Abstract: AACR-555

A novel human TNFR2 antibody (MM-401) modulates T cell responses in anti-cancer immunity

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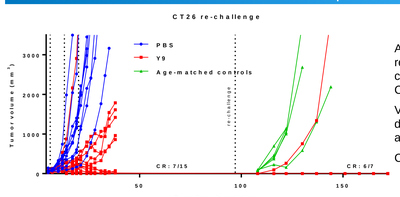
Introduction

Background: Despite the dramatic anti-tumor responses observed for immune checkpoint inhibition in subsets of patients, there remains an unmet medical need for the larger patient population. Combination immunotherapies have been able to improve efficacy, but often at the expense of significant increases in toxicities. Consequently, there is an ongoing need for new cancer immunotherapies that have promising activity but are also well-tolerated. We have identified TNF receptor 2 (TNFR2) as a key regulator of the immunosuppressive microenvironment and have observed significant preclinical anti-tumor responses using a monoclonal antibody targeting that receptor. Here, we report the development of corresponding human agonist TNFR2 antibodies for use in patients.

Materials and methods: Human anti-TNFR2 antibodies were generated from mouse immunization and phage display campaigns and produced as human IgG1. Antibodies were tested for the ability to provide co-stimulation to primary human T cells in an *in vitro* stimulation assay with anti-CD3. The antibodies were tested in a T cell-driven model of xenogeneic graft vs. host disease (GvHD), and the ability of the antibodies to drive anti-tumor efficacy in the presence or absence of PD-1-blocking antibody (Nivolumab) was tested in humanized mouse models.

Results: Ab1 (chimera) and Ab2 (fully human) bind to recombinant TNFR2 and cell surface TNFR2 with sub nanomolar affinity and compete for TNFα binding to the receptor. While Ab1 and Ab2 are not cross-reactive with mouse TNFR2, both antibodies bind the CRD1 domain of human TNFR2 which corresponds to the epitope of the mouse surrogate antibody Y9. Our human anti-TNFR2 antibodies provided co-stimulatory signaling that led to increases in proliferation, activation markers, and cytokines both in CD4⁺ and CD8⁺ T cells *in vitro*. *In vivo*, Ab2 increased survival of mice in the xenogeneic GvHD model. Consistent with the function of the mouse surrogate antibody in syngeneic tumor models, our human anti-TNFR2 antibodies showed robust anti-tumor activity in multiple humanized PDX and CDX models.

Rationale – Anti-mouse TNFR2 led to robust responses in syngeneic tumor models



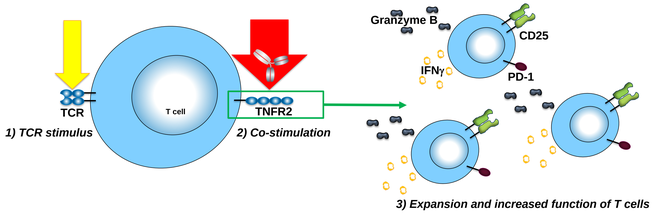
After treatment with Y9, CT26 complete responder mice, as well as age-matched controls (N=5) were re-challenged with CT26 97 days after inoculation.

Vertical lines before re-challenge indicate days of treatment with 300 μg per antibody i.p.

CR = complete response

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

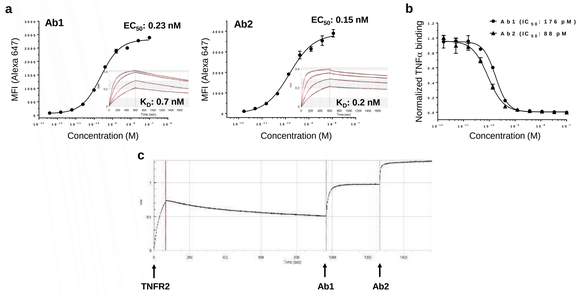


Other TNFR2 posters from Merrimack:

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.

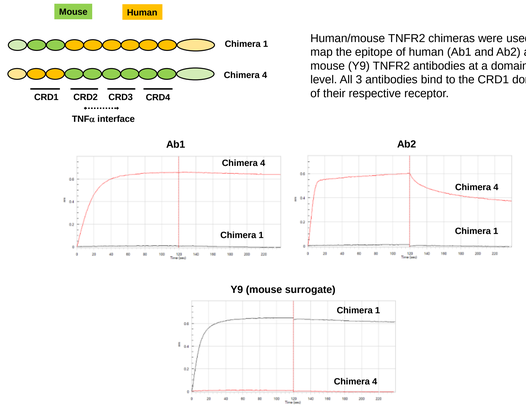
Richards *et al.* MM-401, a novel anti-TNFR2 antibody that induces T cell co-stimulation, robust anti-tumor activity and immune memory. AACR 2019, Abstract #4846.

Development of human anti-TNFR2 antibodies



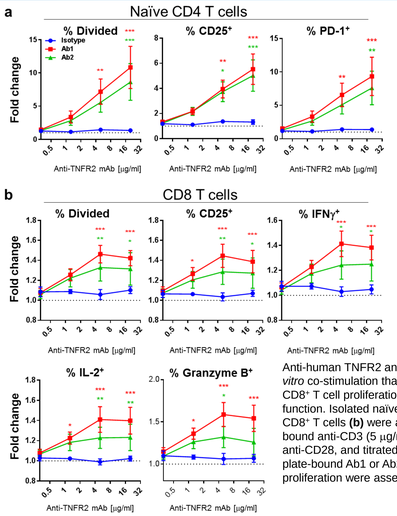
Ab1 is a chimeric antibody derived from mouse immunization and Ab2 is a fully human antibody from a phage display library. **(a)** Antibody binding to TNFR2 on CHO cells and recombinant TNFR2 by biolayer interferometry (BLI) (insert). **(b)** Inhibition of TNFα binding to CHO-TNFR2 cells. **(c)** Epitope binning of antibodies. **(d)** Cross reactivity to cynomolgus and murine TNFR2.

