STRIKE2001-KRAS, a novel CD40-targeting agonistic antibody carrying KRAS peptide cargo via a modular drug cargo loading principle (ADAC™) leading to simultaneous agonism and antigen-presentation in vivo.

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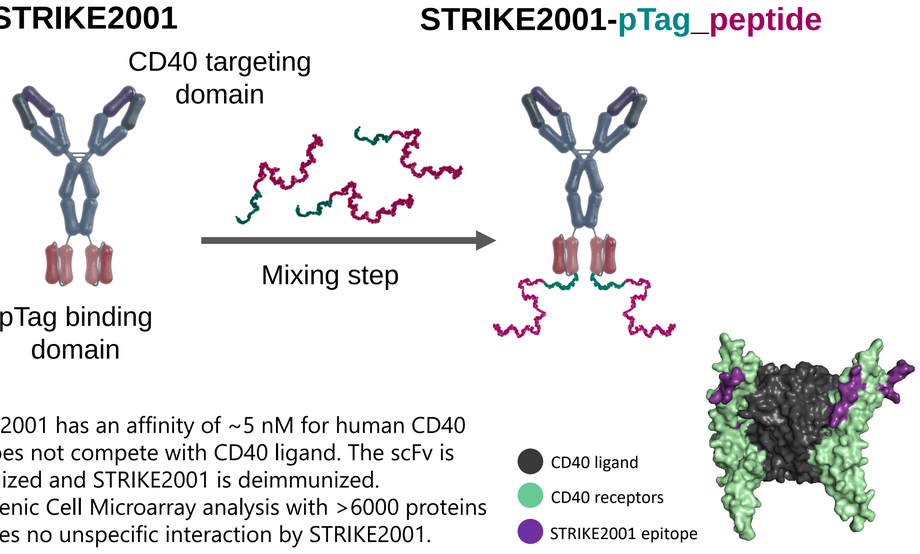
Background

Agonistic CD40 targeting antibodies have demonstrated promising pre-clinical efficacy but only moderate responses in the clinic. This is likely due to the narrow therapeutic window as a result of infusion administration and the absence of a co-administrated T cell antigen, leading to suboptimal T cell priming.

STRIKE2001 is a humanized IgG2 antibody comprising two peptide-binding single chain variable fragments (scFv) linked to the CH3 domain. These scFv bind to a short non-immunogenic peptide tag (pTag). A peptide of choice is then synthesized by solid-phase peptide synthesis along with the pTag, creating a flexible tumor antigen delivery system based on the Adaptable Drug Affinity Conjugate (ADAC) platform. For clinical application, selected mutated KRAS peptides were synthesized to the pTag and this final product, STRIKE2001-pTAG-KRAS, aims to treat patients with KRAS mutated cancer.

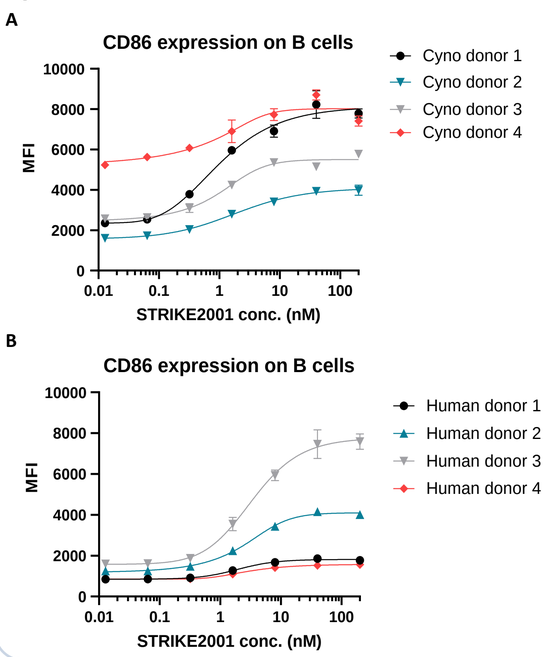
STRIKE2001 **STRIKE2001-pTag_peptide**



STRIKE2001 has an affinity of ~5 nM for human CD40 and does not compete with CD40 ligand. The scFv is humanized and STRIKE2001 is deimmunized. Retrogenic Cell Microarray analysis with >6000 proteins indicates no unspecific interaction by STRIKE2001.

