Istiratumab (MM-141), a bispecific antibody co-targeting IGF-1R and ErbB3, potentiates the activity of immune checkpoint inhibitors

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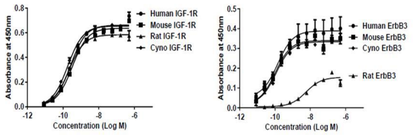
Purpose: Regulatory T cells (Tregs) help maintain immunological tolerance to cancer cells by suppressing T cell effector function. The depletion of Tregs with the CTLA-4 inhibitor antibody ipilimumab has been proven to be a productive therapeutic strategy in melanoma. Additionally, levels of insulin-like growth factor 1 (IGF-1) are often elevated following chemotherapy treatment and have been associated with poor prognosis in multiple malignances. We have demonstrated that IGF-1 can stimulate Treg proliferation *in vitro*. Istiratumab (MM-141), a tetravalent bispecific antibody that targets both the IGF-1 receptor (IGF-1R) and ErbB3, inhibits IGF-1 signaling by blocking ligand binding and inducing rapid receptor internalization. Based on its mechanism of action, we evaluated the activity of MM-141 on Treg proliferation *in vitro* and in combination with immune checkpoint targeting antibodies *in vivo* in syngeneic murine models of cancer.

Experimental Procedures: Splenocytes were harvested from normal mice and from tumor-bearing, PBS- or MM-141-treated mice to evaluate the effect of IGF-1 or MM-141 treatment on Treg expansion and proliferation *in vitro* and *in vivo*. Flow cytometry studies assessed surface receptor expression of T cell populations isolated from murine splenocytes. Efficacy studies using immuno-competent or T cell-deficient mice bearing either IGF-1R/ErbB3-positive (MC38, B16-F10) or IGF-1R/ErbB3-negative (A20) murine tumors determined the activity of MM-141 and immune checkpoint targeting antibodies, alone and in combination, on tumor growth, alongside isotype-matched controls. Mice whose tumors were eradicated by treatment were maintained for several weeks and were subsequently used in tumor re-challenge studies to assess the development of tumor-specific, immunological, long-term memory.

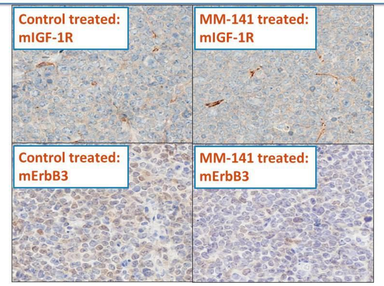
Data Summary: Our T cell analyses indicated that sub-populations of murine CD4+ CD25+ FoxP3+ Tregs express IGF-1R, and that MM-141 can reverse IGF-1-stimulated Treg proliferation *in vitro* and can decrease the % of IGF-1R+ Tregs *in vivo*. In addition, MM-141 monotherapy treatment had significant anti-tumor activity *in vivo* in both IGF-1R/ErbB3-positive and IGF-1R/ErbB3-negative immunogenic murine tumor models grown in immuno-competent and T cell-deficient mice, indicating multiple antitumor mechanisms result from MM-141 treatment. Moreover, MM-141 potentiated the activity of immune checkpoint targeting antibodies in efficacy studies, leading to curative outcomes in a subset of treated animals. These animals developed long-term immunological memory directed against the original tumor cell line.

MM-141 is fully cross-reactive with human and murine IGF-1R and ErbB3

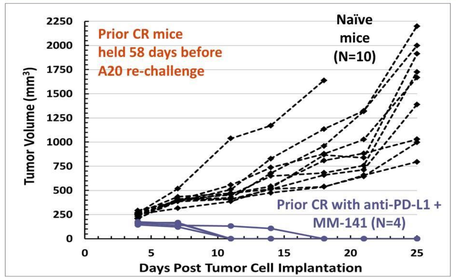


- 96-well ELISA plates coated with species specific ErbB3-His or IGF-1R-His
- MM-141 added in duplicate (500nM and subsequent 2-fold dilutions)
- Bound MM-141 detected with anti-Fc-HRP

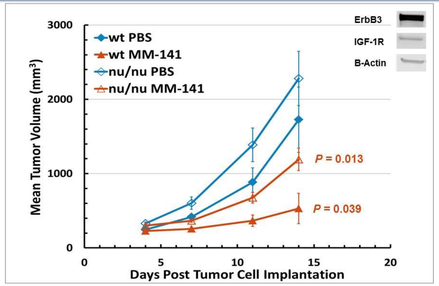
MM-141-responsive A20 model is IGF-1R/ErbB3- *in vivo*



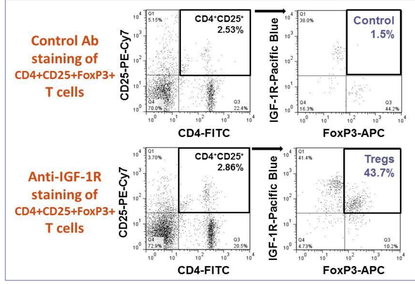
CR mice from treatment with MM-141 + anti-PD-L1 have long-term memory



