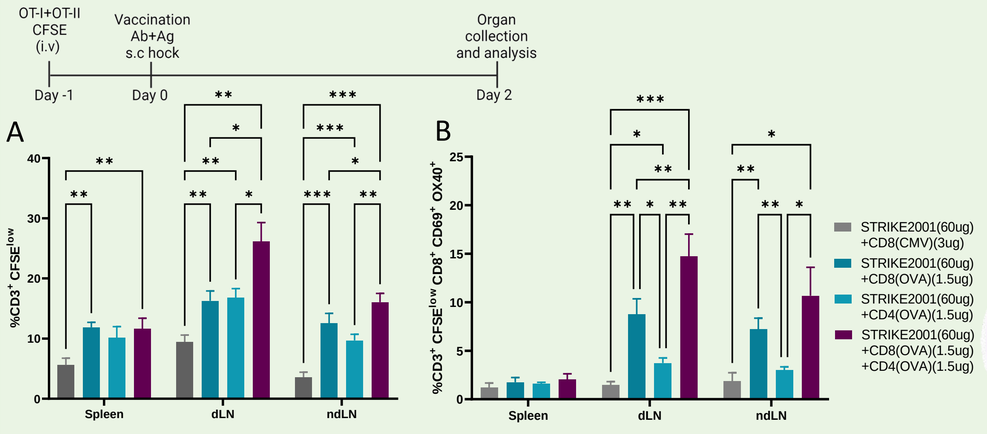


Figure 4. A) The T cell activation and proliferation was evaluated using CD8 and CD4 epitopes alone or in combination. T cells harbouring TCRs recognising the $OVA_{257-264}$ (CD8) and $OVA_{323-339}$ (CD4) were mixed and injected into htgCD40 mice prior vaccination with STRIKE2001 loaded with only control peptide $NLV_{488-510}$ (CMV), mixed with $OVA_{252-264A6K}$ (CD8) and $OVA_{323-339}$ (CD4) or $OVA_{252-264A6K}$ (CD8) mixed with $OVA_{323-339}$ (CD4) and CD8⁺ T cell proliferation (A) was measured by tracking CSFE dilution of the labelled cells and (B) activation markers 48h post vaccination. d=draining, nd=non-draining

5. In vivo anti-tumor response elicited by the ADAC platform together with superior anti-tumor response and lower toxicity compared to selicrelumab