1 Species justification shows cross-reactivity to cynomolgus CD40

Figure 1



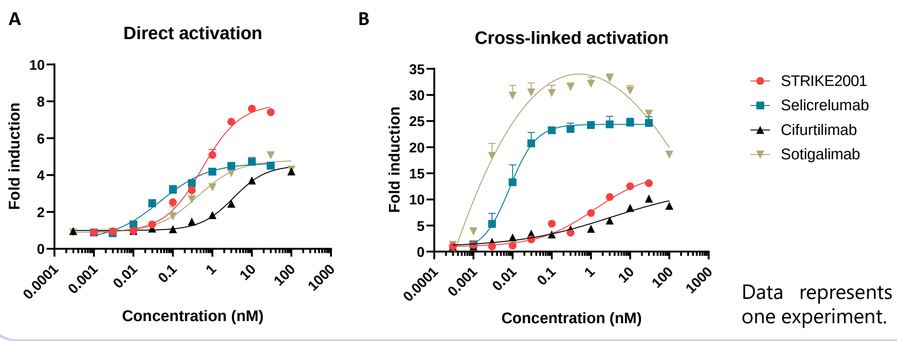
Species justification has shown cross-reactivity of STRIKE2001 to cynomolgus macaque (cyno) CD40 molecules on transfected cells. Cross-reactivity to any other species was not detected. To determine whether cyno could be selected for safety and toxicity testing, a 24-hour stimulation with STRIKE2001 on human and cyno PBMCs was performed. Upon titration of STRIKE2001, both cyno (Figure 1A) and human (Figure 1B) B cells show upregulation of activation marker CD86 in a dose dependent response. Furthermore, STRIKE2001 had similar EC50 values for both human (2.7 nM) and cyno (1.3 nM).

Data represents two experiments, 2 donors per experiment.

2 Intermediate CD40 agonistic antibody STRIKE2001 prevents liver toxicity

Most CD40 agonistic antibodies have a narrow therapeutic window which becomes dose limiting. Since STRIKE2001 not only provides immune stimulation but also delivers peptides, a high administration dose is desired. A dose titration of STRIKE2001 and anti-CD40 antibodies Selicrelumab, Sotigalimab and Cifurtilimab on CD40 reporter cells (Figure 2A), showed that STRIKE2001 induces CD40 activation (EC50 0.6), but only at higher doses than Selicrelumab (EC50 0.05). Furthermore, STRIKE2001 is not affected by FCγRIIb crosslinking (Figure 2B), while Selicrelumab (EC50 0.008) and Sotigalimab (EC50 0.002) transforms into a super agonists by FCγRIIb cross-linking. These data show that STRIKE2001 is an intermediate agonistic antibody which can result in a broader therapeutic window. In addition, the stable agonistic pattern regardless of immune milieu ensures similar therapeutic effect across patients.

Figure 2



Data represents one experiment.

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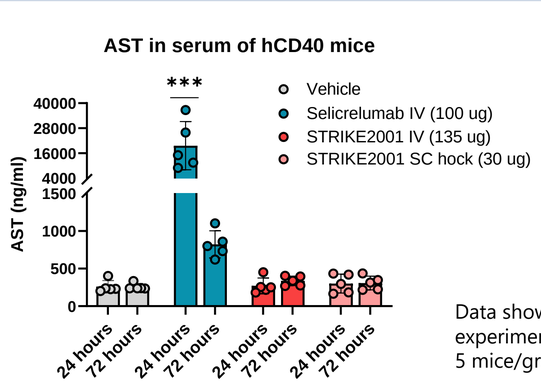


