Poster 3029

FAP-mediated tumor accumulation of a T cell agonistic 4-1BB/FAP DARPin® drug candidate analyzed by SPECT/CT and quantitative biodistribution

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Background

Immunomodulating agents have revolutionized anti-cancer therapy. However, monotherapy is often not sufficient, and development of combination treatments is hampered by cumulative toxicity. In an attempt to overcome this challenge, a tumor-restricted agonistic 4-1BB/FAP DARPin® drug candidate, MP0310, which induces T cell co-stimulation only when clustered via binding to fibroblast activation protein (FAP) expressing cells, has been developed (see also Poster 3752). FAP is a type II membrane-bound glycoprotein abundantly expressed in the stroma of many solid tumors by cancer-associated fibroblasts. As shown previously using *in vitro* and *in vivo* models (HT-29), co-stimulation induced by a FAP-targeted 4-1BB agonistic DARPin® molecule leads to enhanced activation and expansion of CD8⁺ T cells. To support clinical development of the drug candidate, tumor localization and accumulation were studied by whole-body SPECT/CT imaging and quantitative biodistribution using Indium-111 labeled DARPin® molecules in a human colorectal adenocarcinoma (HT-29) xenograft model in CD1 nude mice. For this purpose, a derivative of the therapeutic candidate, MP0310d, cross-reactive with mouse FAP and suitable for radioactive labeling was used.

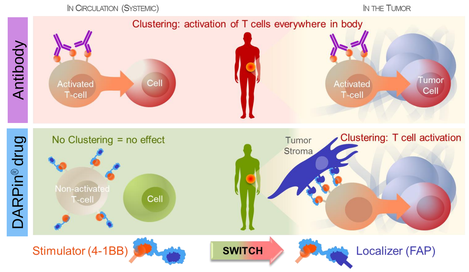

Figure 1. Cartoon illustrating localized immune stimulation concept

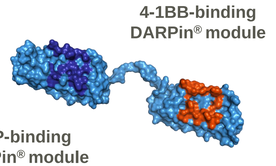

Figure 2: Schematic representation of the immune stimulation-relevant MP0310 DARPin® modules: a conditional 4-1BB agonist, dependent on clustering via the tumor-targeting FAP-binding component. Only simultaneous engagement of both targets, FAP and 4-1BB, results in 4-1BB activation.

Pharmacokinetics of MP0310d in mice

- MP0310d and a non-FAP-binding control DARPin® molecule were analyzed for their PK characteristics in BALB/c mice following single intravenous injection at a dose level of 1 mg/kg.
- Serum concentrations declined in a roughly mono-exponential manner.
- Terminal half-life values were calculated to be approximately 30 h and 40 h for MP0310d and the non-FAP-binding control DARPin® molecule, respectively.

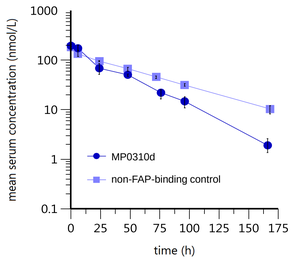

Figure 3: Concentration-time traces of MP0310d and non-FAP-binding control in female BALB/c mice following single 1 mg/kg *i.v.* bolus injection. Concentrations were measured by sandwich ELISA.

FAP density in HT-29 model & human tumors tissues

- In the HT-29 xenograft model, FAP expression is most pronounced in the peripheral areas of the tumor, independent of tumor size
- FAP density in the HT-29 model is sufficient for strong induction of CD8⁺ T cell expansion and anti-tumor activity by MP0310d (see also Poster 3752)
- FAP expression density in most FAP-positive patient samples is higher than in the HT-29 xenograft model

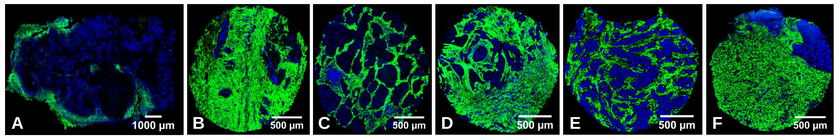

Figure 4: Immunofluorescence staining of FAP in xenograft tumor and human tumor tissue array. In green, FAP staining with polyclonal anti FAP AF3715 (RnD systems); in blue, nuclei are stained with DAPI. **(A)** Whole HT-29 xenograft tumor (~1000 mm³) with medium-high density FAP expression, albeit mostly in the peripheral areas of the tumor. **(B-F)** High density FAP expression detected in human tumor tissue array: **(B)** pancreas adenocarcinoma, **(C)** lung adenocarcinoma, **(D)** breast ductal carcinoma, **(E)** skin carcinoma, and **(F)** ovary cystadenocarcinoma. All pictures were taken with the same microscope and exposures and analyzed with ImageJ software with the same parameters.

