Abstract: AACR-4846

MM-401, a novel anti-TNFR2 antibody that induces T cell co-stimulation, robust anti-tumor activity and immune memory

J. Richards*, C. Wong*, A. Koshkaryev*, R. Fulton, A. Camblin, J. Sampson, L. Luus, J. Suchy, S. Grabow, V. Kurella, S. Kumar, J. Lulo, J. Qiu, Y. Jiao, L. Xu, V. Paragas, M. Razlog, M. Muda, E.M. Tam, A. Raue, D.C. Drummond

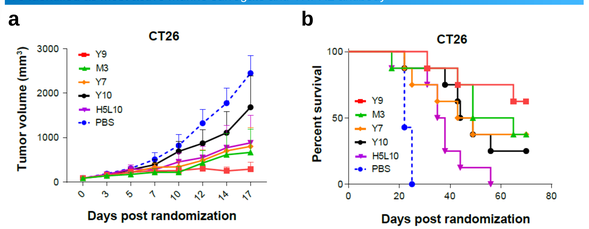
Merrimack Pharmaceuticals, Inc., Cambridge MA, USA

* Contributed equally

Summary

TNFR2 has been implicated as a novel target for cancer immunotherapy. While TNFR2 has been linked to enhanced suppressive activity of regulatory T cells (Tregs) in autoimmune models, the effect of TNFR2-targeted therapy in cancer remains unclear. Here we present a novel monoclonal anti-TNFR2 antibody that provides T cell co-stimulation and yields robust anti-tumor activity in *in vitro* and *in vivo* models. In syngeneic murine tumor models, treatment with a murine surrogate anti-TNFR2 antibody results in robust anti-tumor activity both alone and in combination with checkpoint inhibitor antibodies targeting PD-1 and PDL-1. Complete responders exhibited immunological memory months after initial tumor clearance. Furthermore, significant anti-tumor activity was observed in anti-TNFR2-treated mice even in a model that proved resistant to PD1-targeted antibody treatment. Depletion studies suggest that $CD8^+$ T and NK cells are required for activity, whereas TNFR2 knockout models suggest that TNFR2 expression on cancer cells is not required for activity. Using an antibody with a mutant Fc, we show that activity is dependent on FcγR binding. Studies in FcγR knockout mice, complemented by studies using different antibody-Fc variants, confirm that enhanced agonism via FcγR binding is the dominant mechanism of action. Contrary to antibodies targeting other TNF superfamily receptors, treatment does not lead to strong depletion of TNFR2-expressing cell types such as Tregs. Consistent with its proposed mechanism, long-term dosing of the anti-TNFR2 antibody did not cause toxicity in two inbred mouse strains when compared to an anti-CTLA4 antibody, which caused weight loss, splenomegaly and elevated inflammatory cytokines in serum. Following anti-TNFR2 treatment, we observed a broad reversal of immunosuppression in the tumor characterized by downregulation of suppressive markers and increased cytokine production by $CD8^+$ T cells.

Y9 identified as most active murine surrogate anti-TNFR2 antibody

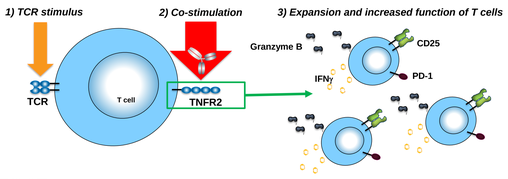


(a) *In vivo* activity of anti-TNFR2 mAbs in the CT26 syngeneic murine tumor model. Each antibody was injected i.p. once at day zero (9 days after tumor inoculation) at 1 mg (N=7 animals per group, data displayed as mean and SEM). **(b)** *In vivo* activity in the CT26 syngeneic murine tumor model at reduced dose. Each antibody was injected i.p. once at day zero (9 days after inoculation) at 300 µg (N=8 animals per group).

