Therapeutic tumor immunity requires the presence and appropriate activation of tumor antigen specific CD8+ T cells and migration of activated tumor-antigen specific CD8+ T cells into a tumor microenvironment where immunosuppressive barriers have been eliminated. Antibody-mediated blockade of CTLA-4 and PD-1 is a clinically effective strategy to dampen tumor-mediated immunosuppression in a minority of patients with advanced cancer, and this approach may be potentiated through vaccination to prime additional CTL clones and through direct stimulation of T cell costimulatory molecules of the TNF superfamily. Here we provide a systematic comparison of anti-tumor vaccination with either heat shock protein gp96-Ig or traditional peptide/adjuvant vaccines given alone or in combination with CTLA-4 or PD-1 blockade and direct T cell costimulation via OX40, 4-1BB and TNFRSF25. Through the tracking of tumor-antigen specific CD8+ and CD8+ T cell responses, these studies demonstrate that both TNFRSF4 and TNFRSF25 independently and additively costimulate vaccine-induced CD8+ T cell proliferation following both primary and secondary antigen challenge. In contrast, the activity of TNFRSF4 and TNFRSF25 were observed to be divergent in the costimulation of CD8+ T cell immunity. Interestingly, antigen-specific cellular and humoral responses were uncoupled upon secondary immunization, which was dramatically affected by the presence of OX40, 4-1BB or TNFRSF25 costimulation. When combined with checkpoint inhibition in therapeutic murine orthotopic tumor models, synergistic anti-tumor activity was observed with various triple combinations with vaccine, checkpoint inhibition and T cell costimulation. These studies highlight the complementary activity of vaccination, checkpoint inhibition and direct T cell costimulation, which may guide the application of combination immunotherapy for effective anti-tumor immunity.

Methodology:

Day 0: C57BL/6 mice were adoptively transferred with a mixed population of ovalbumin-specific CD4+ OT-II and CD8+ OT-I cells by intravenous injection.

Day 1: Mice were inoculated with B16-F10-ova melanoma cells (2.5×10^6 cells, subcutaneously)

Day 9: When tumors were 6-8 mm in diameter, the first treatments were given as indicated:

- ± Mock or 3T3-ova-gp96-Ig (10^6 cells, intraperitoneal injection)
- ± IgG control, OX40 agonist (OX86, 100µg), 4-1BB agonist (3H3, 100µg) GITR agonist (DTA-1, 100µg) or TNFRSF25 agonist (4C12, 100µg)

Day 13: Antibody injections were repeated

Day 17: Antibody injections were repeated

Results and Conclusions

- OX86 alone → weak OT-I response
- Vaccine potentiated OT-I response
- No detectable OT-II expansion
- Vaccine potentiated overall survival

- 4C12 alone → weak OT-I response
- Vaccine potentiated OT-I response
- No detectable OT-II expansion
- Vaccine potentiated overall survival

- 3H3 alone → NO OT-I response
- No effect with vaccine combination
- No detectable OT-II expansion
- Survival not potentiated by vaccine

- DTA-1 alone → weak OT-I response
- Vaccine potentiated OT-I response
- No detectable OT-II expansion
- Survival not potentiated by vaccine

Head-to-Head Ab Alone

- OX40 superior as monotherapy
- OX40 + TNFRSF25 combo best

Treg Response

- TNFRSF25 always ↑Treg
- OX40 + TNFRSF25 additive

CD4/CD8 Ratio

- 4-1BB reverses CD4/CD8 ratio
- Likely antigen non-specific

Take Home Points:

Vaccine Combination OS:
- TNFRSF25 > OX40 > 4-1BB > GITR

Vaccine Combination CD8+:
- TNFRSF25 > GITR > OX40 > 4-1BB

Vaccine Combination Treg:
- TNFRSF25 > 4-1BB > OX40 > GITR

For informational purposes only. TNFRSF25 studies performed in collaboration with Pelican Therapeutics..