# #1121 TAC-003, a TLR9 Agonist Antibody Conjugate for Targeted Immunotherapy of Nectin-4 Expressing Tumors

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## Introduction

- Tumor microenvironment (TME) modulation using TLR9 agonists has emerged as a promising strategy in cancer immunotherapy, with evidence of clinical activity in melanoma when CpG oligodeoxynucleotides (ODNs) are injected intra-tumorally[1]. However, intra-tumoral injection of CpG ODNs is technically challenging, and limited to accessible tumors, whereas systemic delivered CpG ODNs have poor safety and pharmacokinetic profiles.
- A systemically delivered TLR9 agonist with favorable safety profile has potential to provide innate and adaptive anti-tumor immunity across multiple tumor types.
- Nectin-4 is a cell adhesion molecule with limited expression in normal tissues but over-expressed in many solid tumor types and is a clinically validated cancer-associated antigen[2].
- TAC-003 is a clinical candidate Toll-like Receptor 9 Agonist Antibody Conjugate (TRAAC) comprised of a potent CpG ODN (T-CpG) conjugated to a novel Nectin-4 antibody for systemic administration and activation of TLR9 in the immune microenvironment of Nectin-4 expressing cancers.

## Fig. 1: TAC-003 Drives Anti-Tumor Immunity in Nectin-4 Expressing Cancers

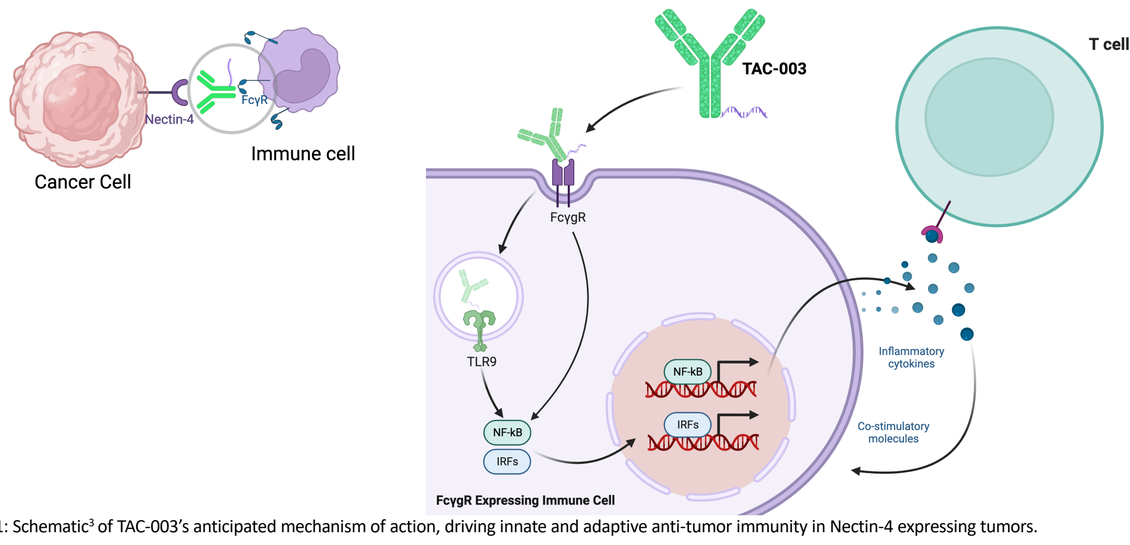



Fig. 1: Schematic[3] of TAC-003's anticipated mechanism of action, driving innate and adaptive anti-tumor immunity in Nectin-4 expressing tumors.

