C056

MIV-818 stimulates an anti-tumor immune response in vitro and enhances the effects of pembrolizumab

Fredrik Öberg, Karin Tunblad, Ana Slipicevic[1], Richard Bethell and Mark Albertella[2]
Medivir AB, Huddinge, Sweden, [1]current address Oncopeptides AB, Stockholm, Sweden, [2]current address Storm Therapeutics LTD, Cambridge, UK
Contact person: Fredrik.Oberg@medivir.com

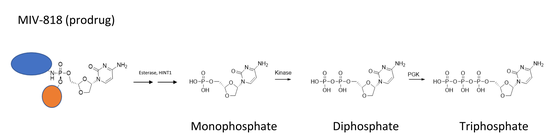
Background

- **MIV-818 is a novel nucleotide prodrug of troxacitabine-monophosphate (TRX-MP), designed as a novel approach to deliver high levels of the chain-terminating nucleotide troxacitabine-triphosphate (TRX-TP) to the liver after oral dosing while minimizing systemic exposure. MIV-818 is currently being evaluated in a phase 1/2 study of cancer in the liver.**
- **Preclinical studies have demonstrated that some DNA damaging agents also can enhance antitumor immune responses in addition to their direct cytotoxic effects.**
- **We therefore investigated whether MIV-818 induces similar immunomodulating effects in complex *in vitro* tumour microenvironment systems.**

Methods

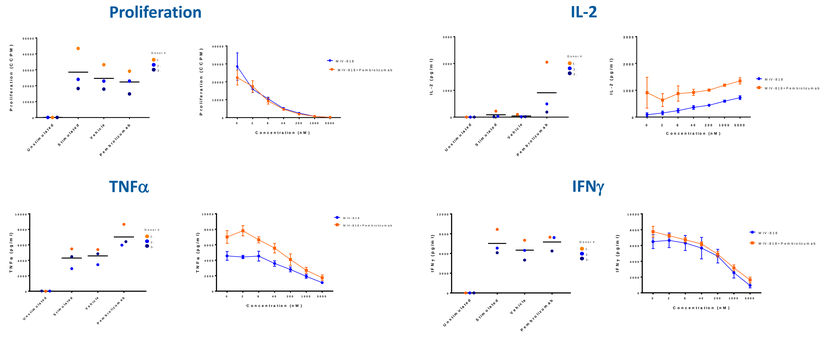
- Human PBMC were isolated from three healthy donors, incubated with the MIV-818, alone or in combination with Pembrolizumab for 1 hour prior to stimulation. Cells were then incubated for five days with Staphylococcal Enterotoxin B (SEB), proliferation was quantified by ^{3}H-thymidine incorporation, and culture supernatants were analyzed for IL-2, TNFα and IFNγ by multiplex assays (Luminex®).
- Immune-mediated tumor cell killing was determined by co-culturing labelled ovarian carcinoma SK-OV-3 cancer cells and PBMC, and quantifying cell numbers over 68 hours using the IncuCyte ZOOM system. Caspase 3/7 dye was used to identify apoptotic tumour cells.
- MIV-818 effects on immune tumor microenvironment (TME) were investigated by the BioMAP CRC oncology panel (DiscoverX) in two complex TME systems. Co-cultures of PBMCs from healthy donors activated by TCR stimulation and HT29 colon adenocarcinoma cells with either primary fibroblasts (**StroHT29**) or endothelial cells (**VascHT29**). Immune, inflammatory, matrix remodelling and angiogenesis biomarkers were profiled in co-cultures treated with MIV-818 (8 to 1000 nM) alone or in combination with pembrolizumab (50µg/mL) for 48 hours.

MIV-818



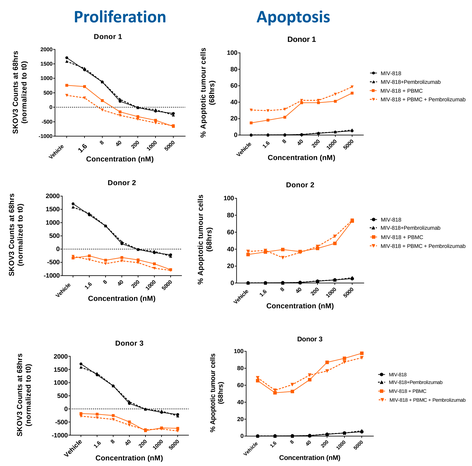
- The MIV-818 prodrug is intracellularly metabolized to troxacitabine (TRX)-monophosphate, which is further sequentially phosphorylated to the active metabolite TRX-TP
- Oral dosing and first-pass uptake, and rapid intracellular conversion to non-permeable charged metabolites, increases effective liver concentration of TRX-TP and reduces systemic exposure
- When incorporated into DNA, TRX-TP causes double strand DNA breaks and cell death
- MIV-818 has demonstrated good efficacy in preclinical HCC models

