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Targeted Immune Cell Activation by Systemic Delivery of Toll-Like Receptor 9 Agonist Antibody Conjugates Induce Potent Anti-Tumor Immunity

Ons Harrabi[1], Amy Chen[1], Emma Sangalang[1], Laura Doyle[1], Danielle Fontaine[1], Bora Han[1], Curt Bradshaw[2], Jaume Pons[3], Janet Sim[1], Tracy C. Kuo[1], Hong I. Wan[1]

[1]Tallac Therapeutics, Inc., Burlingame, CA; [2]Arrowhead Pharmaceuticals, Pasadena, CA; [3]ALX Oncology, Burlingame, CA

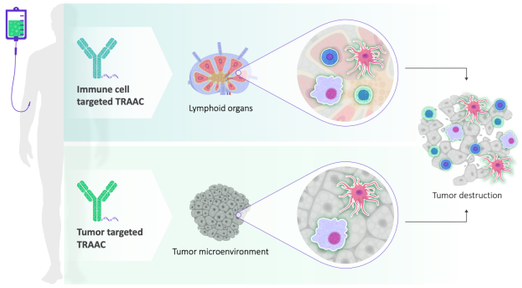
Introduction

- Despite the success of checkpoint inhibitors and adoptive CAR-T therapies, only a fraction of cancer patients benefit. Novel therapies engaging both innate and adaptive immune response may produce more robust and durable anti-cancer immunity [1].
- Toll-like receptors (TLRs) play crucial roles in mounting potent innate immune responses against invading pathogens. Activation of TLR signaling leads to induction of inflammatory cytokines and priming of adaptive immunity [2].
- TLR9 agonists have shown clinical responses in melanoma patients by intra-tumoral injection [3].
- We developed a novel Toll-like Receptor Agonist Antibody Conjugate (TRAAC) platform to deliver a potent TLR9 agonist (T-CpG) for targeted immune activation via systemic administration.
- We evaluated various TRAAC approaches targeting either immune cell receptors or tumor specific antigens, demonstrating robust immune modulation and potent single agent anti-tumor activity in pre-clinical settings.

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

Antibody: Versatile TRAAC targeting
- Specific immune cells, or
- Tumor microenvironment

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



References
1. Kobold., 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.

Immune cell targeting TRAAC

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

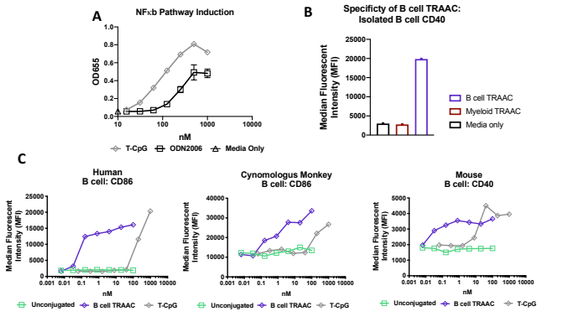
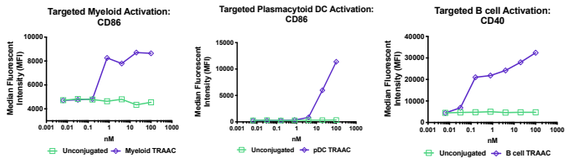


Figure 2: NFκB reporter cells were stimulated with T-CpG or ODN2006 for 18hrs (A). Isolated human B cells were stimulated for 48hrs with B cell or myeloid TRAAC, surface marker expression was assayed by flow cytometry (B). Human, cynomolgus PBMCs, and mouse splenocytes were stimulated with B cell TRAAC, or T-CpG for 48hrs and expression of activating receptors were assayed by flow cytometry (C).

Fig 3: TRAACs directed to human pDC, myeloid and B cells induce potent targeted cellular activation and cytokine production



Cell type	TLR9 activation
pDC	✓
B cell	✓
Myeloid	✓

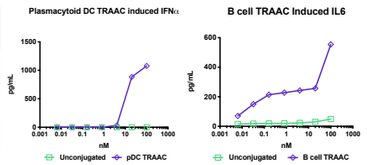


Figure 3. Human PBMCs were stimulated for 48hrs in the presence of pDC, myeloid and B cell-directed TRAACs. Surface marker expression and cytokines was assayed by flow cytometry or ELISA.

Fig 4: TRAACs elicit indirect activation of non-targeted immune cells

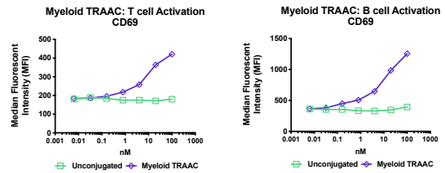


Figure 4. Human PBMCs were stimulated with myeloid TRAAC for 48hrs and assayed for cell markers of activation of non-targeted T cells and B cells by flow cytometry.

