Modular peptide cargo delivery by targeting CD40 enables ligandome driven, precision immunotherapy via the Adaptable Drug Affinity Conjugate (ADAC™) technology

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The ADAC technology

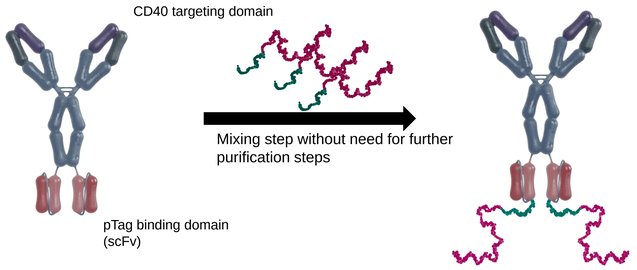
Background

Agonistic antibodies are designed to activate the immune system and aid in T cell priming. However, optimal T cell priming cannot be achieved without considering both antigen presentation and co-stimulation in conjunction. The effectiveness of CD40 agonistic antibodies is supported by in vivo studies showing that anti-tumor responses are mediated by cytotoxic T cells. This highlights the importance of antigen presentation in the step towards drug effectiveness.

To mediate effective anti-tumor responses with the powerful CD40 protein as a target, drug design should incorporate peptide antigens as cargo with the monoclonal antibody. To achieve this, we have developed the **Adaptable Drug Affinity Conjugate (ADAC)** technology. ADAC is a next-generation Antibody Drug Conjugate (ADC) that brings the antibody and peptide cargo together via a non-covalent high affinity (subnanomolar) interaction. This facilitates modular delivery of peptide cargo and improves T cell priming in non-tumor areas to avoid the suppressive tumor microenvironment during drug-mediated T cell priming.

STRIKE2001

STRIKE2001-[pTag]-[T cell antigen]



The ADAC platform comprises a designed tetravalent antibody called STRIKE2001, which consists of a CD40-binding component and a single-chain variable fragment (scFv) that binds to a peptide tag (pTag). This unique combination enables the rapid formation of a drug product as soon as the antibody and peptide are mixed in a 1:3 ratio. The peptide can be either an off-the-shelf product or customized based on the patient's tumor genetic information. This innovative approach offers promising possibilities for more personalized cancer treatments.

Precision immunotherapy using modular peptide cargo and an off-the shelf antibody-carrier

Sequencing and epitope prediction using tumor material and reference normal tissue

Synthetic peptide production small or large scale

In hospital mixing

Subcutaneous dosing

Figure generated by Biorender

STRIKE2001, a lower affinity but potent CD40 agonistic antibody

Figure 1 STRIKE2001 displays robust Fc independent agonistic activity

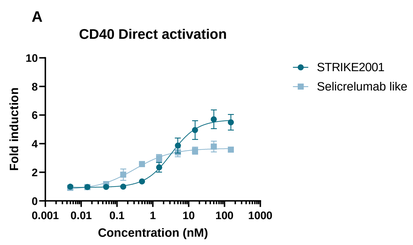
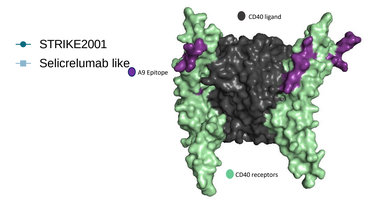


Figure 2



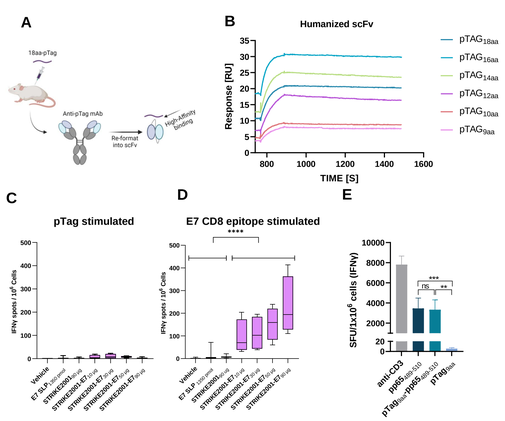
In comparison to selicrelumab, STRIKE2001 binds to CD40 with lower affinity (1.2-2.3 nM) as measured by Fc-CD40, but does not bind to monomeric CD40 (not shown). To compare STRIKE2001 and selicrelumab, we used a reporter assay that expresses CD40 and can be monitored via a luminescence-based readout upon CD40 ligation (CD40 bioassay, Promega). As demonstrated in Yu et al. Nature 2023 [1], lower affinity antibodies retain potent agonistic activity, as shown in Figure 1A, with a calculated EC50 value of 3.3 nM, which is not heavily impacted (EC50 3.9 nM) by the addition of Fc receptor-expressing CHO cells (Figure 1B). Selicrelumab initiates activation at lower doses (EC50 0.3 nM) due to its capability of binding monomeric CD40, but it does not appear to mount as high an activation profile as the lower affinity STRIKE2001. In line with Yu et al. Nature 2023, this could be due to the fact that the lower affinity antibody STRIKE2001 improves CD40 clustering through avidity, while the higher affinity antibody selicrelumab competes out dual-CD40 binding at higher antibody concentrations, leading to poorer CD40 clustering. Selicrelumab shows a high potent CD40 stimulation index (EC50 0.01 nM) when adding Fc receptor-expressing cells, which suggests that its activity could be unpredictable in vivo. The data represents pooled data from 2 independent experiments.
Reference 1: https://doi.org/10.1038/s41586-022-05673-2

