Preclinical identification of the pharmacologically active dose range of the tumor targeted 4-1BB agonist MP0310

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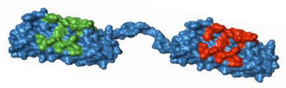
Background

In animal models, agonistic antibodies targeting the T cell costimulatory receptor 4-1BB (CD137) have shown promise as anti-tumor agents, but clinical studies have shown only limited signs of efficacy as well as dose-limiting hepatotoxicity with one of the candidates. To avoid systemic toxicities and to direct immune activation to the tumor, we have generated the tumor-targeted 4-1BB agonist MP0310. MP0310 is a multi-domain DARPin® molecule comprising domains binding to 4-1BB, fibroblast activation protein (FAP), and human serum albumin, the latter for half-life extension. FAP binding targets MP0310 activity to tumors as FAP is highly expressed in many solid tumors and crucially, activation of 4-1BB by MP0310 is dependent on FAP-mediated clustering of 4-1BB.

Schematic representation of a 4-1BB/FAP bi-specific DARPin® drug candidate:

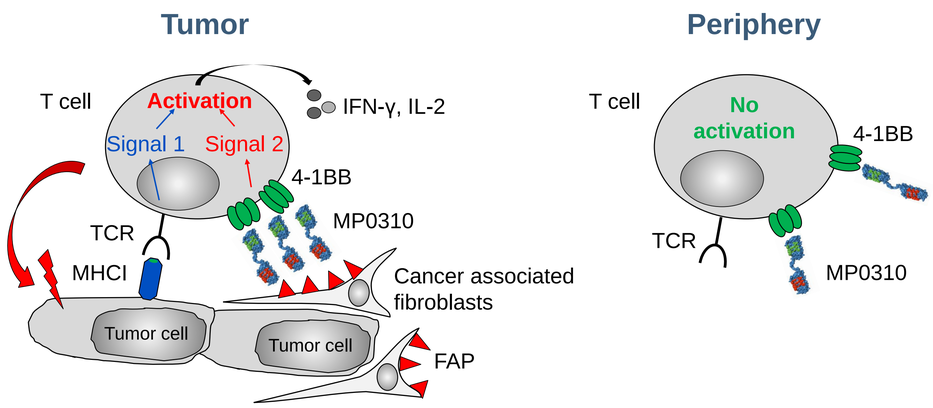
anti-4-1BB DARPin® module



anti-FAP DARPin® module

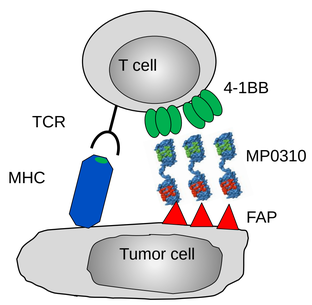
Previously we have shown, *in vitro* and *in vivo*, that MP0310 is at least as potent as the agonistic 4-1BB antibodies but does not induce hepatotoxicity or exacerbate graft versus host disease in humanized mouse models. Also, no systemic T cell activation has been observed in cynomolgus monkeys. The present study, in support of the clinical development of MP0310, is to establish the pharmacologically active dose range of MP0310 in a mouse model based on parameters such as receptor occupancy, CD8+ T cell expansion and anti-tumor activity.

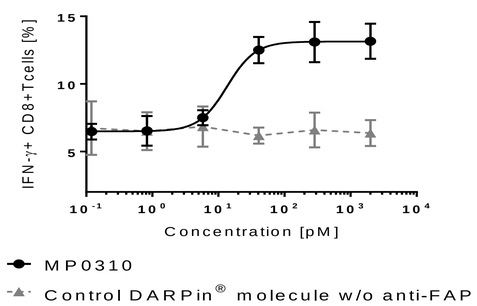
Concept: Tumor-localized activation of 4-1BB (CD137)



- Tumor-localized co-stimulation of T cells by hyper-clustering of the immune receptor 4-1BB by means of binding to FAP.
- FAP is expressed at high levels by stroma of many solid tumors in contrast to healthy tissue.
- High activity of MP0310 in the tumor but reduced systemic activity and toxicity risk due to large differences in FAP expression in tumor versus healthy tissues.

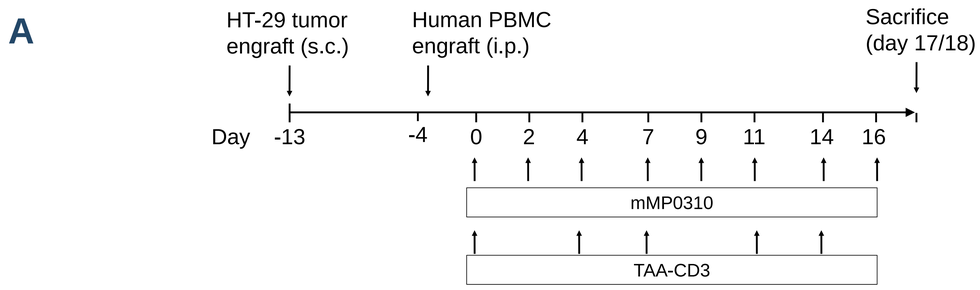
MP0310 is a potent T cell co-stimulator with FAP-dependent activity