MP0310d accumulation in FAP-expressing tumor tissue

- MP0310d shows fast accumulation and prolonged retention by FAP-expressing tumor stroma in the HT-29 xenograft tumor tissue
- Increased tumor accumulation detectable within 24 h
- Significantly increased tumor accumulation of MP0310d over non-FAP-binding control over time (P < 0.0001)

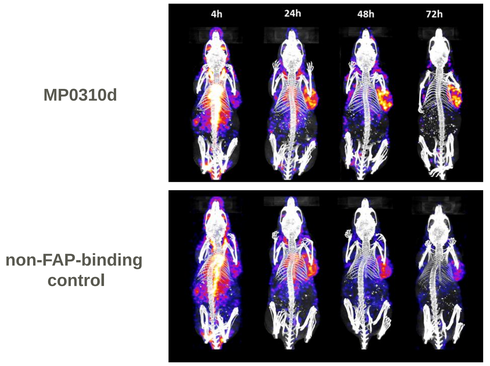

Figure 5: Whole-body SPECT/CT imaging using ^{111}In-labelled DARPin® molecules in a human colorectal adenocarcinoma (HT-29) xenograft model.

Tumor localization method: DARPin® molecules were labeled with ^{111}In (Indium-111) via a bifunctional NODAGA chelator conjugated via NHS-ester to the molecules with an average chelator-protein ratio of 1.3-1.6. Conjugation was confirmed not to affect the molecular properties of the labeled molecules. Female CD1 (nu/nu) mice were inoculated subcutaneously in the right shoulder with 6 x 10^6 HT-29 cells. Tumors were allowed to grow to a diameter of ~500 mm³ and mice were randomized. 1.6 mg/kg ^{111}In-labelled MP0310d-NODAGA (activity: 150 KBq) was intravenously injected in each mouse. Whole body distribution was monitored using a NanoSPECT/CT^{Plus} camera. Maximum intensity projections were generated using a normalized intensity setting (Figure 5, representative picture shown (n=4)). For quantitative biodistribution, organs of interest (tumor and muscle) were extracted, weighed and radioactivity was determined with a γ-counter (Packard Cobra II Gamma D5010, GMI, USA) at 4 h, 24 h, 48 h, 72 h, 96 h and 168 h post-injection. Measured activities are shown as %ID/g (% of the injected dose per gram of organ); mean ± SD).

MP0310d quantitative tissue distribution & kinetics

- MP0310d shows a signal maximum of 15% ID/g (% of the injected dose per gram of tissue) in the tumor 72 h post injection.
- MP0310d shows a two-fold higher accumulation in the tumor than the non-FAP-binding control (t = 72 h)
- Elimination from the blood is in agreement with determined serum half-lives in healthy BALB/c mice (Figure 2)
- The tumor/blood ratio for MP0310d in contrast to control steadily increases over time
- Uptake in liver, spleen and kidney shows no significant difference between FAP-targeted and non-targeted molecules (data not shown)
- No accumulation was observed in the thigh muscle (control tissue)

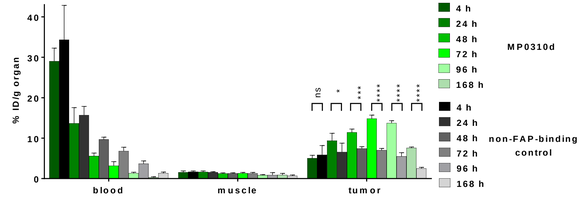

Figure 6: Time course of tissue distribution of ^{111}In-labeled DARPin® molecules in HT-29 tumor-bearing female CD1 (nu/nu) mice. Blood and organs from four mice were analyzed at time points 4 h, 24 h, 48 h, 72 h, 96 h and 168 h post injection. FAP-localized MP0310d is shown in green, non-FAP binding control is shown in grey. Data is shown as mean ± SD from four animals; ns=not significant, * p<0.05, *** p<0.001, **** p<0.0001, compared with control.

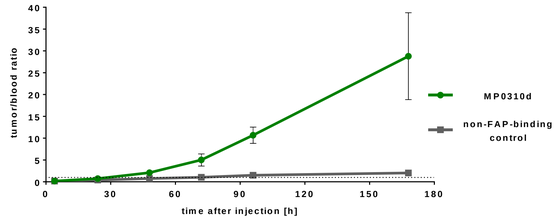

Figure 7: Tumor-to-blood ratios of ^{111}In-labeled DARPin® molecules in HT-29 tumor-bearing female CD1 (nu/nu) mice. Dotted line represents ratio of 1. Data is shown as mean ± SD from four animals.

Conclusion

- FAP-targeting of a 4-1BB agonist DARPin® molecule (MP0310d) resulted in tumor accumulation and retention in the HT-29 xenograft model in whole-body SPECT/CT imaging and biodistribution studies.
- Significantly increased tumor accumulation and tumor-to-blood ratios were observed for MP0310d compared to non-FAP-binding control.
- Tumor-targeting via FAP has the potential to induce T cell activation restricted to the tumor, thereby reducing the risk of toxicities caused by systemic 4-1BB activation.

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