

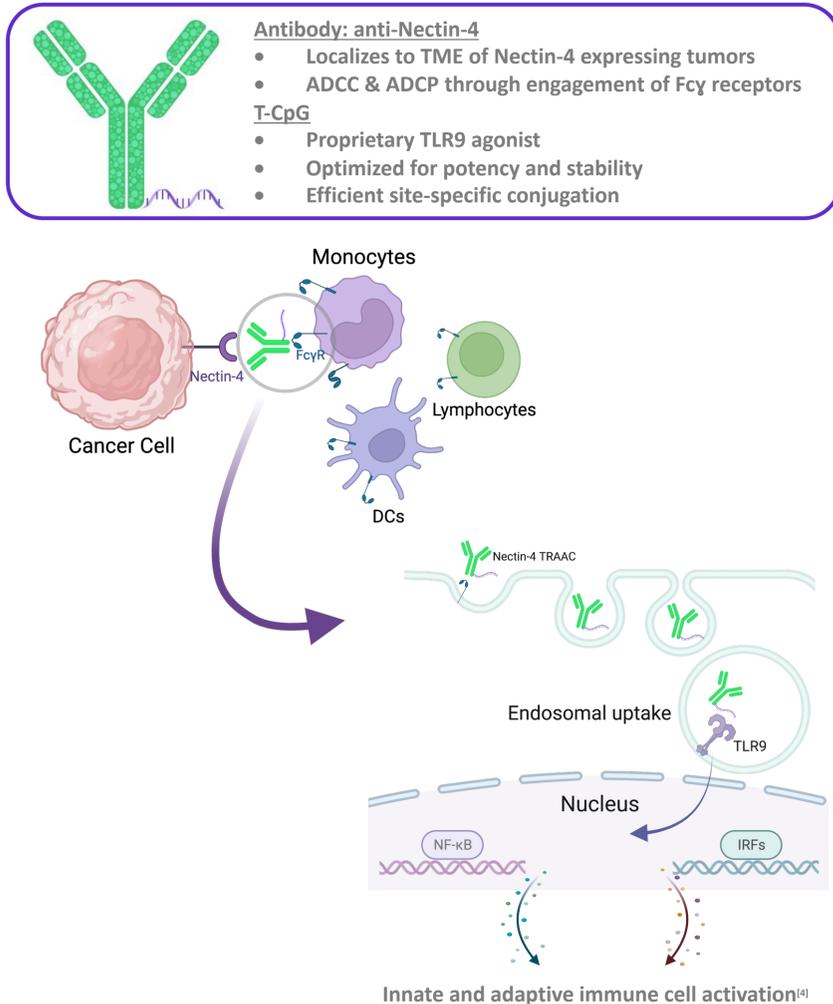
# #1162 A Nectin-4 Targeted TLR9 Agonist Antibody Conjugate Induces Robust Immune Cell Activation and Anti-Tumor Responses

Amy Chen, Min Li, Maja Bonacorsi, Emma R.B. Sangalang, Danielle Fontaine, Mingrui An, Ons Harrabi, Tiffany Chou, Laura Doyle, Janet Sim, Bora Han, Hong I. Wan, Tracy C. Kuo, Maria José Costa, Pavel Strop  
Tallac Therapeutics, Inc., Burlingame, CA

## Introduction

- Novel therapies engaging both innate and adaptive immune response may produce more robust and durable anti-cancer immunity [1].
- Activation of Toll-Like Receptor 9 (TLR9) by unmethylated CpG oligodeoxynucleotides (CpG-ODNs) promotes innate inflammatory responses and the induction of adaptive immunity [1][2]. Several intratumorally injected CpG-ODNs demonstrated clinical responses in melanoma patients [3].
- We developed a Toll-like Receptor Agonist Antibody Conjugate (TRAAC) comprised of an optimized CpG-ODN (T-CpG) conjugated to a novel Nectin-4-targeting antibody for systemic administration that enables delivery of a potent TLR9 agonist to the tumor microenvironment (TME).
- Nectin-4 is a cancer-associated antigen over-expressed in many solid tumors with limited expression in normal tissues. Additionally, Nectin-4 over-expression correlates with poor prognosis [2].
- We present preclinical data demonstrating that Nectin-4 TRAAC triggers TLR9 signaling, induces myeloid and dendritic cell activation, cancer cell phagocytosis, pro-inflammatory cytokine production and lymphocyte activation, altogether resulting in potent anti-tumor efficacy.