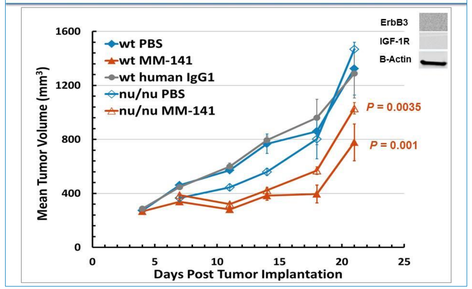
Potential for multiple MM-141 antitumor mechanisms in IGF-1R/ErbB3+ B16-F10 melanoma



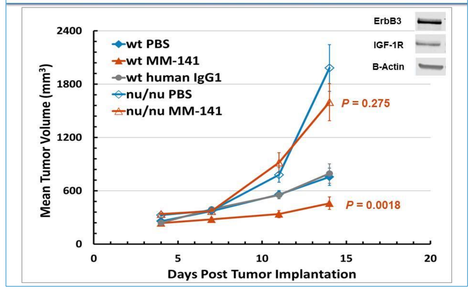
Murine Tregs express IGF-1R



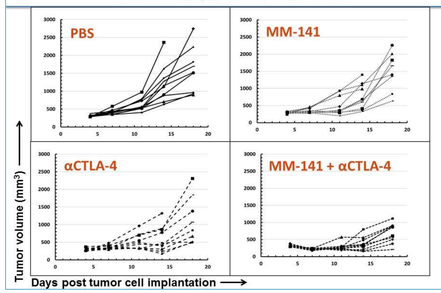
MM-141 recruits innate and adaptive immunity in IGF-1R/ErbB3- A20 B cell lymphoma



MM-141 antitumor activity is T cell-dependent in IGF-1R/ErbB3+ MC38 colon carcinoma



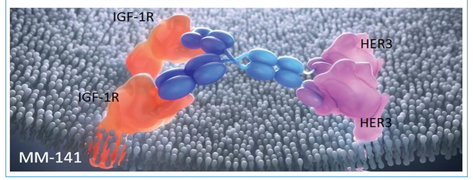
MM-141 potentiates anti-CTLA-4 in IGF-1R/ErbB3+ B16-F10



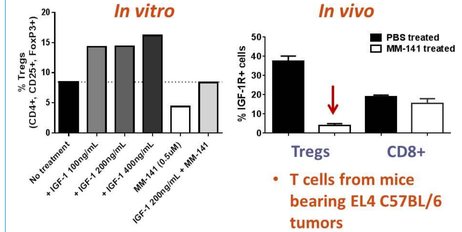
Istiratumab (MM-141) components and direct antitumor mechanisms

Bispecific MM-141 inhibits PI3K/AKT/mTOR by:

- Blocking growth factor induced signaling via IGF-1R and ErbB3
- Degrading receptor complexes of IGF-1R and ErbB3, including their heterodimers with ErbB2 and IR

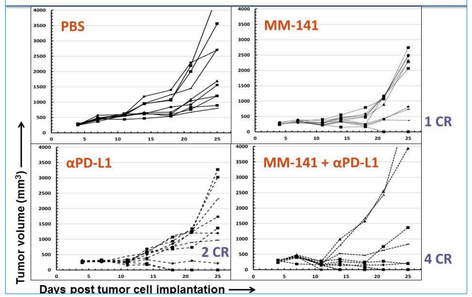


MM-141 inhibits IGF-1-stimulated Tregs expansion *in vitro* and depletes Tregs *in vivo*

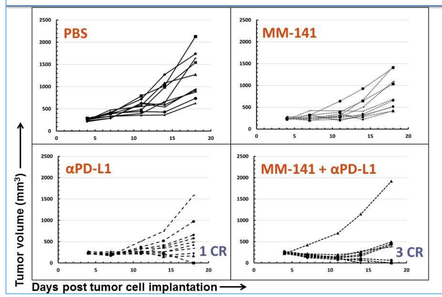


- T cells from mice bearing EL4 C57BL/6 tumors

MM-141 potentiates anti-PD-L1 in IGF-1R/ErbB3- A20



MM-141 potentiates anti-PD-L1 in IGF-1R/ErbB3+ MC38



Summary

Our *in vitro* and *in vivo* preclinical studies demonstrate that istiratumab (MM-141) treatment generates antitumor activity *in vivo* through multiple mechanisms:

- Direct inhibition of tumor cell survival signaling
- Inhibition of host Treg proliferation
- Recruitment of host innate and adaptive immunity

MM-141 potentiates the anti-tumor activity of anti-PD-L1 and anti-CTLA-4 *in vivo* in murine models

Ongoing studies continue to evaluate the potential of MM-141 in immuno-oncology settings.