Mechanism of action summary

Dominant Mechanism:

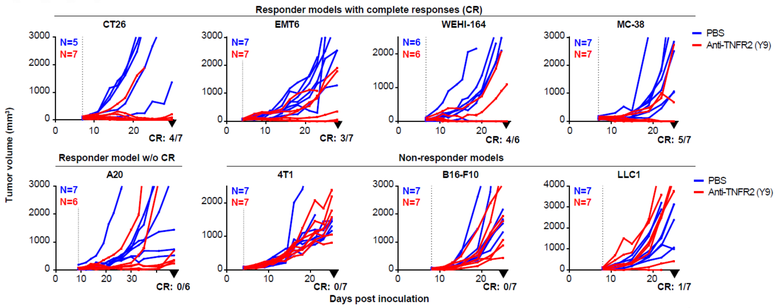
- **Co-stimulatory activity on T cells**
  - ✓ Potent *in vitro* and *in vivo* stimulation of $CD8^+$ and $CD4^+$ T cells
  - ✓ Increases magnitude and effector function of tumor-infiltrating $CD8^+$ T cells
  - ✓ FcγR-dependent efficacy and TNFR2 receptor downregulation
  - ✓ Dependency on inhibitory Fcγ receptors
  - ✓ Comparable activity of mIgG2a, mIgG1, and variants with enhanced binding to inhibitory Fcγ receptors
  - ✓ Fast downregulation of immunosuppressive markers on T cells



Cross-reference

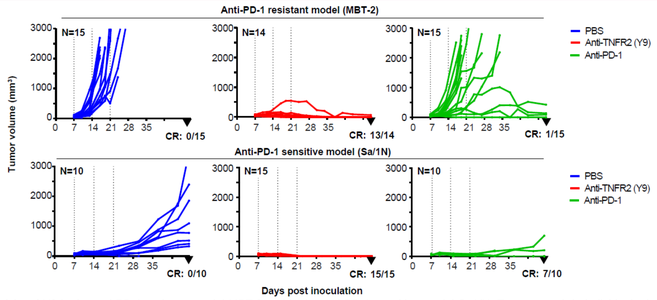
Fulton *et al.* Mechanism of action of a novel agonist TNFR2-antibody that induces co-stimulation of T cells and promotes robust anti-tumor immunity. AACR 2019, Abstract #3270.

Treatment with anti-TNFR2 antibody Y9 leads to complete tumor clearance in multiple syngeneic tumor models



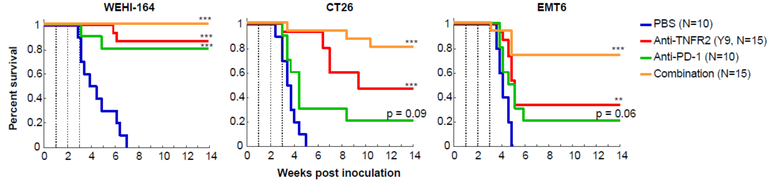
Anti-tumor activity of Y9 in responder and non-responder models. Tumor growth in individual mice is shown. CR; tumors below 60 mm^3 and continued to regress until the end of study. CR indicated for Y9 treatment groups. Vertical dotted lines indicate treatment with a single dose of 300 µg of Y9 antibody. Group sizes are indicated in the figure.

Anti-TNFR2 antibody Y9 is active in PD-1 sensitive and resistant syngeneic tumor models



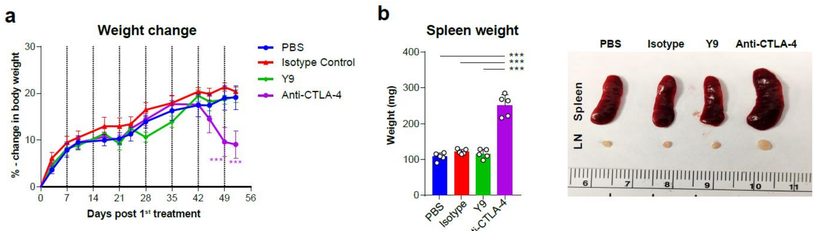
Anti-tumor activity of Y9 in the anti-PD-1-resistant model MBT-2 and in the anti-PD-1-sensitive model Sa1/N. Tumor growth in individual mice is shown. CR; tumors below 60 mm^3 and continued to regress until the end of study. CR indicated for Y9 treatment groups. Vertical dotted lines indicate treatment with 300 µg of Y9 antibody. Group sizes are indicated in the figure.

