A novel viral immunotherapeutic targeting the CD47/SIRPα axis demonstrates potent anti-tumor effects

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Background

- The CD47/SIRPα axis mediates a "don't eat me" signal exploited by tumor cells to escape macrophage-mediated immune surveillance (Fig. 1).
- Anti-CD47 therapies have shown promising clinical results in solid and hematological malignancies; however, efficacy is hindered by systemic toxicity.
- Dual targeting of CD47/SIRPα and PD-1/PD-L1 axes has enhanced efficacy in preclinical studies.
- We hypothesized that local delivery of a therapeutic, able to interfere with the CD47/SIRPα axis within an oncolytic viral chassis, would induce high payload expression paired with oncolytic activity and low systemic exposure, ultimately resulting in improved tumor control.

Figure 1

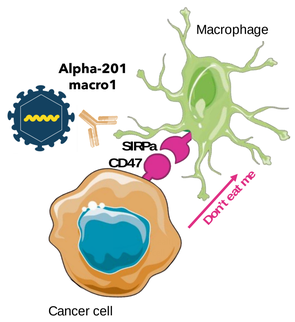


Figure 1: Activation of innate immune surveillance by interfering with the CD47/SIRPα pathway. Image was partly generated using Servier Medical Art [1].

Distinct cellular responses to Alpha series vector infection are observed

Alpha-201 is a viral chassis engineered for enhanced immunostimulatory activity coupled with sustained payload expression and regulated oncolysis. Cellular responses to viral chassis infection were profiled by RNAseq analysis (Fig. 2). Gene Set Enrichment Analysis (GSEA) demonstrated that Alpha series vector infection induces stronger immune responses compared with a Control Vector (Fig. 3).

Figure 2

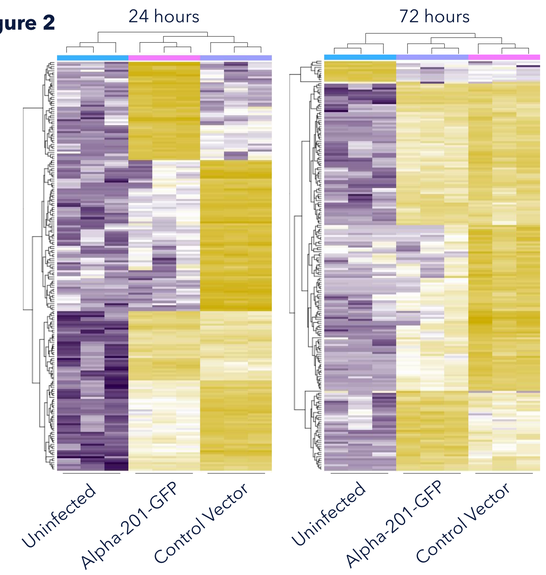


Figure 2: Profiling of cellular responses to infection with the Alpha-201 viral chassis. Hs578T cells were infected with Alpha-201 encoding GFP or a Control Vector (10 PFU/cell, 24-72 h) and then RNA was extracted and RNAseq analysis performed. Control Vector: ICP27mutant.

Figure 3

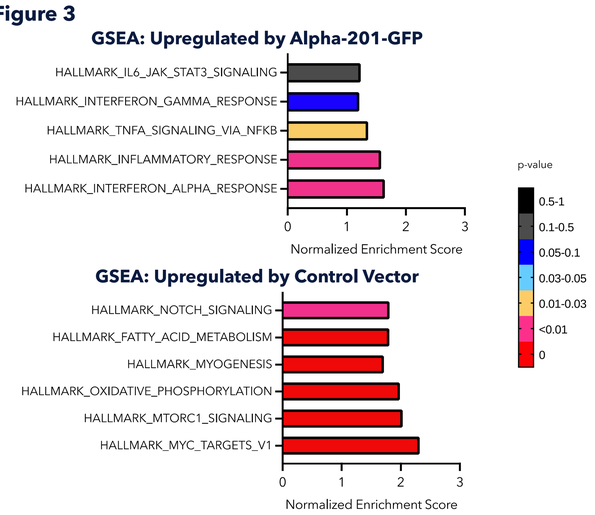
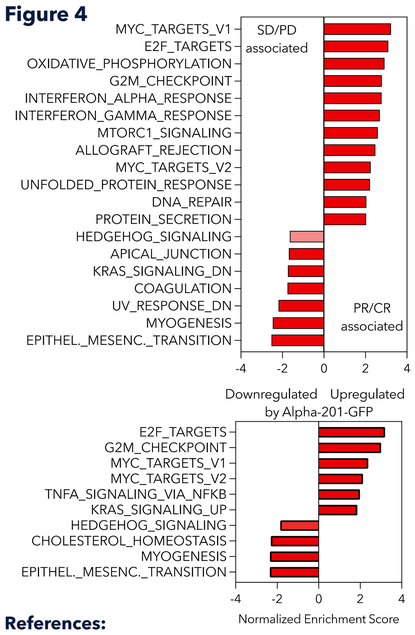