STRIKE2001 has been selected as the final drug candidate (CD), based on the screening of ten different tetravalent formats, with a focus on changes in scFv positioning on the heavy or light chain, along with isotype and scFv linker variation. The final CD was noted to have excellent developability, showing high titer in transient CHO production, and excellent monomeric output (>95%), in line with reference IgGs produced.

The final design of STRIKE2001 is an IgG2 format with scFv positioning on the heavy chain. The CD40 agonistic backbone is based on clone A9, identified in a phage display screening with a focus on affinity, epitope binding (Figure 2), and agonistic activity. The clone does not block CD40L binding, has an affinity of around 1.2-2.3 nM, and has no noted off-target stickiness. STRIKE2001 does not bind to any other proteins than CD40, as per the Retrogenic Cell Microarray analysis performed. The scFv is humanized, and STRIKE2001 is deimmunized.

Retained Activity and No Immunogenicity Risks Identified After Peptide Tag Trimming

Figure 3 Trimmed tag displays no immunogenicity risks

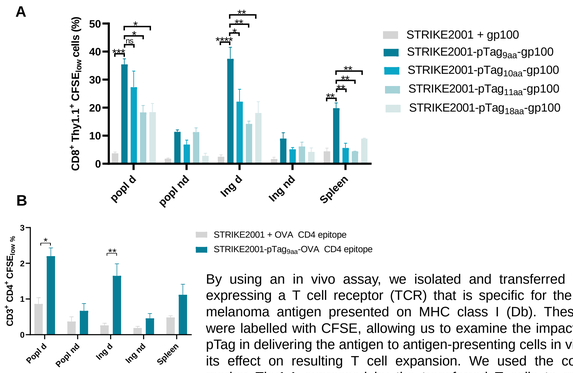


The pTag initially used for binding to STRIKE2001 and facilitating antigen delivery was a longer sequence. However, the tag was subsequently trimmed down to a minimal epitope that allowed for retained binding to the humanized scFv, as shown in Figure 3A. The trimming process enabled binding to a length of 9 amino acids (aa), which was confirmed by surface plasmon resonance (SPR) in Figure 3B.

To assess the immunogenicity of the 9aa sequence, we performed repeated vaccinations (prime/boost/boost) with the peptide-loaded STRIKE2001 at various doses (n=16 exposed mice in total, 4 mice per dose). The peptide was the 9aa pTag linked to the $E7_{44-62}$ derived synthetic peptide known to harbor a strong CD8 epitope (which is capable of inducing T cell responses. An ELISpot assay (Mabtech) was performed, Figure 3C/D. No T cell inducing capability was observed after stimulating isolated lymphocytes with the 9aa tag. In addition, no response was noted in recall evaluations using PBMCs from healthy donors (n=9), as shown in Figure 3E. As a positive control, PBMCs were stimulated with the tagged and non-tagged pp65 SLP.

Furthermore, the trimmed pTag displayed no or reduced binding to endogenous human IgG (data not shown).

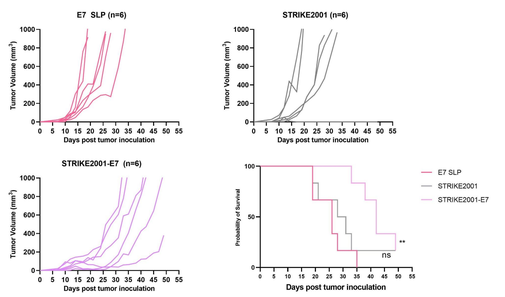
Figure 4 trimmed tag mediates cargo loading on STRIKE2001 that is superior to mixed unloaded product