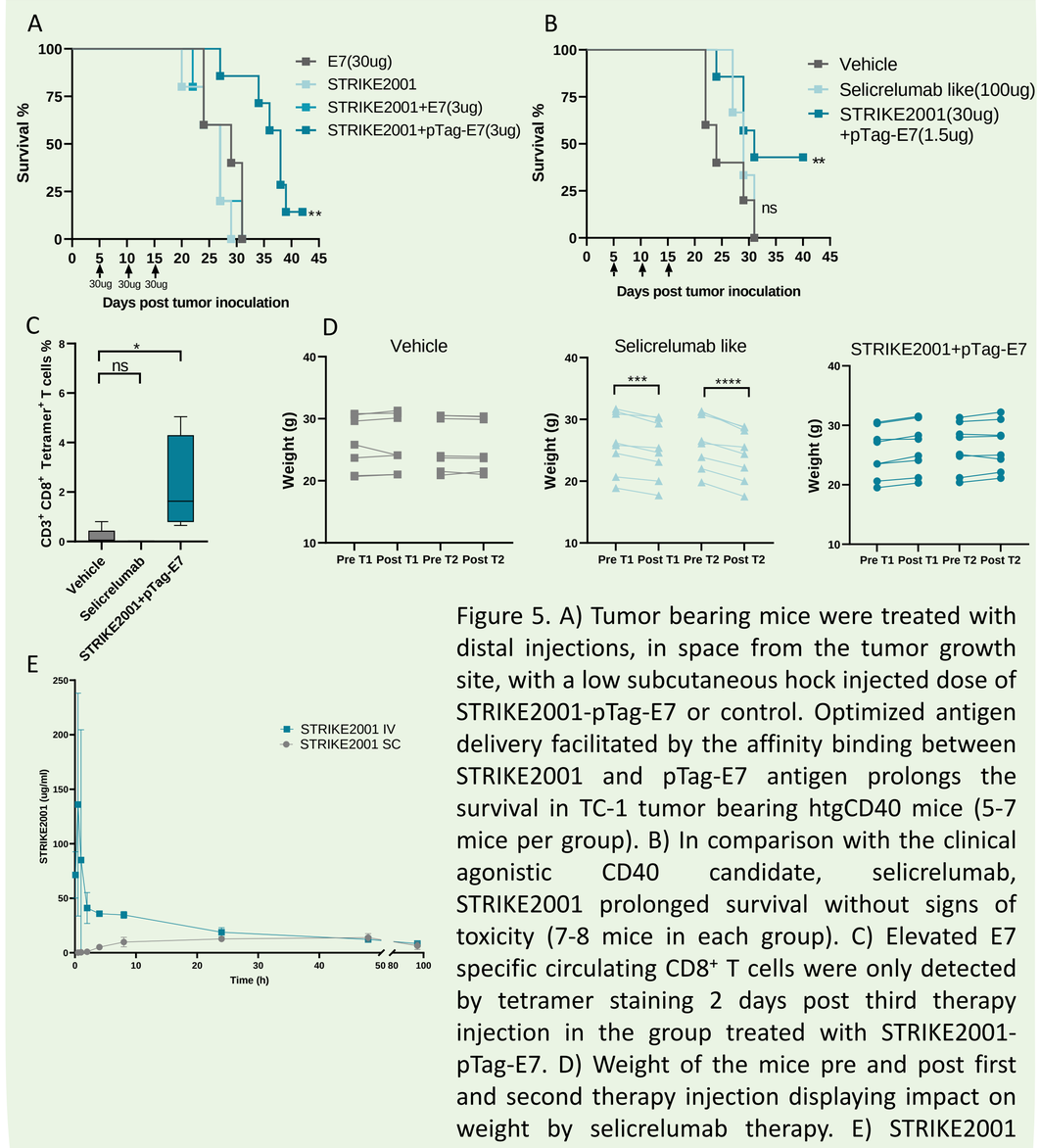
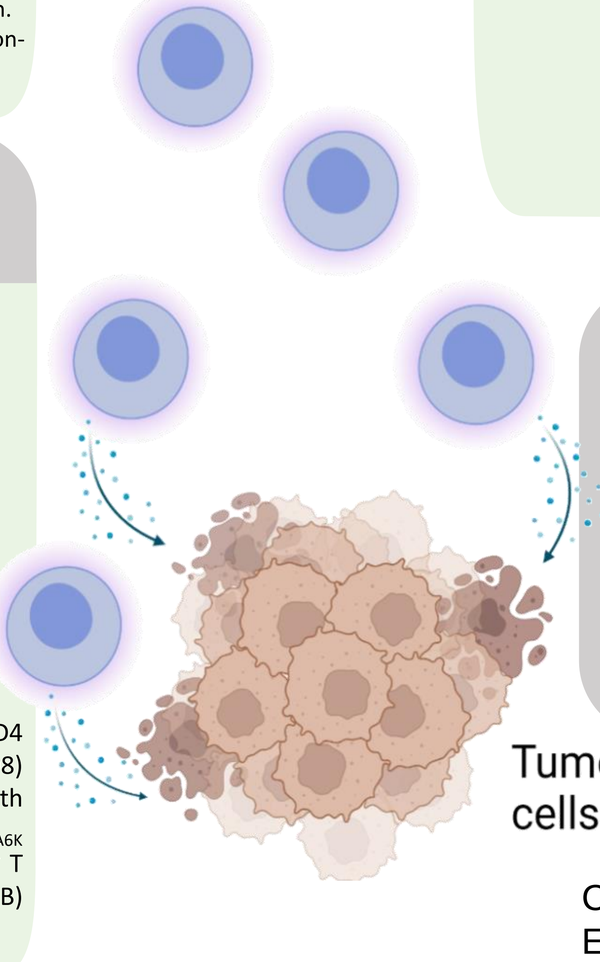


Figure 5. A) Tumor bearing mice were treated with distal injections, in space from the tumor growth site, with a low subcutaneous hock injected dose of STRIKE2001-pTag-E7 or control. Optimized antigen delivery facilitated by the affinity binding between STRIKE2001 and pTag-E7 antigen prolongs the survival in TC-1 tumor bearing htgCD40 mice (5-7 mice per group). B) In comparison with the clinical agonistic CD40 candidate, selicrelumab, STRIKE2001 prolonged survival without signs of toxicity (7-8 mice in each group). C) Elevated E7 specific circulating CD8⁺ T cells were only detected by tetramer staining 2 days post third therapy injection in the group treated with STRIKE2001-pTag-E7. D) Weight of the mice pre and post first and second therapy injection displaying impact on weight by selicrelumab therapy. E) STRIKE2001 concentration in plasma after s.c or i.v injection of 2 mg/kg of antibody at indicated time-points measured by ELISA.



Conclusion

The developed ADAC technology facilitates flexible cargo delivery through a high-affinity interaction and ensures efficient T cell priming through CD40 targeting. The dual effector functions allow, by the bispecific format, local low dose administration with retained specific immune activation without risks of systemic toxicity. The developed ADAC drug candidate, STRIKE2001, is in pre-clinical development and is envisioned to allow for tailored neoantigen delivery for individualized cancer vaccine strategies in the clinic.

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