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# ALTA-002, a SIRPα-Directed TLR9 Agonist Antibody Conjugate, Activates Myeloid Cells And Promotes Anti-Tumor Immunity

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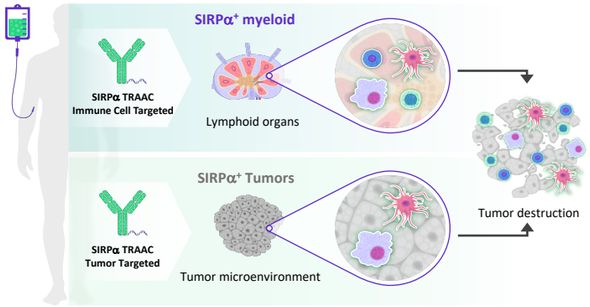
## Introduction

- Novel therapies engaging both innate and adaptive immune responses may engender more robust and durable anti-cancer immunity [1].
- Activation of toll-like receptor 9 (TLR9) by unmethylated CpG oligonucleotides (ODNs) promotes innate inflammatory responses and the induction of adaptive immunity [2]. Several CpG oligodeoxynucleotides have demonstrated clinical response in patients with melanoma by intra-tumoral injection [3].
- We developed a novel Toll-like Receptor Agonist Antibody Conjugate (TRAAC) molecule comprised of a differentiated TLR9 agonist (T-CpG) conjugated to an antibody against SIRPα termed SIRPα TRAAC.
- Signal regulatory protein α (SIRPα) is a myeloid inhibitory receptor that suppresses immune activation following binding of its ligand CD47 [4].
- Blockade of CD47-SIRPα myeloid checkpoint pathway has demonstrated clinical response in patients with solid tumors [5].
- We present preclinical data demonstrating that SIRPα TRAAC delivers T-CpG to myeloid cells via SIRPα and FcγR engagement, triggering TLR9 signaling, cell activation and immune modulation, resulting in robust anti-tumor activity.

### Fig 1: SIRPα TRAAC, is a Toll-Like Receptor Agonist Antibody Conjugate, designed for systemic delivery of T-CpG, a potent TLR9 agonist

**Antibody: anti-SIRPα**
- Targets myeloid cells expressing SIRPα
- Localizes to tumor microenvironment of SIRPα⁺ tumors
- Engages Fc gamma receptors
- Blocks CD47-SIRPα myeloid checkpoint pathway

**Immune Activator: T-CpG**
- Potent TLR9 agonist
- Linear, monomeric, and non-aggregated
- Sequence optimized for potency and stability
- Innovative design enables efficient site-specific conjugation




| Cell type | SIRPα+ | TLR9 |
|---|---|---|
| p(DC) | ✓ | ✓ |
| Myeloid | ✓ | ✓ |
| B cells | — | ✓ |

## Experimental Results

### Fig 2: T-CpG is a potent TLR9 agonist with targeted activation across species

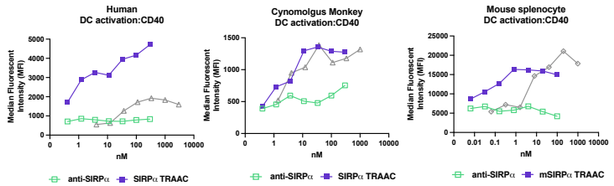



Figure 2: Human, cynomolgus PBMCs, and mouse splenocytes were stimulated with either anti-SIRPα, SIRPα TRAAC, T-CpG, or SIRPα TRAAC conjugated to murine reactive mT-CpG (mSIRPα TRAAC) for 24hr or 48hrs and surface marker expression was assayed by flow cytometry. DC: dendritic cells

### Fig 3: SIRPα TRAAC specifically targets and activates SIRPα⁺ immune cells in human PBMC cultures

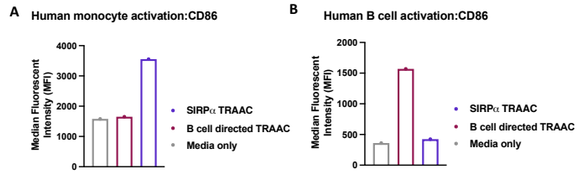



Figure 3: Human PBMCs were stimulated with SIRPα TRAAC, B cell directed TRAAC or media only for 24hr and surface marker expression on monocytes (A) and B cells (B) was assayed by flow cytometry.