## Experimental Results

## Fig. 2: TAC-003 Elicits Activation of Innate and Adaptive Immunity

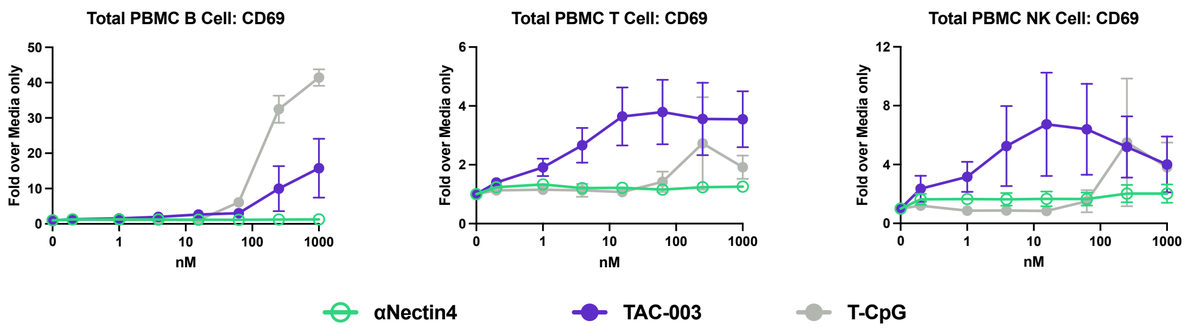



Fig. 2: Co-cultures of human PBMCs with Nectin-4 expressing cancer cells (n=5) were treated overnight with TAC-003, anti-Nectin4 antibody, or T-CpG. Lymphocyte subsets from PBMCs were identified and the expression of the activation marker CD69 analyzed by flow cytometry. Mean CD69 expression levels in each cell type are shown as fold change over media only control. Error bars = Standard Error of Mean (SEM)

## Fig. 3: TAC-003 Triggers Myeloid Cell Pro-inflammatory Differentiation, Activation and Potentiates Phagocytosis




Fig. 3: **(A)** Co-cultures of human PBMCs with Nectin-4 expressing human cancer cells (n=5) were treated overnight with TAC-003, anti-Nectin4 antibody, or T-CpG and assayed for expression levels of various cell activation and differentiation markers by flow cytometry. Data shown as mean fold change over media only control. Error bars = SEM **(B)** Co-cultures of human PBMC (n=3) with CFSE-labeled DLD-1 cells engineered to express various levels of Nectin4 were treated overnight with 1 µM TAC-003, anti-Nectin4 antibody or media only and assayed for percent phagocytic monocytes ($CD14^+CFSE^+$) by flow cytometry. Error bars = SEM

## Fig. 4: TAC-003 Activates TLR9 Signaling in Monocytes, Thereby Inducing Differentiation Towards an APC Phenotype

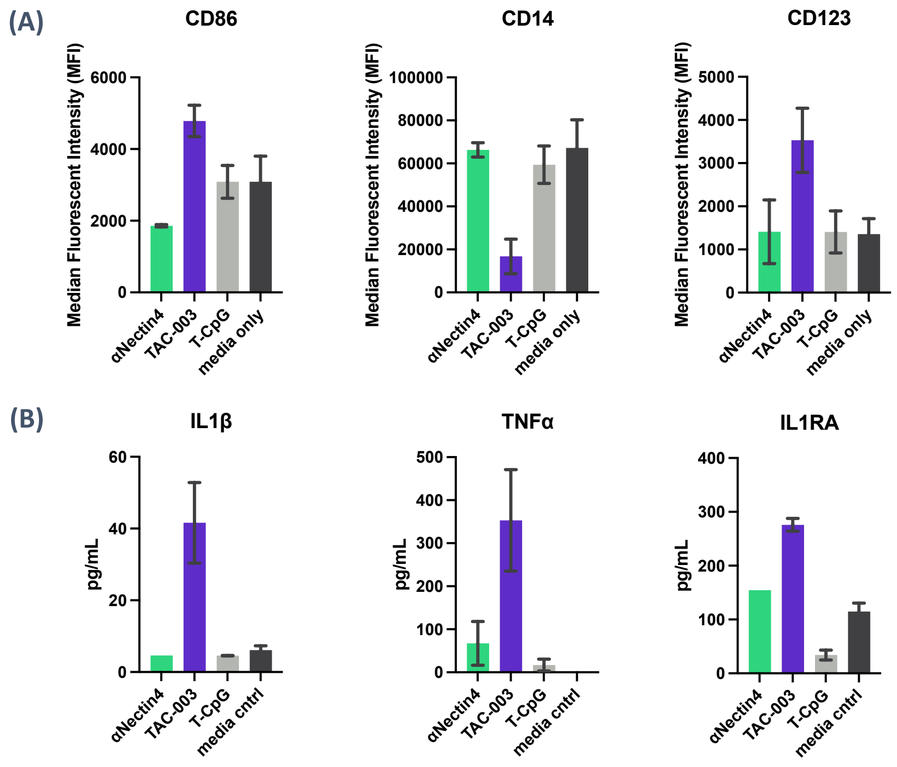



Fig 4: Co-cultures of monocytes isolated from human PBMCs with Nectin-4 expressing human cancer cells were stimulated overnight with TAC-003, anti-Nectin4 antibody, or T-CpG and assayed for **(A)** expression levels of cell surface markers of activation and differentiation by flow cytometry (n=6), and **(B)** levels of pro-inflammatory cytokines in the media supernatant, using cytokine bead array (n=3). Error bars = SEM.