Anti-TNFR2 immunotherapy synergizes with anti-PD-1 in mouse syngeneic tumor models



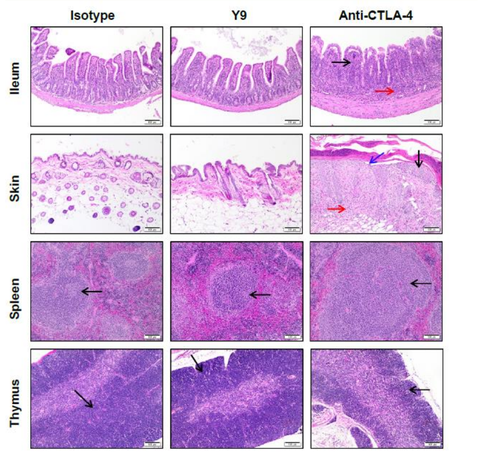
Survival curves for treatment with Y9 alone and in combination with anti-PD-1 in multiple murine models. Statistically significant difference from PBS is indicated. Similar data are available for combination with anti-PD-L1. Vertical dotted lines indicate treatment with 300 µg of Y9 antibody.

Anti-TNFR2 antibody Y9 has a favorable toxicity profile compared to anti-CTLA-4



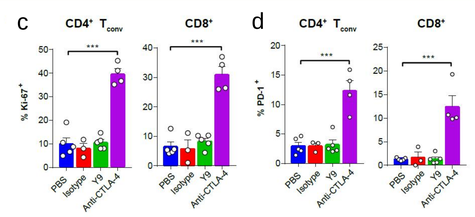
Mice were treated once weekly with PBS or 1 mg murine IgG2a isotype control, Y9, or anti-CTLA-4 (clone 9D9 on mouse IgG2a) (N=5 animals per group). **(a)** Longitudinal percent change in body weight. Vertical dashed lines indicate times of treatment. **(b)** Comparison of spleen sizes and weights 48 h following the final treatment.

Y9 does not cause histological changes in lymphoid and non-lymphoid tissues



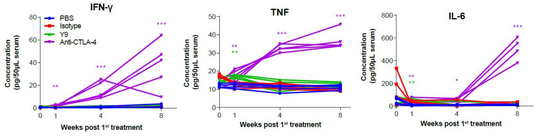
Representative H&E staining from various tissues in BALB/c mice treated with IgG2a isotype, Y9, or anti-CTLA-4. Mice treated with anti-CTLA-4 had increased numbers of inflammatory cells in the lamina propria (red arrow) of the small and large intestine with blunting and fusion of intestinal villi (black arrow). Intestines of Y9-treated mice were comparable to mice treated with isotype control antibody. BALB/c mice treated with anti-CTLA-4 had multifocal epidermal hyperplasia (black arrow) with ulceration (blue arrow), exudation, and mixed cell infiltrate (red arrow) in dermis of the skin, most notably on the ears. There were no lesions in the skin of mice treated with Y9 or isotype control. Spleens from mice treated with anti-CTLA-4 had expansion of the periarteriolar lymphoid sheaths (PALS) with increased follicle size (black arrows) compared to Y9-treated and isotype controls. Compared to mice treated with Y9 or isotype control antibody, mice treated with anti-CTLA-4 had depletion of lymphocytes in the thymic cortex (black arrows, H&E, 100x). Images in are representative of 5 animals per treatment group.

Y9 does not cause spontaneous activation of T cells



Frequency of Ki-67+ **(c)** and PD-1+ **(d)** $CD4^+$ $Foxp3^{neg}$ (T_{conv}) and $CD8^+$ T cells in peripheral blood 7 days after the 4th treatment.

Y9 does not cause chronic elevation of inflammatory serum cytokines



Longitudinal serum cytokines. Data were analyzed using ANOVA with a Dunnett's multiple comparisons post-test comparing treatment groups to PBS. Data plotted as mean ± SEM. Statistically significant difference from PBS control is indicated.

Conclusion

Anti-TNFR2 antibodies showed broad anti-tumor activity in syngeneic mouse models, both alone and in combination with anti-PD-1, and had a favorable toxicity profile compared to anti-CTLA-4 in a long-term exposure study in mice. A human anti-TNFR2 antibody (MM-401) with low nanomolar affinity and binding to the same epitope as the murine surrogate antibody (Y9) has been developed. MM-401 is being developed as a potential novel treatment option for cancer patients.

Cross-reference

Sampson *et al.* A novel human TNFR2-antibody (MM-401) modulates T cell responses in anti-cancer immunity. AACR 2019, Abstract #555.

All data unpublished and on file at Merrimack Pharmaceuticals, Inc.

A PASSION FOR OUTTHINKING CANCER