Epitope of human antibody corresponds with surrogate mouse antibody



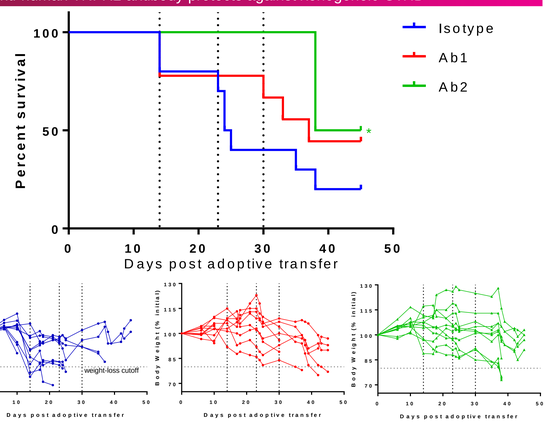
Human/mouse TNFR2 chimeras were used to map the epitope of human (Ab1 and Ab2) and mouse (Y9) TNFR2 antibodies at a domain level. All 3 antibodies bind to the CRD1 domain of their respective receptor.

Anti-human TNFR2 mAbs provide co-stimulation to human T cells



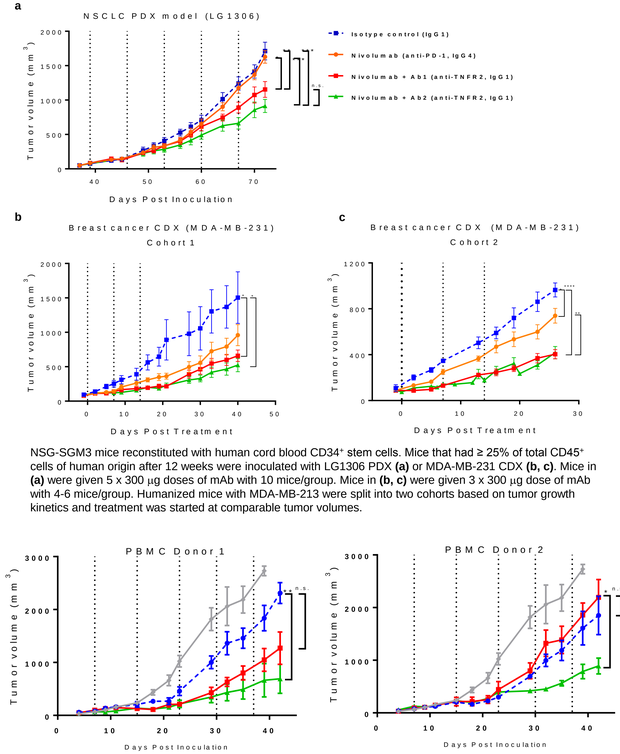
Anti-human TNFR2 antibodies provide *in vitro* co-stimulation that increases CD4⁺ and CD8⁺ T cell proliferation, activation, and function. Isolated naïve CD4⁺ T cells **(a)** or CD8⁺ T cells **(b)** were activated with plate-bound anti-CD3 (5 μg/mL),1 μg/mL soluble anti-CD28, and titrated concentrations of plate-bound Ab1 or Ab2. Activation and proliferation were assessed 4 days later.

An anti-human TNFR2 antibody protects against xenogeneic GvHD



To model GvHD, NSG mice were given 1x10⁷ human PBMCs i.v. 10 mice/group were treated with 3 x 100 μg of human IgG1 isotype, Ab1, or Ab2 (indicated by dashed vertical lines). Mice that lost ≥ 20% body weight or had deteriorating body condition were euthanized.

Treatment with anti-human TNFR2 antibodies leads to reduced tumor volumes in multiple humanized mouse models



NSG-SGM3 mice reconstituted with human cord blood CD34⁺ stem cells. Mice that had ≥ 25% of total CD45⁺ cells of human origin after 12 weeks were inoculated with LG1306 PDX **(a)** or MDA-MB-231 CDX **(b, c)**. Mice in **(a)** were given 5 x 300 μg doses of mAb with 10 mice/group. Mice in **(b, c)** were given 3 x 300 μg dose of mAb with 4-6 mice/group. Humanized mice with MDA-MB-213 were split into two cohorts based on tumor growth kinetics and treatment was started at comparable tumor volumes.

NSG mice administered human PBMCs from 2 different donors and HT-29 CDX cells on the same day. On day 7 when tumors were established, mice were given 5 x 300 μg doses of mAb (vertical dashed lines) with 4-5 mice/group. Mice treated with Ab2 had significantly reduced tumor size compared to those that received isotype control. In this model, Ab1 did not result in significantly reduced tumor size compared to isotype.

Conclusions

- We have developed two high affinity human anti-TNFR2 antibodies Ab1 and Ab2
- For translation, both antibodies have matched epitopes with our exemplary mouse surrogate antibody Y9
- Our anti-TNFR2 antibodies provide strong T cell co-stimulation *in vitro*
- Ab2 protects against xenogeneic GvHD
- Both Ab1 and Ab2 demonstrate anti-tumor efficacy in humanized mouse models

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

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