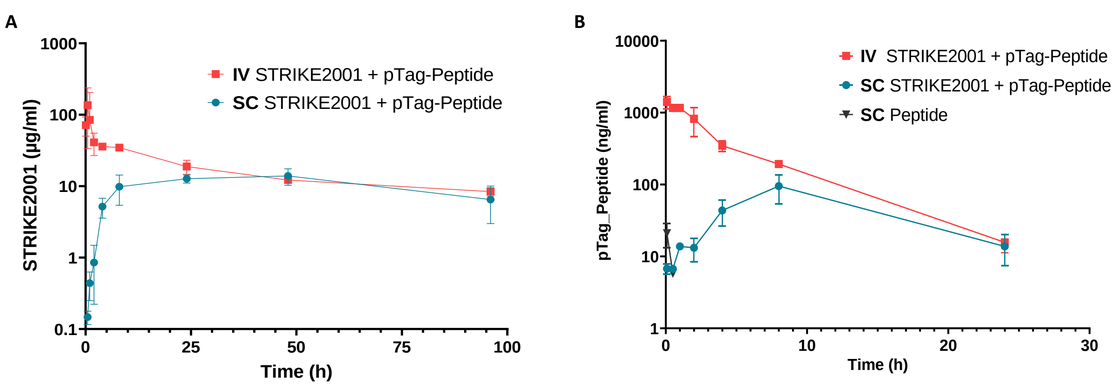
Data shows one experiment, 5 mice/group

Highly agonistic anti-CD40 antibodies can induce hepatoxicity, which limits their use as infusion products. To test toxicity of STRIKE2001, human transgenic CD40 mice were injected with either Selicrelumab IV, STRIKE2001 IV or STRIKE2001 SC (Figure 2C). Serum collected 24- and 72h after injection was analyzed for AST (a sign for liver damage), only Selicrelumab induced high levels of AST, while mice administrated with STRIKE2001 had similar levels to the control group. These data show that STRIKE2001 is safe to administrate, and this is likely a result of its intermediate agonistic characteristic and its independence of Fc receptor cross-linking.

3 STRIKE2001 prolongs the half-life of KRAS peptides in vivo

Mutated cancer-derived peptides are a promising source for cancer vaccines, but peptides are sensitive to proteolytic degradation. By affinity linkage of pTag-peptide to STRIKE2001, peptides are specifically targeted to dendritic cells and are furthermore shielded from degradation. hCD40 mice were injected with either STRIKE2001+pTag-peptide IV, STRIKE2001+pTag-peptide SC or pTag-peptide alone. After IV dosing, apparent elimination half-life of STRIKE2001 was 65 hour (Figure 3A). More interestingly, it shows that binding of STRIKE2001 prolongs the plasma half-life of the peptide, as the peptide could still be detected in plasma after 24 hours, while when administered by itself, the peptide alone could not be detected in the plasma after 30 minutes (Figure 3B).

Figure 3

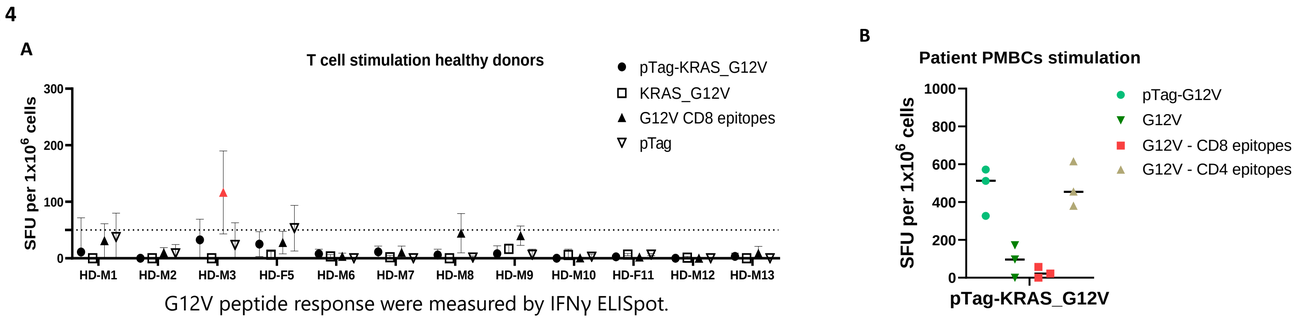


Transgenic hCD40 mice were injected with antibody 2 mg/kg, peptide 0.082 mg/kg. Data represents one experiment, 3 mice per group.