**Fig. 1: Nectin-4 TRAAC: a Toll-like Receptor Agonist Antibody Conjugate designed for systemic and tumor-targeted delivery of T-CpG, an optimized TLR9 agonist**



## References:

- Hamid, O., Ismail, R. and Puzanov I., Intratumoral Immunotherapy-Update 2019. *Oncologist*, 2020 Mar;25(3):e423-e438.
- Chatterjee S., Sinha S., Kundu CN. Nectin cell adhesion molecule-4 (NECTIN-4): a potential target for cancer therapy. *Eur J Pharmacol*. 2021 Nov 15;911:174516.
- Liu, M., O'Connor, R., Trefely, S., Graham, K., Snyder NW., Beatty, GL. Metabolic rewiring of macrophages by CpG potentiates clearance of cancer cells and overcomes tumor-expressed CD47-mediated 'don't eat me signal'. *Nat Immunol*. 2019 Mar; 20(3):265-275.
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## Experimental Results

**Fig. 2: Anti-Nectin-4 antibody displays species cross-reactivity and selective binding to Nectin-4**

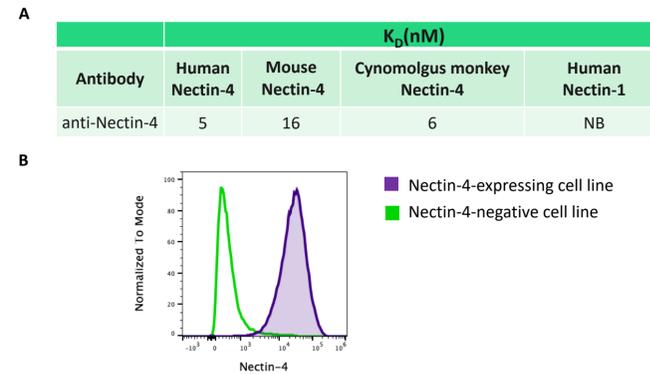


Figure 2: (A) Results of SPR 1:1 binding kinetics fitting using extracellular domains of human Nectin-4, mouse Nectin-4, cynomolgus monkey Nectin-4, and human Nectin-1 proteins. NB = no binding. (B) Results from flow cytometry showing that the novel Nectin-4-targeting antibody binds to a Nectin-4-expressing cancer cell line, whereas no binding is detected on a non-expressing cancer cell line. No binding to human PBMCs was observed in the same assay (data not shown).

**Fig. 3: Nectin-4 TRAAC activates dendritic, myeloid and B cells in human PBMCs co-cultured with Nectin-4+ cancer cells**

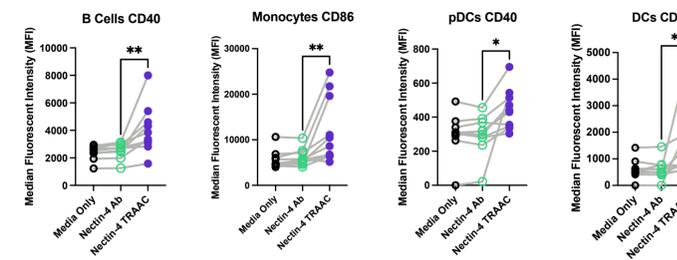


Figure 3: Human PBMCs from 10 healthy donors were co-cultured with Nectin-4 expressing cancer cells and were left untreated (media only) or treated with unconjugated Nectin-4 antibody or Nectin-4 TRAAC. After 24-hour incubation, the expression levels of cell surface markers indicative of cell activation were determined by flow cytometry, \* p<0.05, \*\* p<0.006 (one-way ANOVA).

**Fig. 4: Nectin-4 TRAAC induces monocyte- and macrophage-mediated phagocytosis of Nectin-4+ cancer cells**

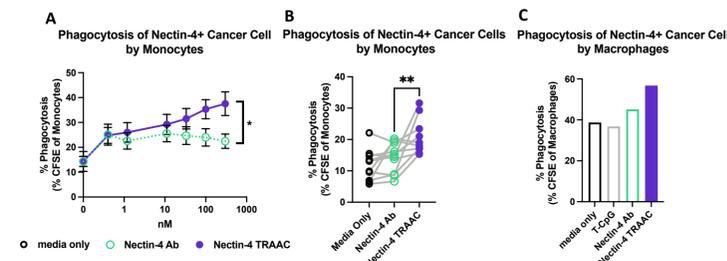


Figure 4: Phagocytosis of cancer cells as assayed by flow cytometry. (A) Human PBMCs were co-cultured with CFSE labeled Nectin-4 expressing cancer cells and incubated with titrated unconjugated Nectin-4 antibody or with Nectin-4 TRAAC for 24 hours. Phagocytic monocytes were identified as CFSE+CD14+ cells. (B) Human PBMCs from 10 healthy donors were co-cultured with CFSE labeled Nectin-4 expressing cancer cells and incubated with media only, unconjugated Nectin-4 antibody or Nectin-4 TRAAC for 24 hours. Phagocytic monocytes were identified as CFSE+CD14+ cells. (C) Monocyte-derived macrophages were co-cultured with CFSE labeled Nectin-4 expressing cancer cells and incubated with unconjugated Nectin-4 antibody, Nectin-4 TRAAC or T-CpG alone for 2 hours. Phagocytic macrophages were identified as CFSE+CD33+ cells, \* p<0.05, \*\* p<0.006 (paired t-test and one-way ANOVA respectively).