Figure 3: Pathway analysis of cellular responses to Alpha-201 viral chassis infection. GSEA analysis of top pathways altered by infection with Alpha-201-GFP and Control Vector (24 h post-infection). Control Vector: ICP27mutant.

Alpha-201 viral chassis regulates pathways associated with responses to ICI

A striking, positive correlation between hallmark GSEA pathways associated with immune checkpoint inhibitor (ICI) response and those regulated by Alpha-201 infection was observed, suggesting that Alpha-201 can orchestrate changes in the tumor microenvironment supportive of ICI response (Fig. 4). The Alpha-201 viral chassis also had a modest anti-tumor effect itself in a syngeneic tumor model similar to that achieved with anti-PD-1 antibody therapy (Fig. 5). Given the interplay between CD47/SIRPα and PD-1/PD-L1 axes, this supported the use of Alpha-201 as the viral chassis for a viral immunotherapeutic targeting the CD47/SIRPα axis.

Figure 4



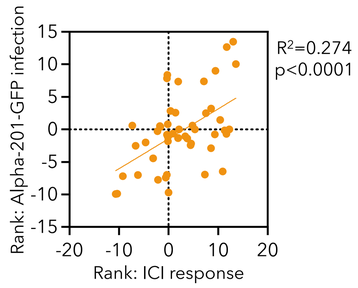


Figure 4: Comparison of Gene Set Enrichment Analysis (GSEA) of RNAseq data from Alpha-201 infected cells (72 h) and patients treated with ICI in the advanced setting. Top pathways identified and correlation analysis for all pathway ranks in each data set are shown. Patient data from the SU2C-MARK cohort [2].
Rank: NES*(-log10(p value)).
PR/CR: partial/complete response to ICI.
SD/PD: stable/progressive disease after ICI treatment.

Figure 5

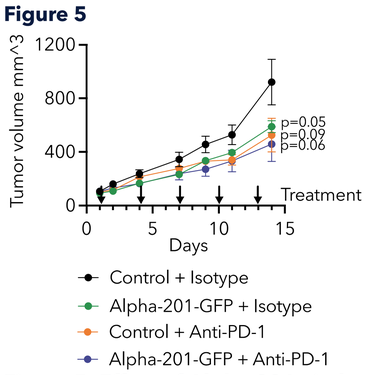


Figure 5: Effect of Alpha-201 viral chassis ± anti-PD-1 antibody therapy on in vivo tumor growth. 5x10⁵ EMT6 cells were injected into the rear flank of Balb/c mice. Treatment (3x10⁷ PFU Alpha-201-GFP, i.t. or 10 mg/kg, i.p. antibodies) began when tumors reached ~100 mm³. N=5-8 mice per group. Anti-PD-1 clone: RMP1-14.

References:
1. Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license
2. Ravi A. et al. Nat Genet 2023;55(5):807-819

Alpha-201-macro1 disrupts binding of SIRPα to CD47 in vitro

Conditioned media from Alpha-201-macro1 infected cells selectively disrupted binding of SIRPα to CD47 in vitro in a payload- (and not vector-) dependent manner (Fig. 6).

