**Abstracts**

**817 ENGINEERED TOXIN BODY TARGETING CTLA-4 (MT-8421) DEPLETES TREGS IN THE TUMOR MICROENVIRONMENT AND SYNERGIZES WITH αPD-1 TO ENHANCE T CELL IMMUNITY**

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**Background** Despite being approved for clinical use over a decade ago, CTLA-4 mAbs remain encumbered by a narrow therapeutic window and relatively severe adverse event (AE) profile. Increasing data support that CTLA-4 mAb efficacy is primarily driven by depletion of CTLA-4+ T regulatory cells (Tregs) in the tumor microenvironment (TME), while AEs have been linked to an overzealous peripheral T cell response mediated by prolonged CTLA-4 blockade. It has been postulated that a more selective approach directly targeting TME-associated Treg cell depletion, while reducing peripheral T cell effects, might improve the tolerability of this class of immune checkpoint inhibitors, yet no CTLA-4 targeted mAb has achieved this. MT-8421 is a potent CTLA-4-targeted engineered toxin body (ETB) that can preferentially deplete high CTLA-4 expressing Tregs in the TME while sparing low CTLA-4 expressing peripheral T cells. In addition, MT-8421 was well-tolerated in a non-GLP non-human primate (NHP) study at doses hundreds-fold higher (450ng/mL) than the IC50 on CTLA-4 expressing cells. Here we demonstrate that MT-8421 synergizes with αPD-1 mAb in a Treg/T cell primary cell co-culture to stimulate T cell proliferative responses through direct Treg cell depletion. Results from animal models including a GLP-NHP study will be described.

**Methods** T cells and Tregs were isolated from PBMCs of healthy donors and were stained to track T cell proliferation. T cells were co-cultured with autologous Tregs at various ratios. Anti-CD3/CD28 was used to drive proliferation of T cells in the presence of 4,000ng/mL of MT-8421, inactive MT-8421, or αPD-1 mAb (20μg/mL) to test for monotherapy activity. In addition, cells were treated with αPD-1 mAb followed by MT-8421 to evaluate combination effects. After 4 days, cells were stained with surface antibodies and analyzed by flow cytometry.

**Results** Co-cultures treated with MT-8421 alone demonstrated release of Treg-mediated CD8 T cell suppression compared to the untreated or inactive MT-8421. In addition, while αPD-1 treatment alone expectedly increased CD4+ and CD8+ T cell proliferation, when followed by MT-8421 treatment, the effects on T cell proliferation were greater than with either treatment alone.

**Conclusions** MT-8421 is a novel approach to CTLA-4 targeting: a potent depletion of TME CTLA-4+ Tregs without long-lasting CTLA-4 blockade in the periphery. This approach may allow for enhanced efficacy over current antibody approaches through the elimination of Tregs in the TME. The lack of prolonged peripheral CTLA-4 blockade may reduce toxicity as seen with antibody approaches to CTLA-4. Clinical studies are expected to start in 2023.

**REFERENCE**