**Fig. 5: Nectin-4 TRAAC triggers TLR9 signaling and elicits pro-inflammatory cytokine production in human PBMCs co-cultured with Nectin-4+ cancer cells**

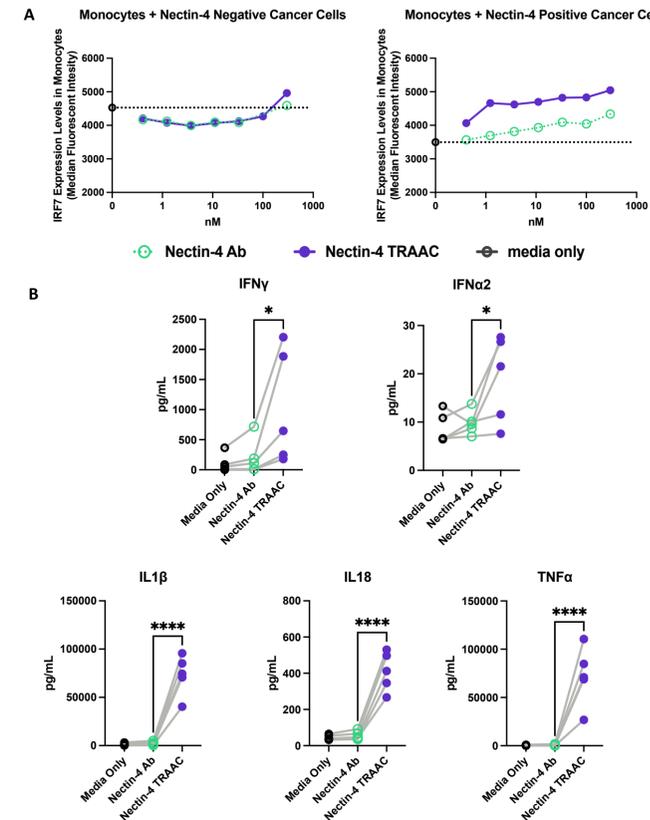


Figure 5: (A) Human PBMCs were co-cultured with Nectin-4 expressing and non-expressing cancer cells and left untreated or incubated with titrated unconjugated Nectin-4 antibody or Nectin-4 TRAAC for 24 hours. IRF7 expression in monocytes was determined by flow cytometry. (B) Human PBMCs from 5 healthy donors were co-cultured with Nectin-4 expressing cancer cells and were left untreated or incubated with unconjugated Nectin-4 antibody or Nectin-4 TRAAC for 24 hours. Cytokine levels were determined by cytokine bead array (flow cytometry), \* p<0.04, \*\*\*\* p<0.0001 (one-way ANOVA).

**Fig. 6: Nectin-4 TRAAC monotherapy exhibits potent efficacy in multiple syngeneic tumor models including checkpoint inhibitor (CPI) refractory model, EMT6**

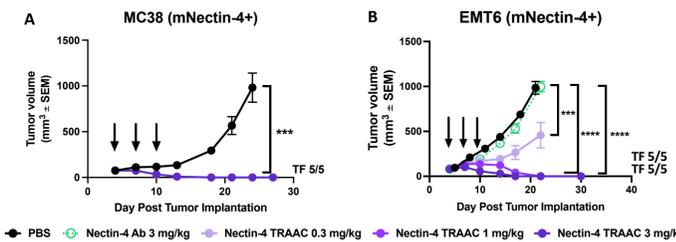


Figure 6: (A) Immune competent mice bearing subcutaneous MC38 expressing murine Nectin-4 were dosed intraperitoneally (i.p.) with PBS or Nectin-4 antibody conjugated to murine reactive T-CpG (mT-CpG). \*\*\* p = 0.0002 (unpaired t-test) (B) Immune competent mice bearing subcutaneous EMT6 expressing murine Nectin-4 were dosed i.p. with PBS, unconjugated Nectin-4 antibody, or various doses of Nectin-4 antibody conjugated to mT-CpG. Arrows indicate doses administered. \*\*\* p = 0.0004, \*\*\*\* p<0.0001 (one-way ANOVA). TF = tumor-free

