Highly potent MCL-1 degraders for haematological malignancies

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BACKGROUND

MCL-1 is a mitochondrial membrane-bound protein well-known for its antiapoptotic activity. It exerts its canonical function by preventing mitochondrial outer membrane permeabilization (MOMP) through many different protein-protein interactions with other proteins from the Bcl-2 family. MCL-1 has been shown to be up-regulated in numerous haematological and solid tumor malignancies, which makes it an important factor in the resistance of some cancer types to conventional cancer therapies. Targeted protein degradation has become an attractive new area of drug development. It has shown great advantages over small molecule inhibitors in overcoming tumour resistance, targeting "undruggable" proteins, and affecting non-enzymatic functions of target proteins.
The results of our MCL-1 project presented here reveal the development of two MCL-1 bifunctional degraders – our lead compounds: CPT-2036 and CPT-908, that are efficacious in *in vivo* leukemia model.

MATERIALS AND METHODS

Compounds' affinity to CRBN, E3 ligase, and their membrane permeability were determined by the cellular NanoBRET Target Engagement assay. Cell viability was assessed by CellTiter-Glo Assay. Apoptosis induction was assessed by flow cytometry using Annexin/PI staining. Targeted protein degradation in cells treated with compounds in the absence or presence of apoptosis inhibitor was assessed by WB. Efficacy studies were conducted in NOD.SCID mice xenografted with MV4-11 cells.

CPT COMPOUNDS TARGETING MCL-1 INDUCE STRONG APOPTOSIS

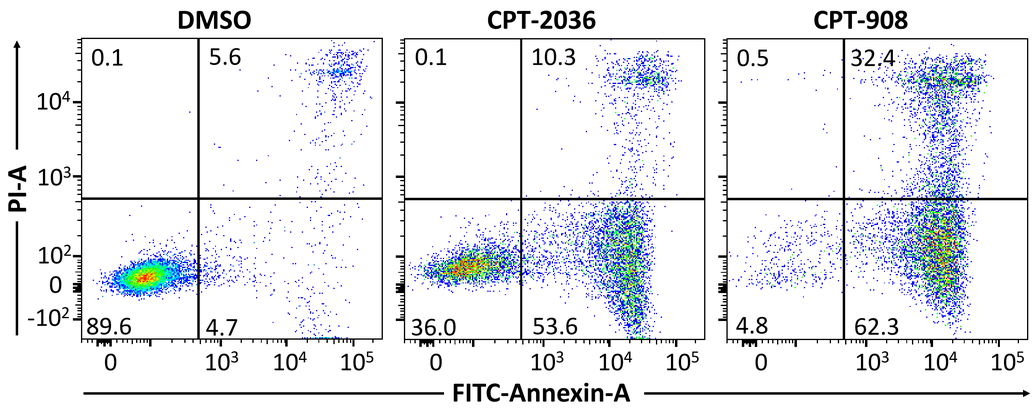


Figure 2. MV4-11 cells were treated for 5 hours with selected compounds and stained with Annexin/PI for the detection of apoptosis using Flow Cytometry.

CPT-908 IS HIGHLY POTENT IN THE AML PDX CELLS AND SHOWS NM ACTIVITY IN CELLS REFRACTORY TO GILTERITINIB AND VENETOCLAX

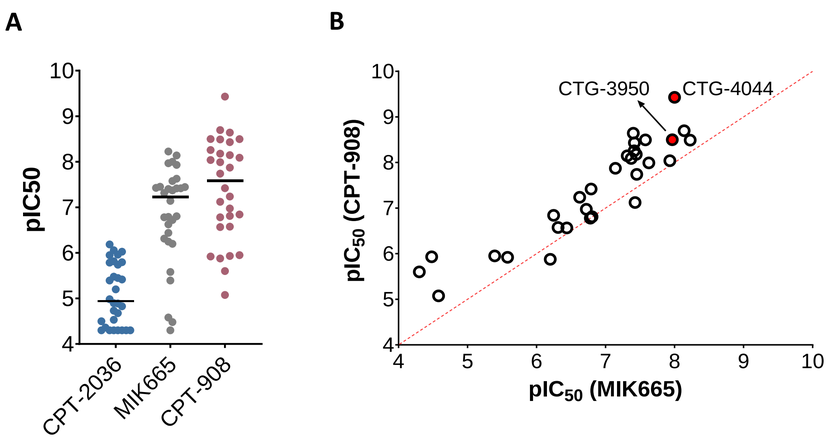


Figure 4. Results of the VitroScreen (Champions Oncology) on 30 AML *ex vivo* models using 48-hour CellTiter-Glo® Assay. **A)** Cytotoxicity of CPT-2036, CPT-908 and MIK665 (Novartis/Servier). **B)** Comparison of MIK665 and CPT-908 activity. CTG-3950 and CTG-4044 - cells resistant to Gilteritinib or Venetoclax, respectively.

