Selection of first-in-human clinical dose range for the tumor-targeted 4-1BB agonist MP0310 (AMG 506)* using a pharmacokinetic/pharmacodynamics modeling approach

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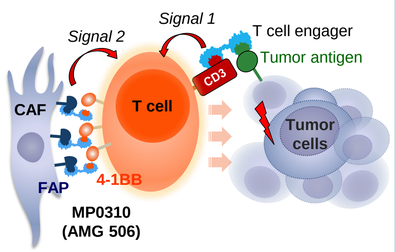
Background - tumor-targeted activation of 4-1BB

Following the clinical success of checkpoint inhibitors, cancer immunotherapy is rapidly expanding into combination treatments to enhance response rates and duration. Agonistic antibodies against the costimulatory receptor 4-1BB (CD137) have been shown to effectively enhance the anti-tumor activity of checkpoint inhibitors and other 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.

Using our modular DARPin® platform, we generated a potent, tumor-targeted 4-1BB agonist MP0310 (AMG 506), which comprises domains binding to 4-1BB and fibroblast activation protein (FAP). MP0310 (AMG 506) triggers 4-1BB activation only if clustered by FAP which is abundantly expressed by cancer associated fibroblasts (CAFs) present in many solid tumors. Costimulation via 4-1BB (signal 2) enhances T cell activation induced by the T cell Receptor (signal 1), here achieved with a bispecific T cell engager, boosting CD8 T cell expansion and anti-tumor efficacy.

Mode of Action
Tumor-localized immune cell activation



We describe here our PK/PD modeling approach to predict the minimum anticipated biological effect level (MABEL), a maximum and the anticipated optimal dose range for the first in human (FIH) clinical study.

PK/PD modeling approach for FIH dose justification

- We established a translational pharmacokinetic-pharmacodynamic (PK/PD) model integrating *in vitro* and *in vivo* data to make dose predictions for the FIH study.
- In addition to a minimal physiologically based pharmacokinetic (mPBPK) model describing MP0310 concentrations over time in mouse and monkey, direct and indirect response models were used to describe pharmacological effects determined in mouse tumor studies:

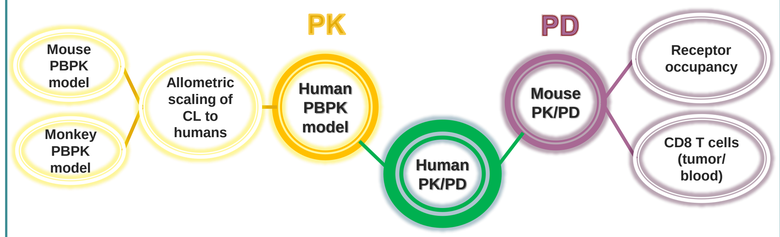
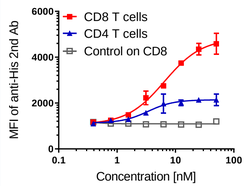


Figure 1. Schematic of translational modeling to support FIH starting dose in two parts: PK and PD.

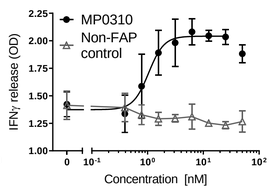
MP0310 stimulates T cell activation dependent on FAP *in vitro*

A MP0310 binding to activated T cells



MP0310	EC_{50} [nM]
CD4 T cells	3.4
CD8 T cells	6.5

B *In vitro* costimulation of human CD8 T cells



	EC_{50} [nM]
MP0310	1.1
Non-FAP control	-

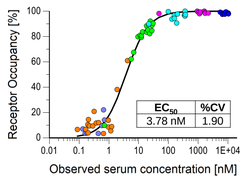
Figure 2. Primary human CD8 T cell binding and activation *in vitro*

A) Binding of MP0310 (his-tagged) to activated primary human CD4 and CD8 T cells assessed by flow cytometry; non-4-1BB binding variant of MP0310 as control.

B) Primary human CD8 T cells were activated by plate-bound CD3 antibody (OKT3) in the presence of increasing concentrations of MP0310 (AMG 506) cross-linked on plate-bound FAP or a non-FAP targeted control. Intracellular IFN-γ in CD8 T cells was measured by flow cytometry.