### Fig 4: SIRPα TRAAC triggers TLR9 signaling in SIRPα⁺ DC and monocytes, eliciting pro-inflammatory and cytotoxic cytokine production in human PBMC cultures

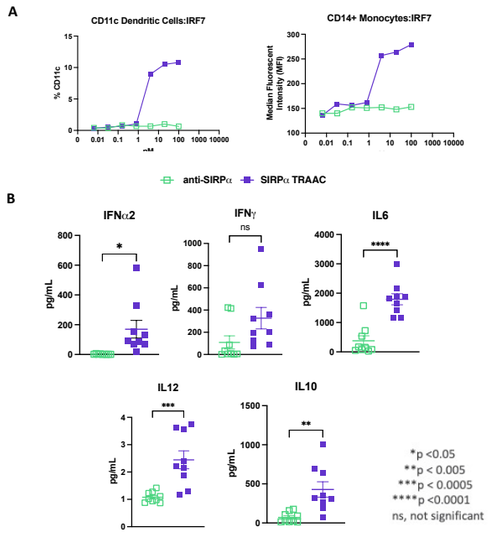



Figure 4. Human PBMCs were stimulated with anti-SIRPα or SIRPα TRAAC for 24hr and assayed for IRF7 induction (A) and cytokine production (B) from 9 healthy donors following 72hr stimulation by flow cytometry. P-values were calculated using unpaired t-test.

### Fig 5: SIRPα expression on tumors potentiates myeloid activation by SIRPα TRAAC

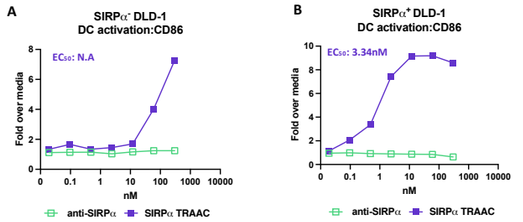



Figure 5. Human PBMCs were co-cultured either in presence of parental DLD-1 (A) or DLD-1 expressing SIRPα (B) and stimulated with anti-SIRPα, SIRPα TRAAC, or media only for 48hr. Surface marker expression was assayed by flow cytometry. Data is presented as fold over media only control.

### Fig 6: SIRPα TRAAC promotes phagocytosis of SIRPα⁻ and SIRPα⁺ tumors by macrophages

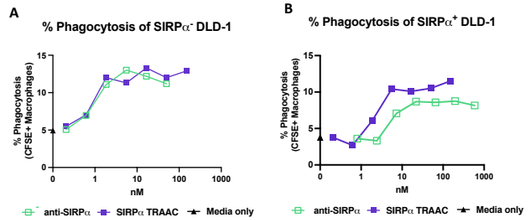



Figure 6. Human monocyte derived macrophages were co-incubated for 2hrs with either parental DLD-1 (A) or DLD-1 expressing SIRPα (B) in presence of anti-SIRPα, SIRPα TRAAC or media only. % Phagocytosis was determined by flow cytometry.

### Fig 7: Localizing mSIRPα TRAAC to SIRPα⁺ tumor demonstrates superior anti-tumor activity in syngeneic model when administrated systemically

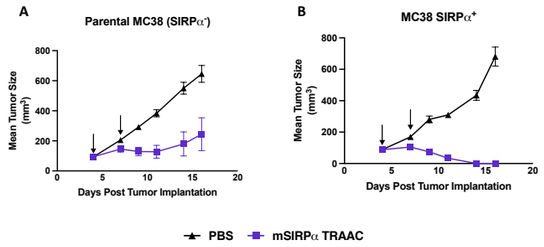



Figure 7. Mice bearing either parental SIRPα⁻ MC38 tumors (A) or expressing SIRPα (B) were dosed intraperitoneally (i.p.) twice, three days apart with mSIRPα TRAAC conjugated with murine reactive mT-CpG at 1mg/kg or PBS control. Arrows indicate doses administered.

