Poster 3752

Preclinical pharmacology of MP0310: a 4-1BB/FAP bispecific DARPin® drug candidate promoting tumor-restricted T cell co-stimulation

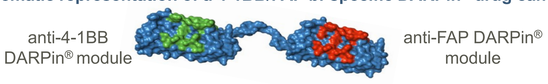
Alexander Link, Julia Hepp, Christian Reichen, Patricia Schildknecht, Ivana Tosevski, Joanna Taylor, Laurent Juglair, Alexander Titz, Mirela Matzner, Ralph Bessey, Christof Zitt, Guy Lemaillet, Jörg Herbst, Keith Dawson, Hong Ji, Victor Levitsky, Dan Snell, Michael T. Stumpp, Andreas Harstrick, Elmar vom Baur

Molecular Partners AG, Zürich, Switzerland

Background

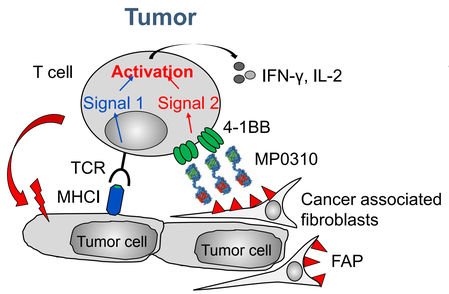
Agonistic antibodies targeting the T cell co-stimulatory receptor 4-1BB (CD137) have proved to be very efficacious anti-tumor agents in preclinical animal models. The clinical development of 4-1BB agonistic antibodies, however, has been hampered by either significant dose-limiting hepatotoxicity or limited clinical efficacy that may be attributable to relatively low potency. Here we describe the characterization of the bi-specific, tumor-targeted, and potent 4-1BB agonist MP0310. For tumor-targeting, MP0310 binds with high affinity to fibroblast activation protein (FAP) which is abundantly expressed by cancer associated fibroblasts in the majority of solid tumors in humans. Simultaneous binding of FAP and 4-1BB results in clustering and immune cell activation.

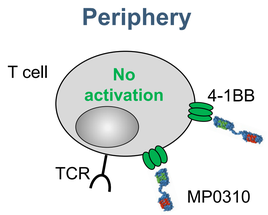
Schematic representation of a 4-1BB/FAP bi-specific DARPin® drug candidate:



In mouse tumor models, a surrogate of MP0310 strongly co-stimulates CD8 T cell activation and leads to tumor regression, while not showing any undesired systemic side effects. Absence of systemic activity was also confirmed in healthy cynomolgus monkeys. Compared to the first generation of 4-1BB targeting antibodies, MP0310 shows highly potent but tumor (FAP)-restricted T cell co-stimulation, potentially providing a new efficacious, immune enhancing treatment option for solid tumors devoid of systemic toxicity (see also poster 3029 on FAP-mediated tumor accumulation and retention).

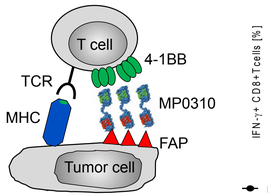
Concept: Tumor-localized activation of 4-1BB (CD137)

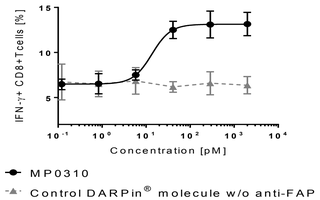




- Tumor-localized co-stimulation of T cells by hyper-clustering of the immune receptor 4-1BB by means of binding to FAP
- FAP is expressed at high levels by stroma of many solid tumors in contrast to healthy human tissue
- High activity of MP0310 in the tumor but reduced systemic activity and toxicity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

MP0310 is a potent T cell co-stimulator

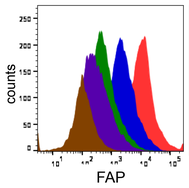




***In vitro* human CD8 T cell activation assay.** Human NLV-peptide specific CD8 T cells were activated by NLV-pulsed HLA-A2- and FAP-positive human WM-266-4 tumor cells in the presence of 4-1BB/FAP or control DARPin® molecules added in increasing concentrations. Intracellular IFN-γ in T cells was measured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

FAP expression level in human tumors are sufficient for potent 4-1BB activation

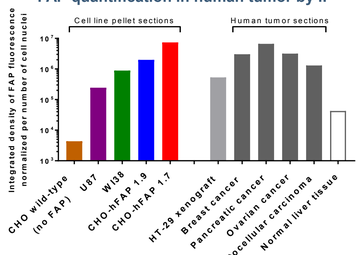
A Quantification of FAP expression by FACS



