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A B-cell targeted TLR9 Agonist Antibody Conjugate Potentiates Cancer Vaccine Efficacy and Rejuvenates Vaccine Responses in the Elderly

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Introduction

- Recent advances of neoantigen cancer vaccines combined with T cell checkpoint inhibitors have shown therapeutic promise [1]. However, there continues to be an unmet need to further potentiate vaccine efficacy to provide durable clinical responses across multiple oncology indications.
- Toll-like receptors (TLRs) play crucial roles in innate immune responses against invading pathogens with activation of TLR signaling leading to induction of inflammatory cytokines and priming of adaptive immunity [2]. TLR9 agonists have been clinically validated as vaccine adjuvants and cancer therapeutics [3,4].
- TAC-001 is a B Cell targeted Toll-like Receptor Agonist Antibody Conjugate (TRAAC) delivering a potent TLR9 agonist (T-CpG) resulting in innate and adaptive immune activation and anti-tumor immunity [5]. TAC-001 is being evaluated as a systemic immunotherapy in a Phase I clinical trial (NCT05399654) in patients with advanced metastatic solid tumors [6].
- Herein, we evaluated TAC-001 mouse surrogate, mCD22TRAAC, as combination therapy in various cancer and infectious disease vaccine models, demonstrating robust humoral and cellular immune activation, resulting in potent anti-tumor activity.

Fig. 1: TAC-001 is a Toll-Like Receptor Agonist Antibody Conjugate (TRAAC) developed for systemic delivery of a potent, B cell targeted TLR9 agonist (T-CpG)

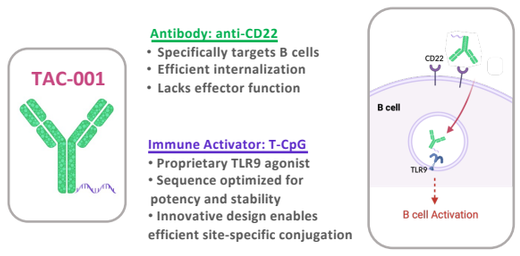
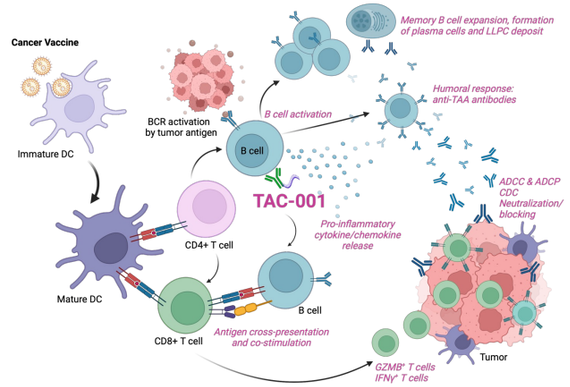


Fig. 1. TAC-001 binds to CD22 on B cell surface, leading to TAC-001:CD22 complex internalization and activation of TLR9 signaling in endosome, thereby resulting in peripheral B cell activation, with co-stimulatory activity and production of pro-inflammatory cytokines and chemokines [6].

Vaccine Associated Mechanism of Action

Fig. 2: Anticipated mechanisms elicited by TAC-001 and cancer vaccine combination



Elderly Vaccine Results

Fig. 6: mCD22 TRAAC rejuvenates primary response with Th1 skewing in elderly mice

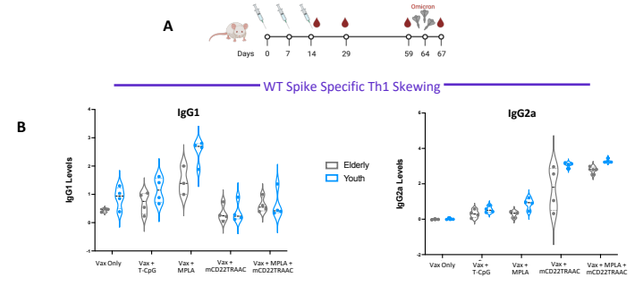


Fig. 6. Young (7-8 wks) and Elderly (12 months) mice were immunized (s.c., i.p.) with wildtype (WT) SARS-CoV2 Spike Trimer (Vax) alone or in combination with 1mpk MPLA, 10mpk mCD22TRAAC or mCD22TRAAC and MPLA. Free T-CpG was dosed equimolar to mCD22TRAAC. **(A)** Immunization, serum collection and omicron challenge schedule. **(B)** Serum anti-WT Spike IgG1 and IgG2a levels measured as optical density on day 29. (ELISA, each symbol is result from an individual mouse).