## Fig. 5: mTAC-003 Hones to Tumor and Induces a Pro-Inflammatory TME

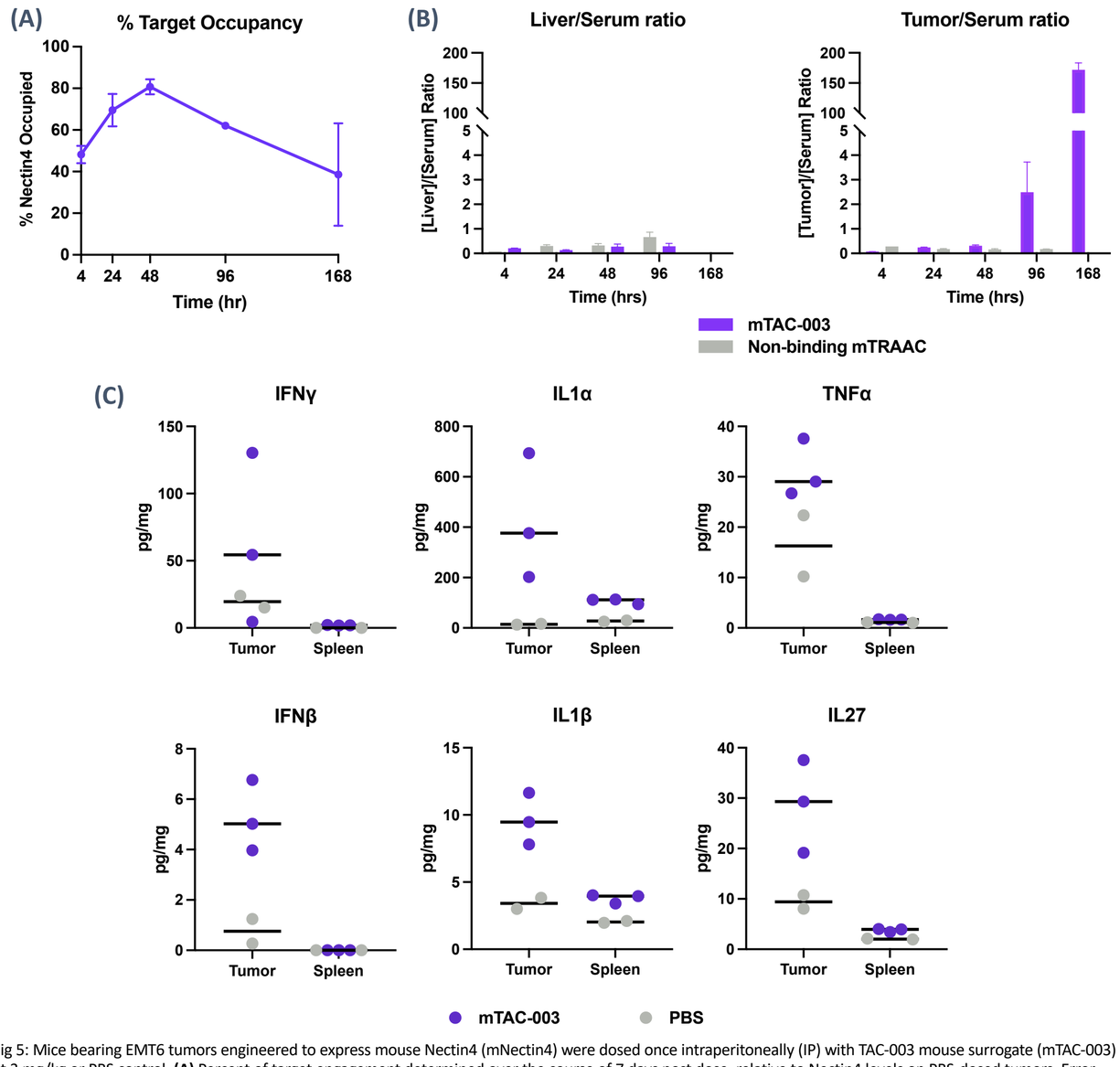
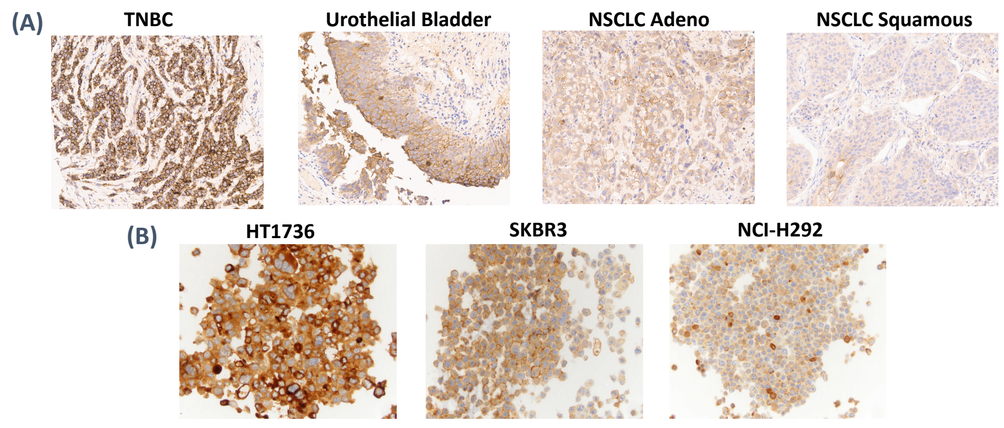