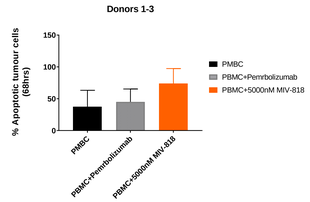
MIV-818 stimulates production of IL-2, which is further enhanced in combination with pembrolizumab



- MIV-818 demonstrated dose-dependent inhibitory effect on proliferation of SEB-stimulated PBMCs.
- Despite this growth inhibitory effect, MIV-818 led to a strong, dose-dependent, increase in IL-2 levels as a single agent which was further enhanced in combination with pembrolizumab
- In contrast, a dose-dependent reduction TNFα and IFNγ levels by MIV-818 was observed
- These data suggest an immunomodulatory role of MIV-818, in the absence of tumour cell killing

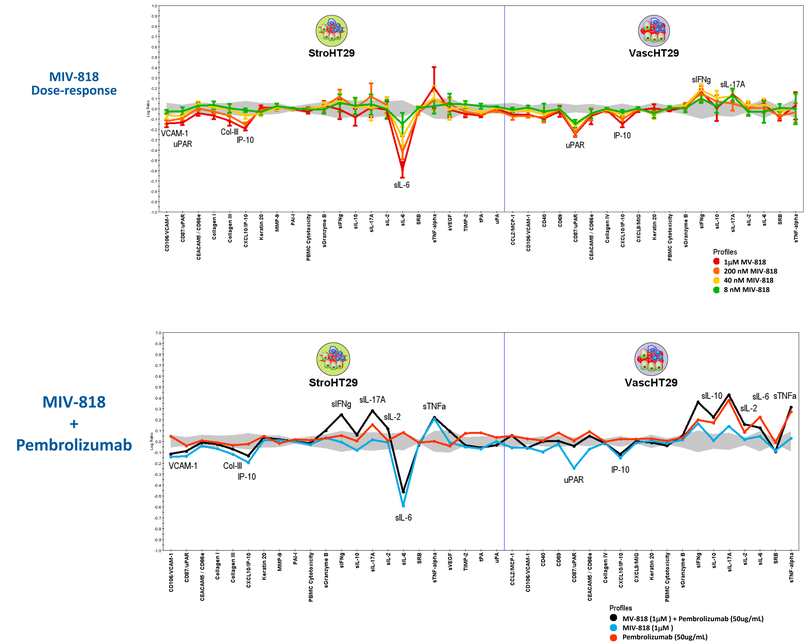
MIV-818 enhances PBMC-mediated cell killing of SK-OV-3 tumour cells





- Co-incubation of SK-OV-3 cells and PBMCs with MIV-818 induced a substantially higher level of apoptosis in the cancer cells (74±25%) compared to co-incubation in the absence of MIV-818 (37±23%).
- MIV-818 inhibited the proliferation of SK-OV-3 cells in the absence PBMCs, but with no significant induction of apoptosis (IC_{50} 7.5nM; ≤5% apoptosis).

MIV-818 modulates immune-related cytokines in the tumour microenvironment



- **MIV-818 treatment impacted immune, inflammatory and angiogenesis-related responses in both the stromal (StroHT29) and vascular (VascHT29) co-culture systems**
- **Key activities modulated by MIV-818 in the stromal cell+PBMC+HT29 co-culture was increased sIL-17A, sIFNγ, TNFα, and decreased sIL-6, IP-10, collagen type III, and VCAM-1**
- **Key activities modulated by MIV-818 in the vascular cell+PBMC+HT29 co-culture was increased IL-17A and IFNγ and decreased uPAR and IP-10**
- **The MIV-818 plus pembrolizumab combination further enhanced an immune-stimulatory effect by increasing IL-2, IL-10, IL-17A and IFNγ, while also modulating immune and inflammation-related cytokines (VCAM-1, IP-10, IL-6, TNFα, Granzyme B).**

Conclusions

- **MIV-818 exerts favorable immunomodulating effects in vitro by increasing immune cytokine levels and PBMC-mediated killing of cancer cells**
- **These immunomodulatory and tumour cell-killing effects are further enhanced in combination with pembrolizumab and support future combination of MIV-818 with PD-(L)1 inhibitors in patients with HCC and other cancers in the liver**
- **The effects of MIV-818 on tumor immune activation markers are being assessed the ongoing phase 1/2 clinical trial**