Fig. 7: In elderly mice, mCD22 TRAAC elicits enhanced memory recall, cross-reactive humoral response, and synergizes with a TLR4 agonist

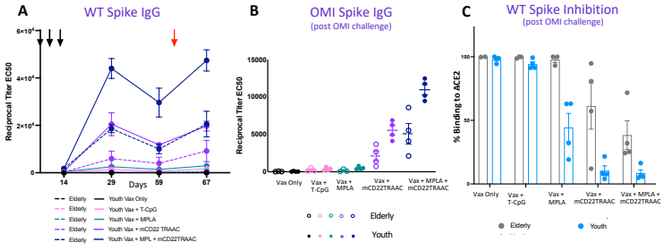


Fig. 7. **(A)** Anti-WT Spike total IgG levels expressed as reciprocal titer EC50 of binding (ELISA, mean ± SEM) on days 14-67 from Fig. 6A. Black arrows indicate treatments administered. mCD22TRAAC induced robust primary response (day 29) and secondary response (day 67) post omicron (OMI) challenge (red arrow). **(B)** Anti-Omicron Spike total IgG levels expressed as reciprocal titer EC50 of binding on day 67 (ELISA, each symbol is result from an individual mouse, lines are mean ± SEM of cohort). **(C)** Serum inhibition of WT Spike post Omicron Spike challenge (ELISA, serum diluted 1/100; each symbol is result from an individual mouse, bars are mean ± SEM of cohort).

Cancer Vaccine Results

Fig. 3: mCD22 TRAAC potentiates Nectin-4 vaccine associated humoral immunity with Th1 phenotype

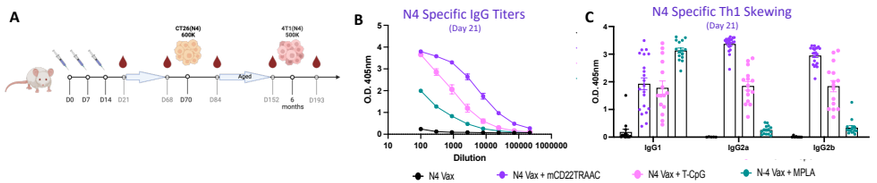


Fig. 3. Mice (8wk) were immunized with human Nectin-4 ectodomain protein (N4, neoantigen-like with 94% homology to mouse ortholog) as a vaccine (N4 vax) alone or co-administered with 200 µg mCD22TRAAC, or 8 µg T-CpG (equimolar) or 50 µg MPLA (TLR4 agonist). **(A)** Immunization (s.c., i.p.), serum sampling and tumor challenge schedule. **(B)** Anti-N4 total IgG serum titers (ELISA, mean ± SEM). Serum IgG confirmed to bind CT26 transduced with N4 (CT26N4, not shown). **(C)** mCD22TRAAC mediates a homogeneous, N4, effector function subtype antibody response (ELISA, serum diluted 1/2,700, each symbol is result from an individual mouse, bars are mean ± SEM of cohort).

Fig. 4: mCD22 TRAAC enhances Nectin-4 vaccine specific anti-tumor immunity with robust memory recall in aged mice