By using an in vivo assay, we isolated and transferred T cells expressing a T cell receptor (TCR) that is specific for the gp100 melanoma antigen presented on MHC class I (Db). These cells were labelled with CFSE, allowing us to examine the impact of the pTag in delivering the antigen to antigen-presenting cells in vivo and its effect on resulting T cell expansion. We used the congenic marker Thy1.1 expressed by the transferred T cells to measure proliferation. The results showed that proliferation was improved in all draining lymph nodes when the antigen (pTag-$gp100_{20-39}$ SLP) was delivered via the STRIKE2001-pTag formulation. Furthermore, there was a slight improvement in proliferation upon pTag trimming (Figure 4A). In addition, CD4 T cell proliferation, measured using CFSE-labelled $OVA_{323-339}$-specific transgenic CD4 T cells, was also enhanced by STRIKE2001 antigen delivery compared to mixing the antibody and the antigen without providing the ADAC mediated interaction (Figure 4B).

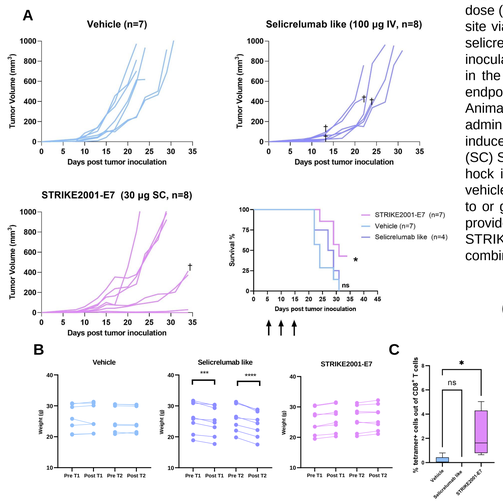
Subcutaneous Low-Dose STRIKE2001-$E7_{44-62}$ Therapy Induces Circulating Antigen-Specific T Cells and Improves Anti-Tumor Responses Compared to Selicrelumab Infusion

Figure 5 STRIKE2001 peptide formulated therapy is superior to higher dose of peptide or STRIKE2001 alone



The $E7_{44-62}$-derived synthetic peptide, which contain one potent CD8 epitope and two CD4 epitopes, or STRIKE2001 alone, when dosed in the hock opposite to where the tumor was inoculated, did not lead to tumor growth inhibition. However, when the tagged E7 peptide was delivered via STRIKE2001, it greatly improved anti-tumor responses and significantly prolonged the survival of mice with TC1-inoculated tumors compared to the peptide alone group. STRIKE2001 was administered on days 5, 10, and 15 at doses of 50 µg, 30 µg, and 30 µg, respectively, while the E7 peptide alone was administered at a dose of 30 µg. The combination therapy was dosed on the same days and at the same doses as STRIKE2001 alone, but employed only 3 µg of tagged E7 peptide. The TC-1 cell-line was kindly provided to Uppsala University by TC Wu at Johns Hopkins University.

Figure 6 Low dose peptide cargo-loaded STRIKE2001 reduces tumor growth without any noted adverse events



The formulated STRIKE2001-E7, consisting of 30µg of STRIKE2001 and a low dose (1.5µg) of pTag-E7 SLP (at a 1:3 molar ratio), was injected into the non-tumor site via hock injection. This was compared to an intravenous infusion of 100µg of selicrelumab, with all injections administered on day 5, 10, and 15 after tumor inoculation (Figure 6A). Mice that experienced weight loss greater than 10% (n=2 in the selicrelumab group) or had wounds were sacrificed according to humane endpoints (marked with a dagger) and were not included in the survival analysis. Animals treated with selicrelumab showed significant weight loss after the administration of doses 1 and 2 (Figure 6B). Additionally, selicrelumab did not induce circulating antigen-specific T cells at day 17, while low-dose subcutaneous (SC) STRIKE2001-E7 therapy did (Figure 6C). Notably, STRIKE2001-E7 dosed via hock injection at the non-tumor site significantly improved survival compared to vehicle-treated animals (n=7). The experimental endpoint was set at tumors equal to or greater than 1 cm3. The H-2Db RAHYNIVTF tetramer produced and kindly provided to Uppsala University by NIH Tetramer Core Facility. Dose-optimization of STRIKE2001 and peptide will be performed ahead as well as assessment with combination treatment strategies. NOTE: *Experiment is still ongoing*.

Our study shows that it is possible to develop a new antibody-cargo format (ADAC) that can be adapted to the genetic makeup of tumors, without the need to create a new drug entity for each cargo selection. This approach has the potential to streamline the drug development process and pave the way for more personalized and effective cancer treatments.