Cell Line	FAP density (SABC*)
CHO-FAP 1.7	610'000
CHO-FAP 1.9	150'000
WI38	44'000
U87	19'000
CHO wt	not detected

*specific antibody binding capacity

B FAP quantification in human tumor by IF



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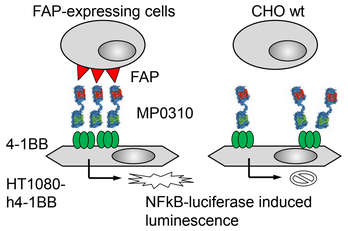

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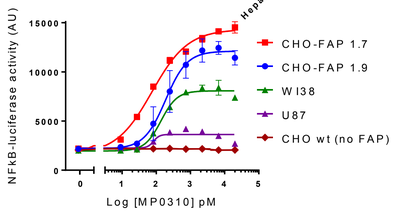
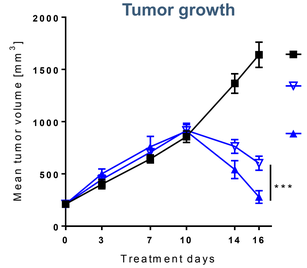
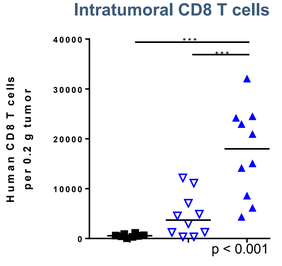


FAP expression levels in human solid tumors are sufficient for FAP-dependent 4-1BB activation by MP0310. **(A)** Quantification of FAP surface expression on various cell lines, either naturally expressing or transfected with FAP, using a flow cytometry QIFIKIT® bead assay. **(B)** Quantification of FAP expression levels in human tumors and xenografts by immunofluorescence (IF) in comparison to FAP-positive cell pellets. **(C)** NFkB-Luciferase reporter cell assay schematic to assess the effect of FAP expression levels on 4-1BB activation by MP0310. **(D)** In vitro correlation of FAP expression levels with 4-1BB activation by MP0310. Relatively low FAP density is sufficient in a mouse xenograft model for activity of the 4-1BB/FAP DARPin® molecule (see next section). The majority of tested human tumor samples express FAP at significantly higher density, suggesting adequate FAP expression in target tumors for MP0310 functional activity.

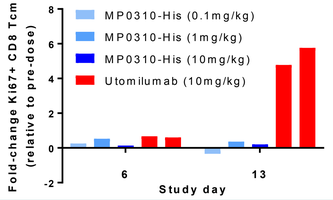
Enhanced anti-tumor efficacy and increased tumor CD8 T cell expansion in human PBMC engrafted xenograft model





Immunodeficient (NSG) mice implanted with HT-29 xenograft tumors and human PBMCs treated with 4-1BB/FAP DARPin® molecule. Combination of the 4-1BB/FAP DARPin® molecule 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 a strong expansion of intra-tumoral CD8 T cells and enhanced tumor regression. Mean tumor volumes (±SEM, n=10). Tumor infiltrating human CD8 T cells were analyzed by flow cytometry. Median + individual values, one-way ANOVA, Tukey.

Safety: No systemic activity compared to antibody in cyno



MP0310 does not induce systemic CD8 memory T cell proliferation in cynomolgus monkeys. Different doses of MP0310-(HIS) or the control clinical anti-4-1BB antibody MOR-7480 (utomilumab) were administered intravenously. Ki-67+ CD8+ central memory T cells (Tcm, CD95+/CD28+) were measured by FACS 6 and 13 days following administration. Fold changes were calculated relative to baseline pre-dose samples collected prior to the start of dosing. In contrast to utomilumab, MP0310 showed no systemic expansion of CD8 memory T cells.

Conclusion

- MP0310 is a potent second generation 4-1BB agonist with tumor-targeted activity produced by binding to the tumor-associated protein FAP with expected excellent safety profile:
  - MP0310 mediated 4-1BB activation is restricted to tissues expressing FAP at densities commonly found in human solid tumors
  - 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 avoids strong systemic activation of CD8 T cells and reduces the risk of systemic side effects and toxicities seen with anti-4-1BB antibodies
- Tumor-targeted activation of 4-1BB may be a safe and effective way to enhance tumor immunotherapy. MP0310 is in preparation to enter clinical development.

Molecular Partners AG, Wagistrasse 14, 8952 Zurich-Schlieren, Switzerland – www.molecularpartners.com – info@molecularpartners.com – phone: +41 44 755 77 00