**Fig. 7: Nectin-4 TRAAC monotherapy exhibits efficacy in a tumor model with low Nectin-4 expression levels**

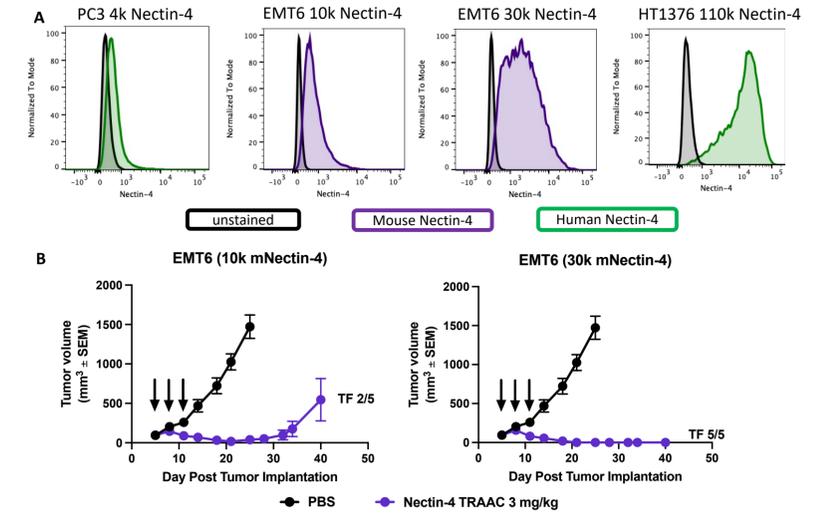


Figure 7: (A) Nectin-4 expression levels and average copy number in EMT6 cells engineered to express various levels of murine Nectin-4 in comparison to levels of Nectin-4 endogenous expression in human cancer cells. Results are from flow cytometric analysis using species cross-reactive antibody. (B) Mice (n=5/group) bearing EMT6 tumors expressing murine Nectin-4 as shown in (A) were dosed i.p. with PBS, or Nectin-4 antibody conjugated to mT-CpG. Arrows indicate doses administered, TF = Tumor-free.

**Fig. 8: Nectin-4 TRAAC monotherapy results in durable responses and anti-tumor immunological memory**

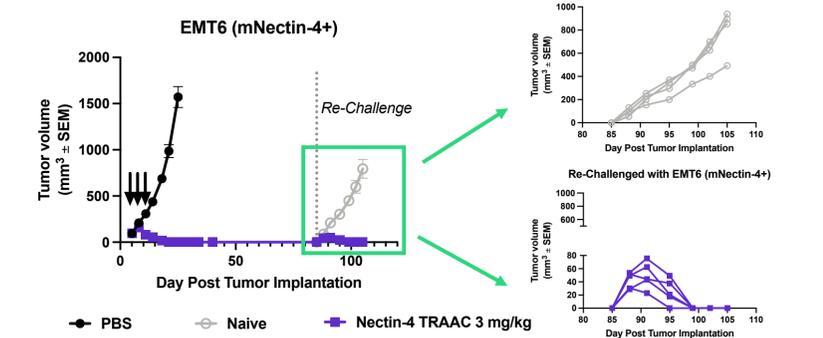


Figure 8: Tumor-eradicated mice from EMT6 over-expressing murine Nectin-4 following 3 mg/kg 3q3 systemic treatment (arrows) with Nectin-4 antibody conjugated to mT-CpG were re-challenged ~70 days post initial tumor clearance. Tumor and treatment naïve, age-matched, mice were used as a control for tumor growth.

## Summary and Conclusions

- We generated high-affinity, species cross-reactive, antibodies specifically targeting Nectin-4. The Nectin-4 antibodies were conjugated with our optimized TLR9 agonist to generate Nectin-4 TRAAC.
- Nectin-4 TRAAC targets both innate and adaptive immune cells via Fcγ receptor engagement leading to robust pro-inflammatory activity in cancer cell-PBMC co-cultures.
- Nectin-4 TRAAC promotes phagocytosis of Nectin-4-expressing cancer cells.
- Systemic administration of single agent Nectin-4 antibody conjugated to mT-CpG produces curative efficacy in multiple Nectin-4-expressing syngeneic tumor models, including those refractory to checkpoint inhibitors and tumors with low Nectin-4 expression levels, and triggers anti-tumor immunological memory.
- The preclinical data of Nectin-4 TRAAC supports its development for solid tumor malignancies that express Nectin-4.