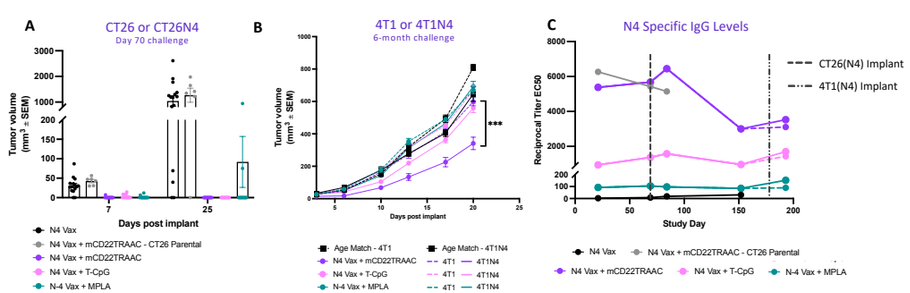


Fig. 4. **(A)** Immunized mice from Fig. 3 were challenged with parental CT26 or CT26 transduced with human Nectin-4 (N4) on day 70. On days 7 and 25 post implantation, mice treated with mCD22TRAAC, T-CpG and MPLA demonstrate effective anti-tumor protection. Each symbol is result from an individual mouse; bars are mean ± SEM of cohort. **(B)** Surviving mice from each of the treatment groups were aged 6 months and split into 2 groups, then challenged with a high number (500K cells) of either parental 4T1 or 4T1 transduced with N4 (4T1N4). Age-matched mice were used as control. Only mCD22TRAAC treated mice continued to demonstrate vaccine specific anti-tumor inhibition (***p=0.0001, 2-way ANOVA). **(C)** Anti-N4 total IgG levels expressed as reciprocal titer EC50 of binding (ELISA).

Fig. 5: mCD22 TRAAC significantly increases vaccine specific cytotoxic T cell activity

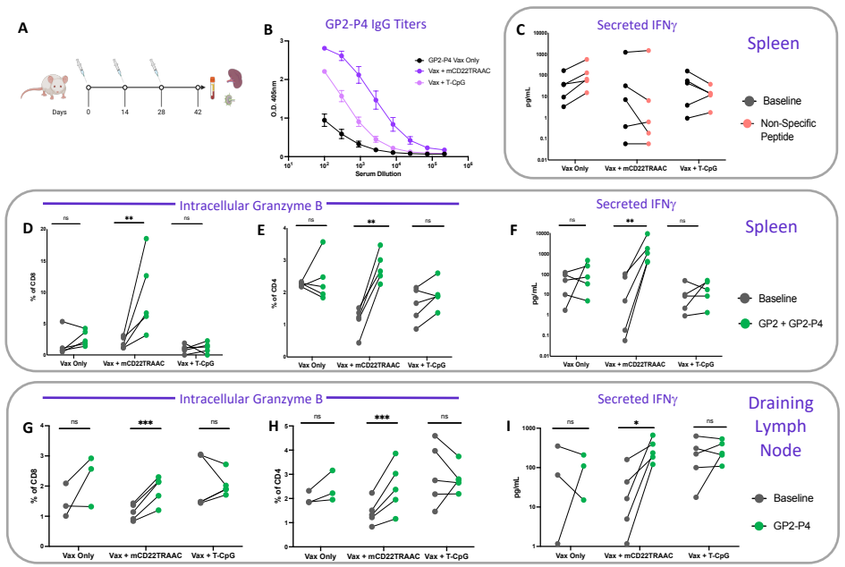


Fig 5. Mice (10wk) were immunized (s.c., i.p.) with MHC class I restricted HER2 peptide (GP2) fused with B cell HER2 epitope peptide (P4) and conjugated to KLH, as a vaccine (GP2-P4) alone or co-administered with 200µg mCD22TRAAC or 8µg free T-CpG. **(A)** Immunization and tissue collection schedule. **(B)** Anti-GP2-P4 total IgG titers on day 42 (ELISA, mean ± SEM). **(C-F)** Splenocytes were cultured with media only (baseline), GP2 and GP2-P4 peptides **(D-F)** or non-specific peptide **(C)** for 72 hrs to test for vaccine recall. **(G-I)** Draining lymphocytes were cultured with media only or with GP2-P4. Secreted IFNg levels **(C, F, I)** and intracellular Granzyme B expressing T cells **(D, F, G, H)** were quantified by flow cytometry. Each line is result from a different mouse; *ps0.02, **p ≤0.006, ***p ≤0.001, ns= non-significant (ratio paired *t* test).

Conclusions

Combination of mCD22 TRAAC, a TAC-001 murine surrogate, with various cancer and infectious disease vaccines enhances humoral and cellular vaccine specific immunity

Specifically, we demonstrate that mCD22 TRAAC:

- Induces robust vaccine specific IgG titers
- Generates durable IgG response indicative of vaccine specific, long-lived plasma cells (LLPC)
- Skews humoral response to a Th1 phenotype
- Induces robust and durable functional antibodies (antigen neutralizing, Fc-dependent effector function) in both youth and elderly, with rejuvenation of vaccine responses in elderly mice
- Synergizes with MPLA, a TLR4 agonist for potent rejuvenation of response in elderly
- Significantly elicits vaccine specific cytotoxic T cell activity
- Enhances neoantigen-like cancer vaccine specific anti-tumor immunity with superior memory recall

References

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