### Fig 8: mSIRPα TRAAC elicits potent single agent anti-tumor response in RENCA, a SIRPα⁺ model refractory to anti-PD-1

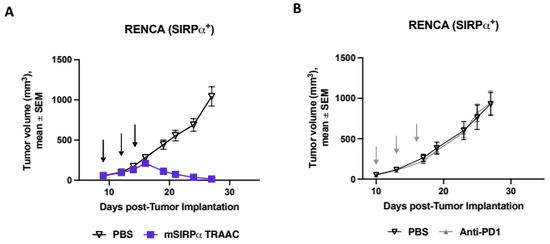



Figure 8. Mice bearing RENCA tumors were dosed intraperitoneally (i.p.) three times, three days apart with mSIRPα TRAAC at 10mg/kg or PBS (A). A separate cohort was dosed with anti-PD1 at 10mg/kg three times, three days apart or PBS (B). Arrows indicate doses administrated.

### Fig 9: The combination of suboptimal dose of mSIRPα TRAAC and anti-PD-1 elicits enhanced anti-tumor response in CT26, a SIRPα negative tumor model

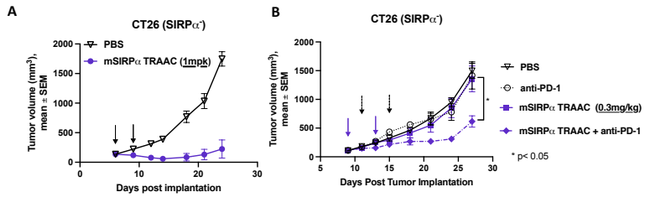



Figure 9. Mice bearing CT26 tumors were intraperitoneally (i.p.) treated with mSIRPα TRAAC at 1mg/kg or PBS (A) or mSIRPα TRAAC at 0.3mg/kg, anti-PD-1 at 10mg/kg, in combination, or PBS control (B). Arrows indicate doses administrated. P-values comparing combination group vs. anti-PD-1 in were calculated using unpaired t-test.

## Conclusions

- SIRPα TRAAC is a SIRPα-directed TLR9 receptor agonist antibody conjugate designed for systemic administration.
- SIRPα TRAAC specifically targets myeloid cells triggering TLR9 signaling via SIRPα and FcγR engagement leading to robust cellular activation and cytokine induction in cultured immune cells.
- SIRPα expression on tumors potentiates the activation of myeloid cells by SIRPα TRAAC.
- SIRPα TRAAC promotes phagocytosis of tumors independent of SIRPα expression.
- Localization of mSIRPα TRAAC to SIRPα+ tumors demonstrates robust and curative single agent activity in multiple models including anti-PD-1 refractory tumors.
- mSIRPα TRAAC enhances tumor regression in combination with anti-PD-1 in syngeneic tumor model.
- **ALTA-002, our SIRPα TRAAC candidate, is in preclinical development for the treatment of patients with various types of malignancies**

References:
1. Kobold et al., Innate and adaptive immunity combined for cancer treatment. *PNAS* 2019, 116 (4) 1087-1088.
2. Dowling et al., Toll-like receptors: the swiss army knife of immunity and vaccine development. *Clin. Transl. Immunology*. 2016 5(5), e85–10.
3. Hamid et al., Intratumoral Immunotherapy—Update 2019. *The Oncol.* 2019; 25:343–359.
4. Kuo, T et al., Targeting the myeloid checkpoint receptor SIRPα potentiates innate and adaptive immune responses to promote anti-tumor activity. 2020. J Hematol *Oncol* 13, 160.
5. Lee KW et al., ALX148, a CD47 blocker in combination with standard chemotherapy and antibody regimens in patients with gastric/gastroesophageal junction (GC) cancer and head and neck squamous cell carcinoma (HNSCC); ASPEN-01. SITC 2020.