Fig 5: Mice bearing EMT6 tumors engineered to express mouse Nectin4 (mNectin4) were dosed once intraperitoneally (IP) with TAC-003 mouse surrogate (mTAC-003) at 3 mg/kg or PBS control. **(A)** Percent of target engagement determined over the course of 7 days post dose, relative to Nectin4 levels on PBS-dosed tumors. Error bars = SEM **(B)** Concentration levels of human IgG detected in tumor and liver tissue homogenates were compared to levels detected in serum, using ELISA. Error bars = SEM **(C)** Median cytokine levels (normalized by tissue weight) in tumor and spleen tissue homogenates at 24 hours post dose, as measured by cytokine bead assay.

References:
1. Hamid, O., Ismail, R. and Puzanov I., Intratumoral Immunotherapy-Update 2019. Oncologist, 2020 Mar;25(3):e423-e438.
2. 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.
3. Created with Biorender.com
4. Olivier Cabaud, Ludovic Berger, *et al*; Overcoming Resistance to Anti–Nectin-4 Antibody-Drug Conjugate. Mol. Cancer Ther. 1 July 2022; 21 (7): 1227–1235.
5. Chen A, Li M, Bonacorsi M, *et al* 1162 A Nectin-4 targeted TLR9 agonist antibody conjugate induces robust immune cell activation and anti-tumor responses. JITC. 2022;10

## Fig. 6: TAC-003 is Expected to Be Efficacious Across a Range of Nectin-4 Expression Levels




(C)

| Tumor Type | N | Nectin-4 H-score (Avg) | Nectin-4 Copy # |
|---|---|---|---|
| Human Triple Negative Breast Cancer (TNBC) | 12 | 151.1 | - |
| Human Urothelial Carcinoma of the Bladder | 9 | 157.5 | - |
| Human NSCLC Adenocarcinoma | 8 | 43 | - |
| Human NSCLC Squamous Cell Carcinoma | 6 | 6.9 | - |
| HT1376 Human Cell Line | 1 | 240 | 110k |
| SKBR3 Human Cell Line | 1 | 90 | 59k |
| NCI-H292 Human Cell Line | 1 | 45 | 35k |
| EMT6 mNectin4 Mouse Cell line | 1 | - | 30k |
| EMT6 hNectin4 Mouse Cell Line | 1 | 60 | 60k |
| MC38 mNectin4 Mouse Cell line | 1 | - | 76k |

Fig. 6: Representative images of endogenous Nectin-4 expression levels as determined by immunohistochemistry (IHC) on **(A)** untreated human tumor tissue specimens, and **(B)** human cancer cell lines. **(C)** Nectin-4 expression H-score of human tumor tissue specimens and cell line pellets; Nectin-4 copy numbers, as determined by flow cytometry with a human/mouse Nectin-4 cross-reactive antibody, are also shown for various human and mouse cell lines utilized in these studies.