Figure 6

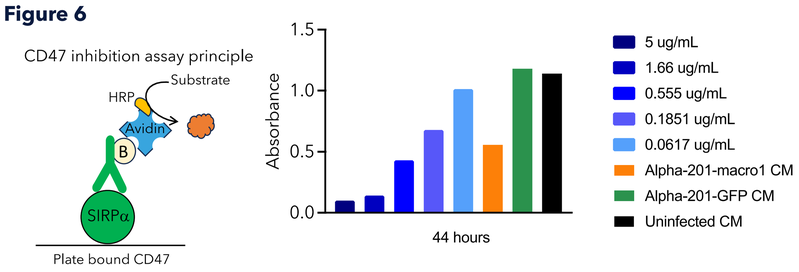


Figure 6: Effect of conditioned media (CM) from Alpha-201-macro1 infected cells in a CD47 displacement assay. Vero cells were infected with Alpha-201 encoding macro1 or GFP, and CM was harvested 44 h post-infection. CM was tested for its ability to disrupt the interaction between SIRPα and CD47 in vitro, and results were compared to an anti-CD47 antibody.

Alpha-201-macro1 induces macrophage-mediated phagocytosis ex vivo

Conditioned medium from Alpha-201-macro1 infected cells resulted in a dose-dependent increase in macrophage-mediated target cell phagocytosis that was greater than the effect of an anti-CD47 antibody (Fig. 7). Moreover, pretreatment with CM from Alpha-201 vectors encoding irrelevant payloads did not significantly affect macrophage phagocytosis, demonstrating the specificity of the therapeutic payload response.