CPT MCL-1 DEGRADERS ARE EFFICACIOUS *IN VIVO*

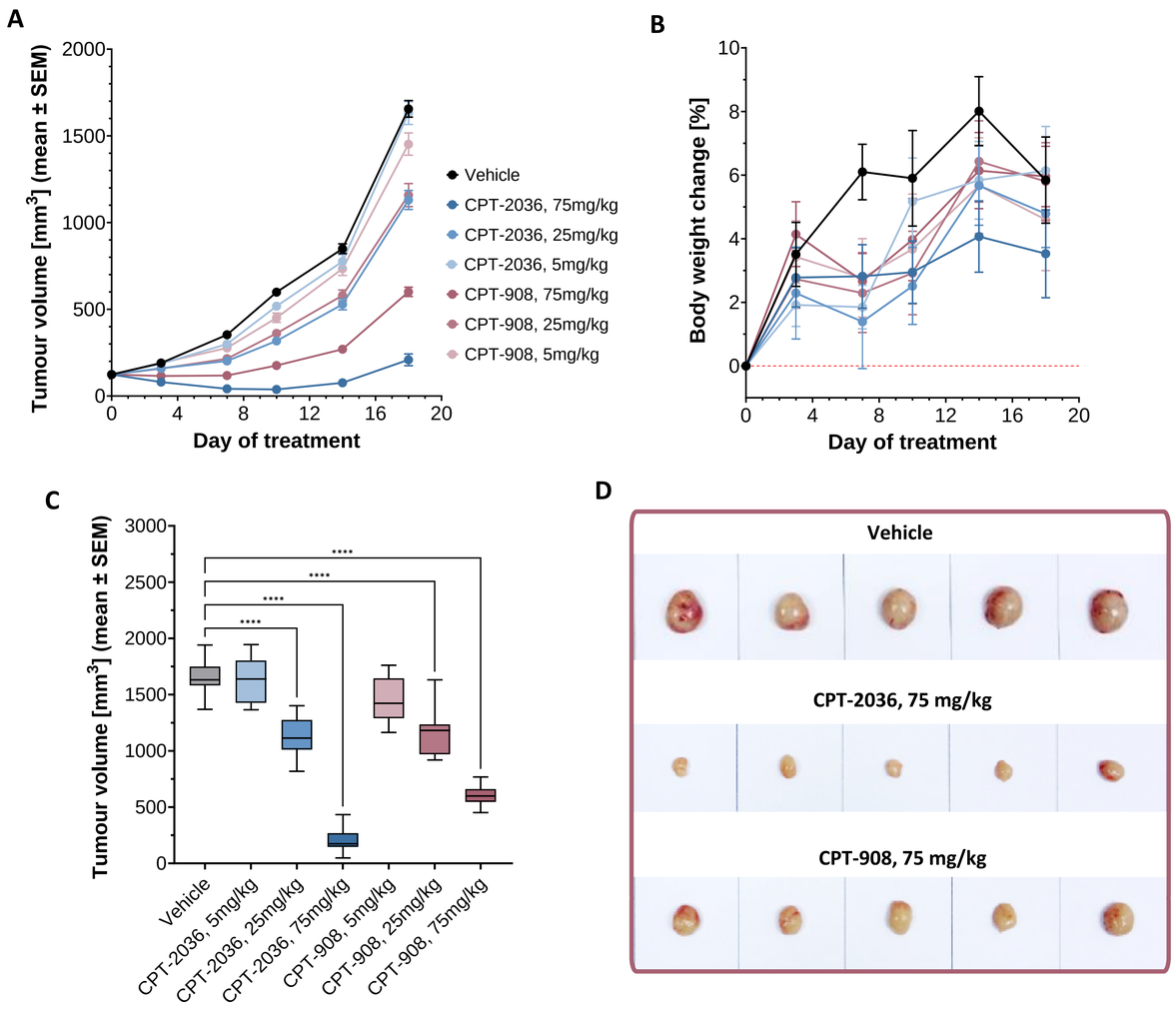


Figure 5. NOD.SCID mice (N=10/group) with ~150 mm³ MV4-11 tumours were treated with tested compounds every 3 days (by IV route). Tumour volume and body weight were measured twice per week, and the results are shown in figures **A** and **B,** respectively. **C)** ANOVA of differences in the tumour volume at the end of the study between all groups. **D)** Pictures of tumours from mice treated with CPT-2036, CPT-908 or with the vehicle alone.