## Fig. 7: mTAC-003 Monotherapy Shows Potent Dose Dependent Efficacy and Unleashes Anti-Tumor Immunological Memory

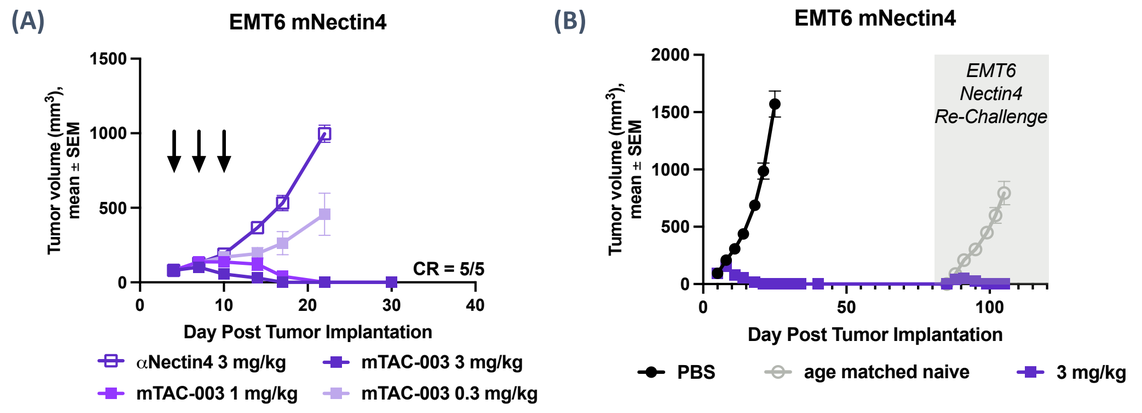
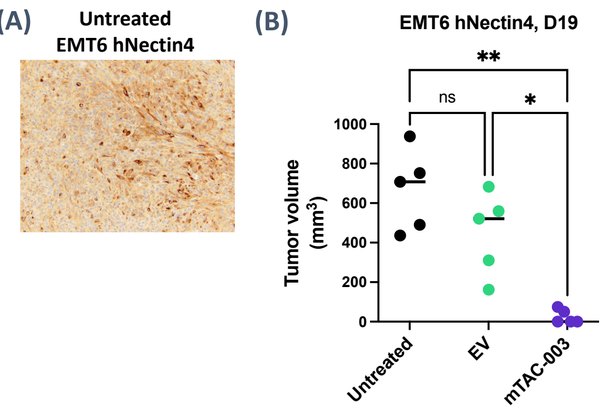



Fig. 7: **(A)** Mice (n=5/group) implanted subcutaneously with EMT6 tumors engineered to express murine Nectin-4 (EMT6 mNectin4) were dosed IP with anti-Nectin4 antibody, or various dose levels of mTAC-003. Arrows = dosing days, CR = Complete Response. **(B)** Mice that eradicated EMT6 mNectin4 tumors following 3 mg/kg mTAC-003 3q3 treatment were re-challenged 64 days post tumor clearance. Tumor and treatment naïve, age-matched, mice were used as control for tumor growth.

## Fig. 8: mTAC-003 Monotherapy Demonstrates Improved Efficacy Over Nectin4 ADC




(C)

| Treatment | Tumor Volume ($mm^3$)[a] (D19) | TGI%[b] (D19) | CR[c] (D19) | DCR[d] (D39) |
|---|---|---|---|---|
| PBS | 665.0±91.3 | - | 0/5 | 0/5 |
| EV | 447.3±93.2 | 37.9 | 0/5 | 0/5 |
| mTAC-003 | 25.0±15.8 | 111.4 | 3/5 | 4/5 |

[a] Mean ± SEM
[b] TGI: tumor growth inhibition; TGI (%) = 100 × [1 − (Vtreat-t − Vtreat-1) / (Vcontrol-t − Vcontrol-1)], where Vtreat-1 and Vcontrol-1 are mean tumor volumes of the treated and control groups on grouping day, and Vtreat-t and Vcontrol-t are mean tumor volumes of the treated and control groups on a given day
[c] CR: complete response; number of animals with no measurable tumors on day 19.
[d] DCR: durable complete response; number of animals with no measurable tumors at end of the study (day 39).