Figure 7

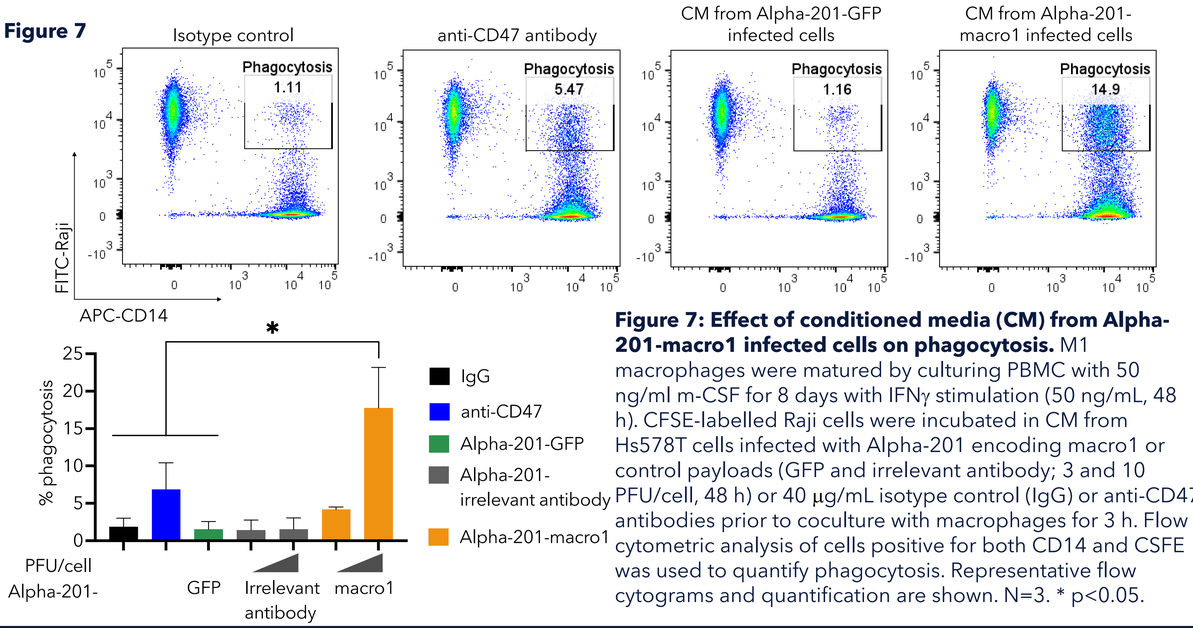


Figure 7: Effect of conditioned media (CM) from Alpha-201-macro1 infected cells on phagocytosis. M1 macrophages were matured by culturing PBMC with 50 ng/ml m-CSF for 8 days with IFNγ stimulation (50 ng/mL, 48 h). CFSE-labelled Raji cells were incubated in CM from Hs578T cells infected with Alpha-201 encoding macro1 or control payloads (GFP and irrelevant antibody; 3 and 10 PFU/cell, 48 h) or 40 μg/mL isotype control (IgG) or anti-CD47 antibodies prior to coculture with macrophages for 3 h. Flow cytometric analysis of cells positive for both CD14 and CSFE was used to quantify phagocytosis. Representative flow cytograms and quantification are shown. N=3. * p<0.05.

Alpha-201-macro1 demonstrates anti-tumor efficacy in vivo

In vivo, treatment with Alpha-201-macro1 resulted in tumor growth inhibition compared to the vehicle control group (Fig. 9). This effect was dependent on payload expression, as Alpha-201-GFP did not show statistically significant anti-tumor activity. There was a trend towards greater efficacy with local Alpha-201-macro1 delivery compared to systemic anti-CD47 antibody therapy. No changes in macrophage infiltration in the tumors were observed (Fig. 10). Alpha-201-macro1 was well-tolerated based on body weight (Table 1) and clinical observations.

Figure 8

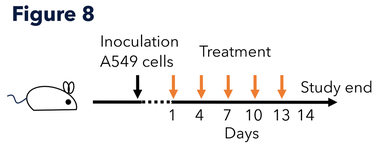


Figure 8: A549 in vivo tumor study design. 5x10⁶ A549 cells were injected into the rear flank of Balb/c nude mice. Treatment (3x10⁷ PFU Alpha-201 vectors, i.t. or 10 mg/kg, i.p. antibodies) began when tumors reached ~100 mm³. N=8 mice per group.

Table 1: Body weight change at endpoint

Group	Body weight change (% ± SD)
Control	8.7 ± 3.6
Alpha-201-GFP	5.2 ± 3.7
Alpha-201-macro1	2.9 ± 4.2
IgG	6.5 ± 2.7
Anti-CD47 Ab	6.7 ± 3.4

Figure 9

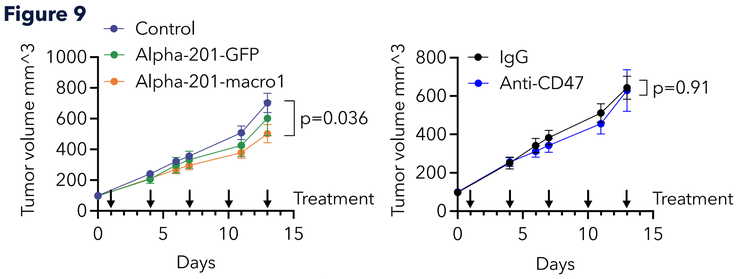


Figure 9: Tumor growth curves for Alpha-201-macro1 or anti-CD47 antibody treated mice.

Figure 10

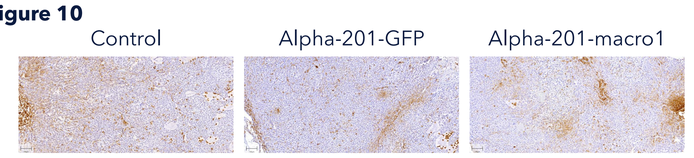


Figure 10: Immunohistochemical assessment of macrophage infiltration of tumors. A549 tumor sections collected at day 12, were stained with F4/80 to visualize intratumoral macrophages. 10X magnification.

Conclusions

- Alpha-201-macro1 disrupts binding of SIRPα to CD47, enhances macrophage phagocytosis ex vivo, and exerts anti-tumor efficacy in vivo, effects which exceed those of anti-CD47 antibody therapy.
- Further in vivo studies of Alpha-201-macro1 and modified, multi-payload versions of this vector, in combination with immune checkpoint inhibitors, are ongoing.

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