CONCLUSIONS

We developed highly potent MCL-1 bifunctional degraders that are active both *in vitro* and *in vivo* in haematological malignancies and solid tumours models, and have a low cardiotoxic potential. Our results show that compound-mediated MCL-1 degradation represents an exciting new therapeutic strategy for cancer treatment.

CPT-2036 AND CPT-908 INDUCE MCL-1 DEGRADATION IN MV4-11 CELLS

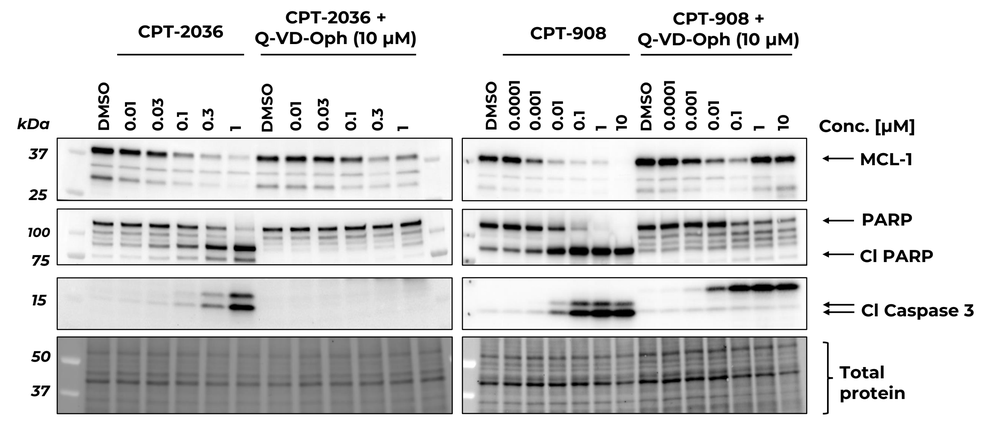
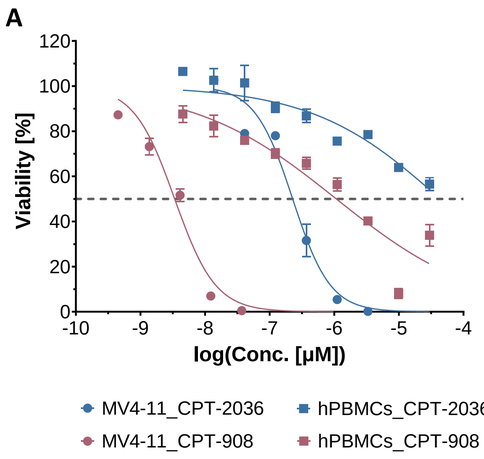


Figure 1. Western Blot analysis of MV4-11 cells treated with two different bifunctional degraders for 6 hours. Apoptosis level was measured by detection of cleaved: PARP (Cl PARP) and Caspase 3 (Cl Caspase 3). Degradation of MCL-1 in the presence of the pan-caspase inhibitor (Q-VD-Oph) indicates proteasomal degradation mediated by CPT-2036 and CPT-908.

MCL-1 DEGRADERS SHOW POTENT CYTOTOXIC ACTIVITY IN CANCER CELLS BUT NOT IN PRIMARY CELLS



B

Cell line	pIC50 CPT-2036	pIC50 CPT-908
MV4-11	6.5 ± 0.1	8.5 ± 0.2
MV4-11 Ven-resistant	-	11.5
MV4-11 Ven-resistant + Venetoclax	-	12.0
WSU-DLCL-2	5.4 ± 0.2	7.6 ± 0.1
DMS 114	6.2 ± 0.3	7.8 ± 0.1
OPM-2	6.6 ± 0.2	>8.3 ± 0.1
Hep3B	< 4.53	-
hPBMC	4.9 ± 0.7	6.3 ± 0.5
hiPSC-CM	4.8 ± 0.8	5.8

Figure 3. A) Cytotoxicity of CPT-2036 and CPT-908 in the AML cell line – MV4-11 and in primary cells – human peripheral blood mononuclear cells (hPBMCs) using 72-hour CellTiter-Glo® Assay. **B)** Summary of viability assay results (CellTiter-Glo® Assay) on different cancer cell lines and primary cells. hiPSC-CM – human induced pluripotent stem cells-cardiomyocytes

PLASMA STABILITY AND CARDIAC SAFETY PROFILE OF CPT-2036 AND CPT-908

ASSAY	Parameter	CPT-2036 mouse	CPT-2036 monkey	CPT-2036 human	CPT-908 mouse	CPT-908 monkey	CPT-908 human
Plasma stability	Remaining @120 min [%]	**59.2**	87.7	80.4	**0.2**	83.3	95.4
	T-half [min]	**> 120**	**>240**	**> 240**	**< 15**	**> 240**	**> 240**