Fig. 8: **(A)** Micrograph of human Nectin-4 expression in EMT6 hNectin4 syngeneic subcutaneous tumor model, detected by IHC. Mouse EMT6 cells were engineered to express human Nectin4 and implanted in syngeneic mice. **(B and C)** Mice (n=5/group) were inoculated with $2x10^6$ syngeneic EMT6 hNectin4 cells (same model as in A). When tumors reached ~90 $mm^3$, mice were treated with 3 mg/kg EV (4q3, intravenously), 3 mg/kg mTAC-003 (3q3, IP), or PBS (untreated). **(B)** Median EMT6 hNectin4 tumor size on day 19 post treatment, * $p = 0.03$, ** $p = 0.003$; ns = non-significant (RM one-way ANOVA, Tukey's multiple comparison). **(C)** Summary of tumor growth inhibition and complete tumor regression results.

## Fig. 9: mTAC-003 with anti-mPD1 Elicits Enhanced Anti-Tumor Response

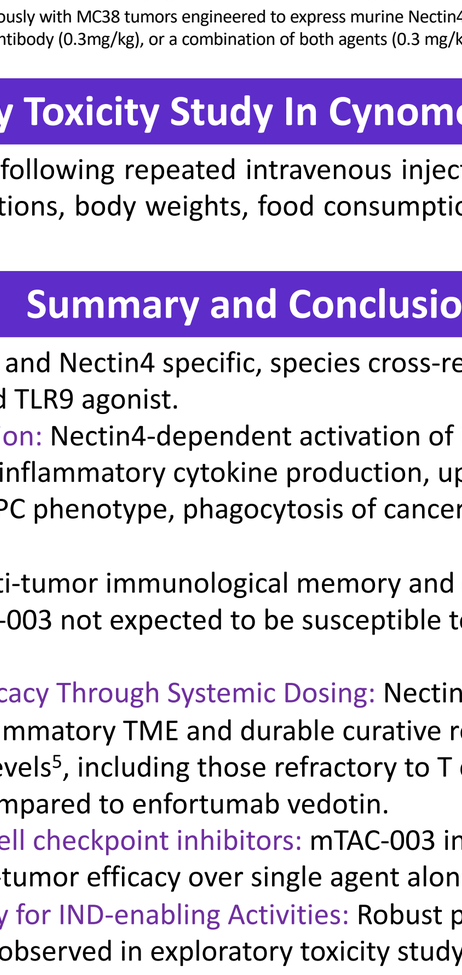



Fig. 9: Mice (n=10/group) implanted subcutaneously with MC38 tumors engineered to express murine Nectin4 (MC38 mNectin4) were dosed IP with sub-optimal dose levels of mTAC-003 (0.3mg/kg), or anti-mPD-1 antibody (0.3mg/kg), or a combination of both agents (0.3 mg/kg each). PBS dosed mice were used as control. Arrows = dosing days.

## Exploratory Toxicity Study In Cynomolgus Monkeys

TAC-003 was well tolerated following repeated intravenous injections in cynomolgus monkeys, as assessed by clinical observations, body weights, food consumption, hematology, serum chemistry and histopathology.

## Summary and Conclusions

- TAC-003: A high-affinity and Nectin4 specific, species cross-reactive, fully human antibody conjugated to optimized TLR9 agonist.
- Robust Immune Activation: Nectin4-dependent activation of immune cells via Fc$\gamma$R and TLR9 engagement elicits pro-inflammatory cytokine production, up-regulation of co-stimulatory molecules, enhanced APC phenotype, phagocytosis of cancer cells and triggering of adaptive immunity.
- Differentiated MoA: Anti-tumor immunological memory and efficacy not limited to $Nectin4^{High}$ tumors. TAC-003 not expected to be susceptible to same resistance mechanisms of cytotoxic ADC[4].
- Potent Single Agent Efficacy Through Systemic Dosing: $Nectin4^+$ tumor targeted localization of TAC-003 drives pro-inflammatory TME and durable curative responses in models with a range of Nectin4 expression levels[5], including those refractory to T cell checkpoint inhibitors, with improved efficacy as compared to enfortumab vedotin.
- Increases Efficacy of T cell checkpoint inhibitors: mTAC-003 in combination with anti-PD1 antibody increases anti-tumor efficacy over single agent alone.
- Clinical Candidate Ready for IND-enabling Activities: Robust preclinical package, including favorable safety profile observed in exploratory toxicity study in cynomolgus monkeys.