Poster 1733

MP0317, a CD40xFAP targeting multi-specific DARPin® therapeutic, drives immune activation and reverts myeloid-mediated T-cell suppression in vitro and ex vivo

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Tumor-localized activity for improved therapeutic window

DIRECT

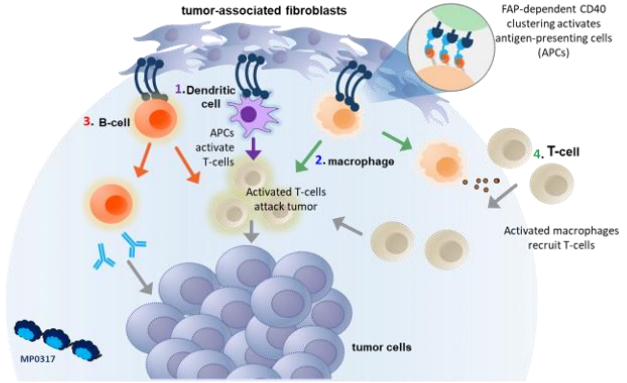
1. Dendritic cells:
Enhanced antigen processing and presentation

2. Macrophages:
Repolarization of immune-suppressive M2 environment into pro-inflammatory M1 environment

3. B-cells:
Activation of B-cells

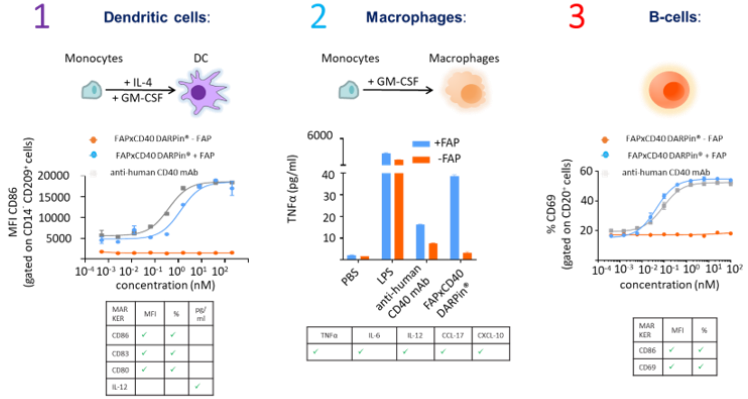
INDIRECT

4. T-cells
Activation of T-cells to attack tumor



Validated mode of action

MP0317 activates APCs in a FAP-dependent manner *in vitro*



MP0317 repolarizes M2 macrophages and reverts T-cell suppression

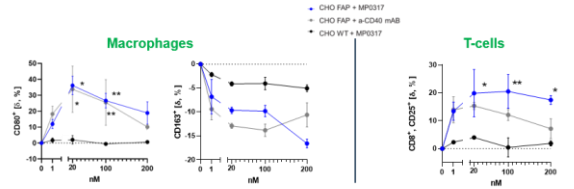


Figure 1. MP0317 leads to downregulation of CD163 and upregulation of CD80 on human M2-like macrophages that consequently results in CD8 T-cell activation. Monocyte-derived macrophages were generated *in vitro* and polarised towards a M2 phenotype. They were then co-cultured with cell lines expressing physiological FAP levels or no FAP in the presence of either MP0317 or an anti-human CD40 monoclonal antibody for 48 h, with a portion stained for CD163 and CD80. The remaining macrophages were co-cultured with autologous T-cells and dynabeads for 5 days and CD25 expression was assessed on T-cells. Results are plotted as Δ% of positive cells for each marker after subtracting the average % of positive cells of the untreated control from the experimental values.

Patient-derived Cancer Associated Fibroblasts Drive MP0317-mediated B-cell Activation

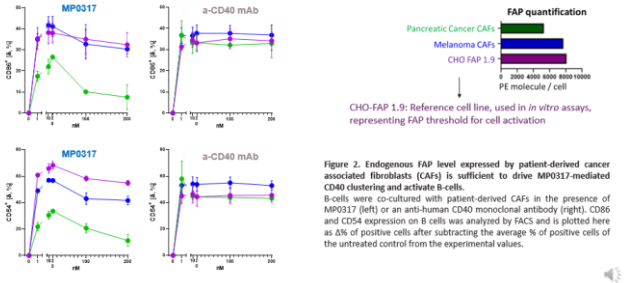


Figure 2. Endogenous FAP level expressed by patient-derived cancer associated fibroblasts (CAFs) is sufficient to drive MP0317-mediated CD40 clustering and activate B-cells. B-cells were co-cultured with patient-derived CAFs in the presence of MP0317 (left) or an anti-human CD40 monoclonal antibody (right). CD86 and CD54 expression on B cells was analyzed by FACS and is plotted here as Δ% of positive cells after subtracting the average % of positive cells of the untreated control from the experimental values.

MP0317 Induces CD40 Clustering & Immune Cell Activation in Human *ex vivo* Ovarian Cancer Tissue

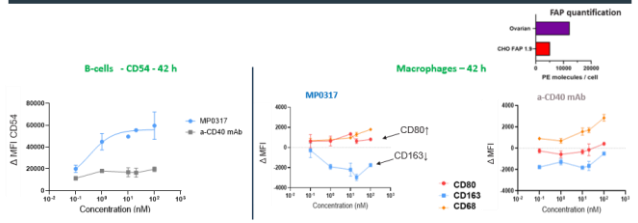
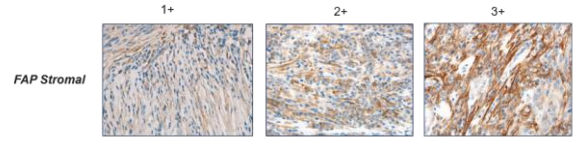


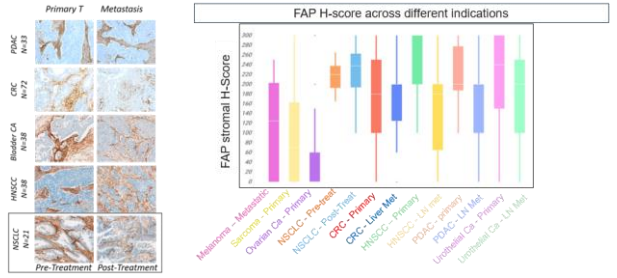
Figure 3. MP0317 activates B-cells and repolarises macrophages in a high FAP ex vivo tissue sample of ovarian cancer. A fresh ovarian tumour tissue sample was dissociated and single cells were then cultured in the presence of MP0317 or an anti-human CD40 mAb at indicated concentrations for 42 h. CD54 expression was evaluated on B cells and CD80, CD163, CD68 expression on macrophages and plotted here as ΔMFI, after subtracting the average MFI of the untreated control from the experimental MFI values.

Immunohistochemistry (IHC) Analysis – FAP Scoring



H score = [1 × (% cells 1+) + 2 × (% cells 2+) + 3 × (% cells 3+)]

IHC analysis reveals high FAP levels across different tumor indications



Summary and Conclusion:

- We provide novel *in vitro* data showing that MP0317 repolarizes M2 human macrophages towards an M1 phenotype, which consequently reverses T-cell suppression and enables T-cell activation
- Endogenous FAP level expressed by patient-derived CAFs led to MP0317-mediated clustering of CD40 and B-cell activation
- *In vitro* findings were validated *ex vivo* in a human ovarian tumor sample, where MP0317 led to B-cell activation and macrophage repolarization
- IHC analysis of FAP expression in human tissue samples from several indications revealed high FAP expression, which would enable MP0317-mediated tumor-localized CD40 activation leading to enhanced therapeutic window

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