ASSAY	CPT-2036	CPT-908
NanoBRET (permeability) [RBA*]	**4.2**	**0.006**
hNav1.5 (inhibition @10 µM) [%]	21	6
hERG (inhibition @10 µM) [%]	18	10
hCav1.2 (inhibition @10 µM) [%]	6	N/A
3D Cardiac Microtissue	no effects up to 30 µM	N/A
cTnI** [ng/mL]	BLQ<0.078***	BLQ<0.078***

*RBA – Relative Binding Affinity – ratio of compound's binding to CRBN in live and permeabilized cells
**Cardiac Troponin-I conc. in monkey serum from animals treated with 10 mg/kg of a compound
***BLQ – below limit of quantification

CPT-908 AND CPT-2036 PHARMACODYNAMIC EFFECT IN NHP

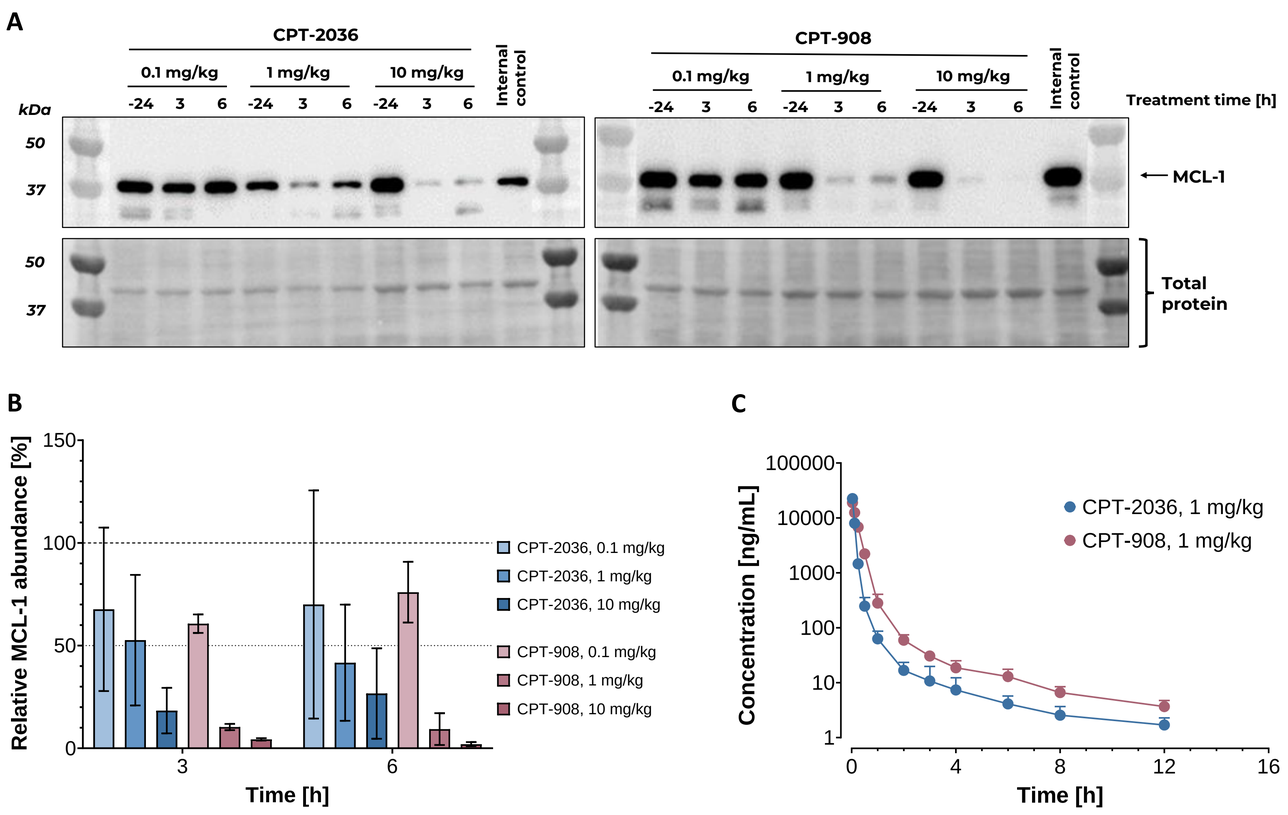


Figure 6. Cynomolgous monkeys (N=3/group) were treated with 0.1, 1 and 10 mg/kg doses of CPT-2036 or CPT-908 administered IV; at the indicated timepoints blood samples were collected and PBMCs were isolated using density gradient centrifugation. **A)** Purified PBMCs were analyzed for MCL-1 degradation using WB. **B)** Densitometric analysis of WB results. **C)** Mean (±SD) CPT-2036 and CPT-908 plasma concentration following IV administration.

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