4 Anti-KRAS responses are low in healthy donors or patient bearing a KRAS mutation

KRAS mutations are often detected in lung, pancreas and colon cancer, the G12V and G12D mutations being among the most frequent. However, anti-KRAS responses are often not present. In a 10-day DC-T cell co-culture using healthy donors, only one donor responded to mutated G12V peptide re-stimulation (Figure 4A), one donor to mutated G12D (data not shown). Furthermore, a 10-day peptide stimulation of PBMCs from a patient with confirmed G12V mutation, only showed responses against CD4 peptides after re-stimulation (Figure 4B). These data indicate that KRAS-peptide vaccination is needed to activate the KRAS-specific T cells.

Figure 4

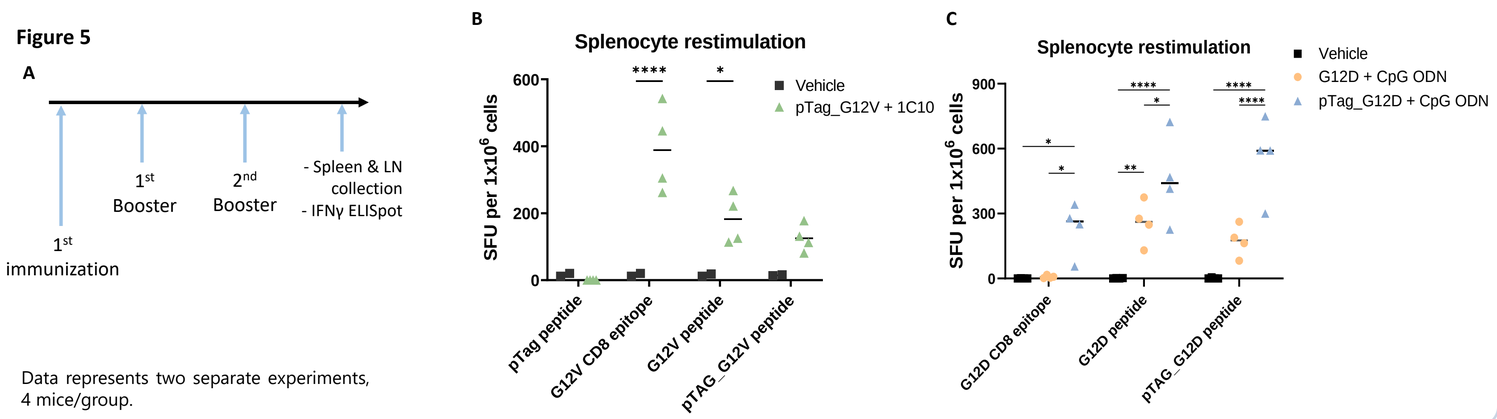


G12V peptide response were measured by IFNγ ELISpot.

5 Immunizing HLA.A11 mice with mutated KRAS peptides yields peptide-specific CD8+ T cells

The lack of anti-KRAS CD8+ T cells responses suggest that vaccination with mutated KRAS peptide is necessary. To this end, HLA.A11 expressing mice were immunized with either the G12V or pTag_G12V peptide together with an anti-mouse CD40 antibody (clone 1C10) or the mice were immunized with G12D or pTag_G12D peptide together with CpG ODN (Figure 5A). After three times immunizations, splenocytes and lymph nodes were collected and re-stimulated with the KRAS peptides in an IFNγ ELISpot. Immunization with both the pTag_G12V (Figure 5B) and pTag_G12D peptides (Figure 5C) resulted in significant CD8+ T cell responses. These data show that the generation of anti-KRAS responses is feasible when vaccination is applied. Furthermore, the addition of the pTag sequence to the peptides did not affect the G12V or G12D peptide presentation (Figure 5B and 5C)

Figure 5



Data represents two separate experiments, 4 mice/group.

Conclusion

STRIKE2001 provides a new cargo drug delivery platform based on the ADAC which can transform the field of agonistic antibody and peptide drug efficacy.

STRIKE2001 induces CD40 activation while avoiding liver toxicity. Furthermore, by the affinity linkage of pTag-peptides to STRIKE2001, the peptides are targeted to CD40 expressing antigen presenting cells and are protected from degradation.

STRIKE2001 is aimed to treat patients with KRAS-mutated cancer. Through its ability to stimulate the immune system and targeted peptide delivery, STRIKE2001 can boost the poor pre-existing anti-KRAS responses in patients.

For more information on the ADAC platform, visit our website.