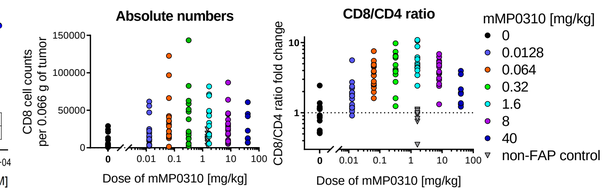
MP0310 enhances the activity of a bispecific T cell engager in a humanized mouse tumor model

A 4-1BB receptor occupancy on blood CD8 T cells



EC_{50}	%CV
3.78 nM	1.90

B Intratumoral CD8 T cells



C Tumor growth

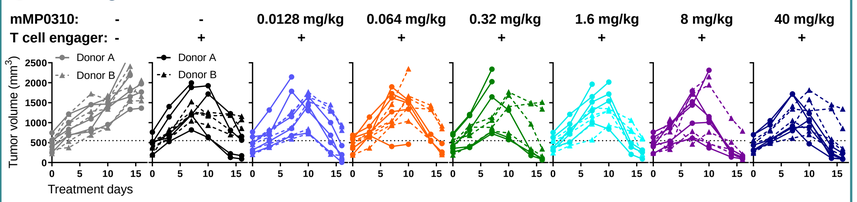


Figure 3. Combination treatment of a MP0310 surrogate with a bispecific T cell engager. Immunodeficient (NSG) mice, inoculated with HT-29 colorectal adenocarcinoma cells and human PBMCs, were treated either with a mFAP x h4-1BB surrogate of MP0310 (mMP0310) over a broad dose range or with a non-FAP binding control in combination with a fixed dose of a bispecific T cell engager against a tumor-associated antigen. **A)** 4-1BB receptor occupancy on blood CD8 T cells was assessed by flow cytometry using two different 4-1BB detection reagents, one competing and one non-competing with mMP0310. **B)** Tumor infiltrating human CD8 T cells were analyzed by flow cytometry and are shown as total number per tumor mass and as CD8/CD4 ratio. **C)** Individual tumor growth curves shown (n=5) for 2 out of 4 PBMC donors. Combination treatment with mMP0310 showed a trend towards increased tumor regression; however, due to the potent anti-tumor activity of the T cell engager alone and the high variability among individual animals and PBMC donors, the changes of the CD8/CD4 ratio were used as a pharmacodynamic marker for the PK/PD modeling.

Pharmacokinetics

- FAP-dependent tumor accumulation was assessed by biodistribution studies in mice
- Slight non-dose proportional systemic PK behavior observed in mouse and monkey
- A minimal PBPK model with linear systemic and nonlinear tissue clearance was applied to describe such nonlinearity
- Predicted human half-life is 5.9 to 14 days across a broad dose range

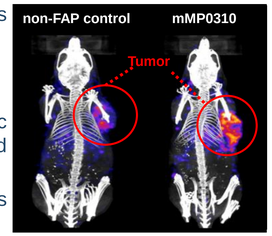


Figure 4. FAP-dependent tumor localization. Whole-body SPECT/CT imaging using ^{111}In-labeled DARPin® molecules in the HT-29 model. *Cancer Res. 2018 78(13 Suppl.):3029.*

PK/PD model extrapolation for human dose selection

Predictions from the combined PK/PD model provided

i. a FIH starting dose with minimal expected systemic PD effects (based on 20% receptor occupancy at 0.015 mg/kg),
ii. the anticipated therapeutic optimal dose range (0.5 to 5 mg/kg), and
iii. a dose level at which the max. therapeutic effect may be exceeded (12 mg/kg) for optimal dose range confirmation

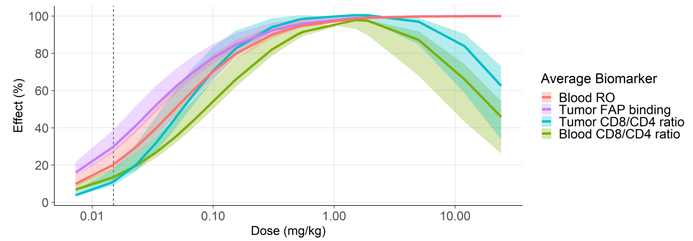


Figure 5. Prediction of various PD markers versus dose in human. Exposure values (C_{av}) derived from the established minimal PBPK models (based on *Zhao et al., 2015*) were used to translate PD effects from the mouse tumor studies (all as % of max. effect) and predict the dose-effect relationships in humans. Prediction intervals (shaded areas) are based on lower and upper bounds set during scaling of clearance to humans. *Note*: Predicted systemic CD8 T cell activation and expansion is based on the humanized PBMC mouse model. No systemic T cell activation was observed in healthy NHP.

Conclusions

- MP0310 (AMG 506) is a potent, tumor-targeted 4-1BB agonist promoting T cell stimulation *in vitro* and *in vivo* while minimizing the risk of undesired systemic immune activation.
- A PK/PD-modeling approach was used to select FIH starting dose and optimal dose range.
- Based on the predictions of the therapeutic dose range, a FIH dose-escalation trial was initiated in H2/2019 in cancer patients (ClinicalTrials.gov Identifier: NCT04049903).
- In the ongoing FIH dose escalation study, all model-relevant readouts and other exploratory pharmacodynamic markers are being assessed and used to update the PK/PD model in support of dose decisions for further development.

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