Fig 5: Systemic administration of immune cell targeted TRAACs exhibit potent, dose-dependent, single agent activity in syngeneic tumor models

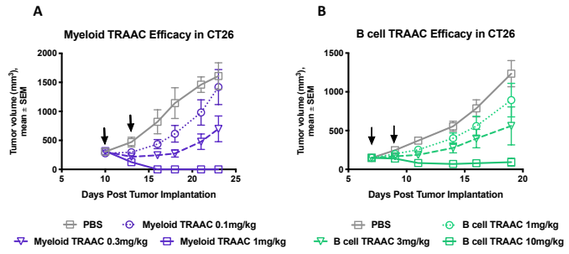


Figure 5. Mice bearing CT26 were intraperitoneally (i.p.) treated with PBS or myeloid TRAAC at 0.1mg/kg, 1mg/kg or 3mg/kg (A) or B cell TRAAC at 1mg/kg, 3mg/kg or 10mg/kg (B). Murine TRAACs are conjugated to mouse reactive T-CpG. Arrows indicate doses administrated.

Fig 6: Durable anti-tumor memory response in tumor free syngeneic model following administration of immune cell targeted TRAAC

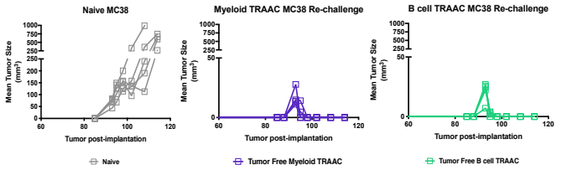


Figure 6. Tumor eradicated mice from MC38 bearing mice following 10mg/kg 2q3 systemic treatment with either myeloid TRAAC or B cell TRAAC were re-challenged 60-70 days post-initial tumor clearance. Naïve age-matched mice were used as control for tumor growth.

Fig 7: TRAAC evokes rapid innate and adaptive immune signatures within tumor microenvironment

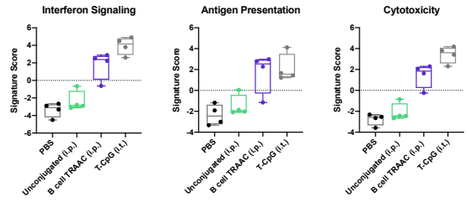


Figure 7. Mice bearing CT26 were dosed intraperitoneally (i.p.) once with B cell TRAAC or unconjugated antibody at 10mg/kg. Mouse reactive T-CpG was injected directly into the tumor at 50ug/mouse. Tumors were harvested 8hrs post-dose for nanostring analysis. Raw data was normalized and visualized using nsolver software by Nanostring.

Tumor Targeting TRAAC

Fig 8: HER2-directed TRAAC induces tumor antigen dependent myeloid activation

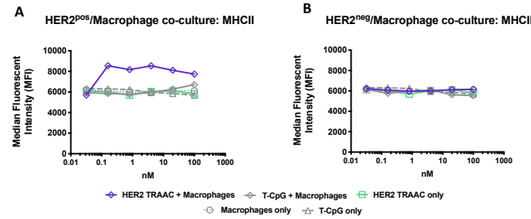


Figure 8. Bone marrow-derived mouse macrophages were co-cultured in the presence of HER2^{pos} (A) or HER2^{neg} tumors (B) and treated with HER2 TRAAC or mouse reactive T-CpG for 24hrs. Median MHCII was assessed by flow cytometry.

Fig 9: HER2-directed TRAAC demonstrates potent and durable anti-tumor response in syngenetic tumor model

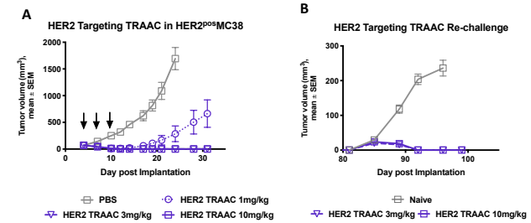


Figure 9. Mice bearing HER2^{pos} MC38 were intraperitoneally (i.p.) treated with HER2-directed TRAAC at 1mg/kg, 3mg/kg or 10mg/kg (A). Arrows indicate doses administrated. Tumor eradicated mice were rechallenged with HER2^{pos}MC38 60 days post-initial tumor clearance, naïve mice were used as control for tumor growth (B).

Exploratory Studies in Cynomolgus Monkey

- Immune targeting TRAACs were well tolerated following repeated intravenous injections in cynomolgus monkeys, as assessed by clinical observations, body weights, food consumption, hematology, serum chemistry and histopathology.

Conclusions

- TRAAC is a novel immune activating platform that engages innate and adaptive immunity.
- TRAAC delivers T-CpG, a potent differentiated TLR9 agonist, to targeted immune cells or tumor microenvironment leading to immune activation.
- TRAACs demonstrate robust and durable tumor regression in multiple syngeneic tumor models, as a single agent.
- TRAACs exhibit favorable tolerability and safety profiles in cynomolgus monkeys.

- **Designed as a systemic therapy, TRAACs demonstrate potent targeted immune activation, robust anti-tumor activity and favorable tolerability in pre-clinical settings.**
- **TRAAC platform has broad therapeutic potentials against multiple solid tumor malignancies.**