5101: Defining exposure-PD and efficacy relationships with the novel liver-targeting nucleotide prodrug MIV-818 for the treatment of liver cancers

Mark Albertella1, Biljana Rizoska2, Alastair Kyle3, Andrew Minchinton2, Annelie Linqvist2, Sanja Juric1, Susanne Sedig1, Karin Tunblad1, Fredrik Öberg1, Björn Classon1, Anders Eneroth1, John Öhö1 and Richard Bethell1
1Medivir AB, Huddinge, Sweden; 2Capenda, Vancouver, BC, Canada

Background
- Many systemic chemotherapeutics have failed due to lack of cell-specific toxicity.
- Technologies for determining therapeutic 5T-KP levels are being developed for use in clinical studies.
- We investigated MIV-818 and troxacitabine using several in vitro models to identify therapeutic levels of TRX.

Methods
- In vitro studies: Human hepatocytes (HepG2) and Hep3B cells were treated with 2.5, 10 and 25 mg/kg troxacitabine i.p. BID x 5 d. For MIV-818, a dose of 5 mg/kg on days 1, 2 and 5 was administered. Toxicity was assessed up to 168 hrs after last dose of troxacitabine.
- In vivo studies: Hep3B and HepG2 tumour xenograft models were used to determine antitumour activity of MIV-818 and troxacitabine. Tumour growth delay, efficacy relationships, and PK were studied. A high dose of MIV-818 (5 mg/kg) or troxacitabine (25 mg/kg) was administered i.p. BID x 5 d to mice.
- PK studies: Plasma and tissue concentrations were determined using high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS). Tissue concentrations were normalized to tissue mass.

In vitro studies
- MIV-818 has a superior in vitro profile to troxacitabine:
  - 10x increased potency of inhibition of HCC cell line growth
  - 4x increased conversion to its active metabolite TRX
  - Optimized for oral bioavailability and liver targeting, including permeability and intracellular stability
  - Stable in human, dog and cynomolgus whole blood, even at up to 200 times human plasma concentration

Table 1: Effect of MIV-818 and Troxacitabine on HepG2 cells (EC50 in µM).

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In vivo studies
- Dose-response effects of troxacitabine on PD end-points were evaluated in the Hep3B, Huh-7 and HepG2 models.
- Troxacitabine was administered at doses 2.5, 10 and 25 mg/kg i.p. BID for 5 days. Tumours were dissected after the last dose and processed for historical analyses of markers of proliferation (Ki67 and DNA damage; pH2AX, Fig. 3E-O).

Table 2: TGI in HepG2, Hep3B and Huh-7 cells after 5 days of treatment with troxacitabine (25 mg/kg).

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<td>25</td>
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- Tumour growth inhibition
  - Efficacy studies to determine therapeutic TRX-TP levels were performed using troxacitabine due to the instability of MIV-818 in mouse blood.
  - Dose- and TRX-TP exposure-dependent tumour growth inhibition (TGI) was demonstrated in the Hep3B, Huh-7 and HepG2 xenograft models.

- TGI = 100% - (T/G) x 100

- TGI was assessed at start of treatment; time to tumour regression was complete expressed as mean (days).

Exposure-PD/Efficacy Relationships
- TRX-TP exposures required for pronounced anti-tumour effects are informing a comprehensive understanding of PD-PD efficacy relationships for the active metabolite of MIV-818 and are expected to guide dosing and tissue selection in preclinical studies.

- MIV-818 is currently in nonclinical development for the identification of clinical trials in patients with advanced HCC and other liver cancers.

Conclusions
- Toxicology in HCC cells vs. hepatocytes
  - MIV-818 shows high selectivity for HCC cell lines relative to primary human hepatocytes in proliferation assays (>200x).
  - MIV-818 demonstrates a large selectivity index in terms of DNA damage response (phosphate-p53, Fig. 3; pH2AX, not shown) in HepG2 compared to human hepatocytes.
  - Low toxicity in human hepatocytes suggests potential for tumour selectivity

Effects on pharmacodynamic (PD) end-points
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Conclusions

Fig. 3: Representative photomicrographs stained for BrdU (A) and pH2AX (B-D) exposed for different times. A: Control; B: treated with troxacitabine (10 mg/kg) for 4 days; C: treated with MIV-818 (10 mg/kg) for 4 days; D: treated with MIV-818 (10 mg/kg) for 4 days. Data expressed as fold increase vs. vehicle (25 mg/kg).

Fig. 4: Effects of troxacitabine on tumour growth inhibition in the Hep3B (A), Huh-7 (B) and HepG2 (C) models. Horizontal dashed line indicates tumour volume at start of treatment; vertical dashed line indicates time at which study day 24 was used for the TGI calculations below.

Fig. 5: Time-response effects after a single dose.

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- The largest effects were observed in the HepG2 model (Fig. 4C) with a complete tumour regression for a prolonged period of time