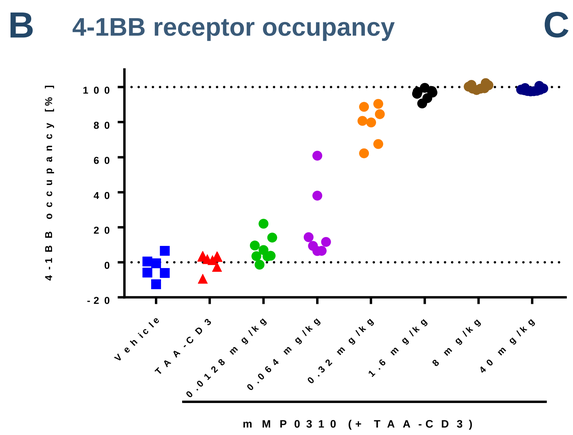


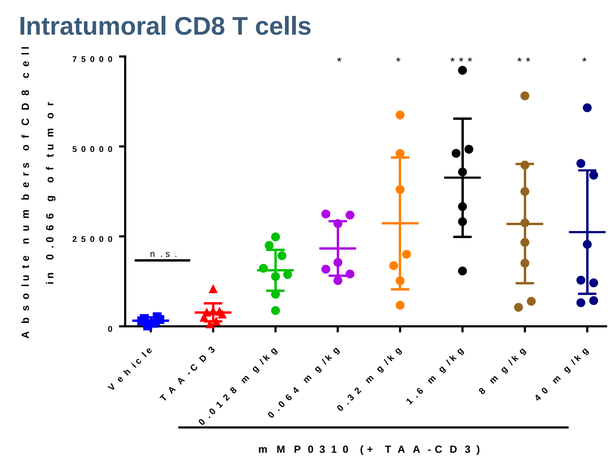


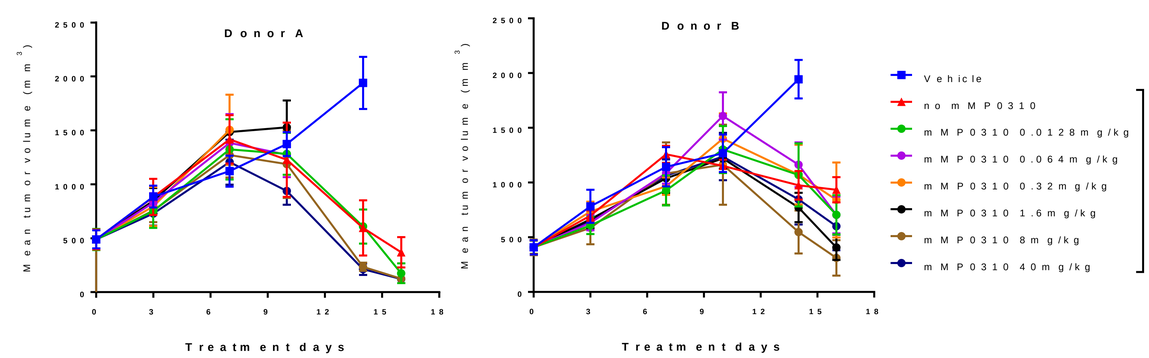
***In vitro* human CD8 T cell activation assay.** Human NLV-peptide specific CD8 T cells were activated by NLV-pulsed HLA-A2- and FAP-positive human WM-266-4 tumor cells in the presence of 4-1BB/FAP or control DARPin® molecules added in increasing concentrations. Intracellular IFN-γ in T cells was measured by flow cytometry.

mMP0310 enhances tumor T cell infiltration and anti-tumor efficacy in human PBMC engrafted xenograft model over a broad dose range









Immunodeficient (NSG) mice implanted with HT-29 xenograft tumors and human PBMCs treated with several doses of mMP0310 molecule in combination with a TAA-CD3 bispecific antibody. Combination of mMP0310 (human FAP binding domain was exchanged with mouse cross-reactive binder) with a bispecific T cell engager against a tumor-associated antigen (TAA) to induce T cell receptor triggering in tumor resident T cells leads to expansion of intra-tumoral CD8 T cells and enhanced tumor regression. (**A**) Study design, (**B**) Flow cytometric analysis of receptor occupancy of 4-1BB on blood CD8 T cells, (**C**) Tumor infiltrating human CD8 T cells were analyzed by flow cytometry. Individual values with mean and 95% CI. Kruskal-Wallis test and Dunn's multiple comparisons test was used for statistical analysis (* $p<0.05$, ** $p<0.01$, *** $p<0.001$). (D) Mean tumor volumes from mice engrafted with two PBMC donors (±SEM, n=4-5 per donor).

Conclusions

- MP0310 is a potent second generation 4-1BB agonist with tumor-targeted activity produced by binding to the tumor-associated protein FAP.
- Activation of 4-1BB by MP0310 results in strong CD8 T cell activation and expansion *in vitro* and *in vivo,* and enhances the anti-tumor efficacy of bispecific T cell engagers *in vivo.*
- MP0310 demonstrates a broad window of pharmacological activity with the maximal effect on intra-tumoral CD8 cells correlating with peripheral 4-1BB RO close to 100%.
- Tumor-targeted activation of 4-1BB has the potential to be a safe and effective way to enhance tumor immunotherapy. MP0310 is in preparation to enter clinical development.