Preclinical evaluation of novel MCL-1 degrader in *in vitro* and *in vivo* cancer models

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BACKGROUND

MCL-1 protein belongs to the Bcl-2 family consisting of both pro- and anti-apoptotic proteins. It serves as a master pro-survival factor by inhibiting apoptosis in a broad range of human malignancies. MCL-1 is involved in cancer resistance to different types of therapies; thus its targeting appears very attractive. Although several MCL-1 inhibitors have been studied in clinical trials, none has been approved for clinical use so far. Degradation of a target protein offers several advantages over traditional inhibitors, e.g., potential to overcome resistance to inhibitors, greater response even at a lower dose, extended pharmacodynamics, better selectivity and many others. This approach has recently emerged as a novel therapeutic modality in drug discovery. In this report, we present an *in vitro* and *in vivo* characterization of a newly-developed compound capable of degrading MCL-1 protein.

MATERIALS AND METHODS

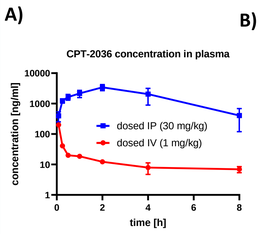
A series of biophysical methods (FP, SPR, AlphaLisa) have been utilized to characterize compound interactions with MCL-1 protein and E3 Ligase. The biological properties of the reported molecule have been determined using cancer cell culture models (cell viability assessment using Cell Titer-Glo Assay), molecular biology techniques (western blots to confirm target protein degradation, apoptosis induction and the MoA) as well as a MV-4-11 xenograft *in vivo* model.

SUMMARY OF BIOPHYSICAL AND *IN VITRO* ADME PROPERTIES OF CPT-2036

Cpd	pK_d (SPR) hMCL-1	pK_i (FP) hMCL-1	pK_i (FP) hCRBN	pEC_{50}, (AlphaLisa) hMCL-1, h CRBN
	(binary complex formation)			(ternary complex formation)
CPT-2036	8.22	>8.62	5.68	8.13

ASSAY	SPECIES	VALUE
CYP inhibition *IC_{50} [μM]*	1A2	>30
	2C9	>30
	2D6	>30
	3A4	>30
	2C19	>30
Plasma protein binding *[% bound]*	Mouse	>99
	Rat	>99
	Human	>99
Plasma stability *[% remaining @ 120 min]*	Mouse	59
	Rat	56
	Human	56
Liver hepatocyte stability *$T_{1/2}$ [min]*	Mouse	25
	Human	78
	Monkey	41

CPT-2036 PHARMACOKINETIC PROFILE IN CD-1 MICE



B)

BASIC PK PARAMETERS FOR CPT-2036			
dosed IV (1mg/kg)		dosed IP (30 mg/kg)	
Dose (mg/kg)	1,00	Dose (mg/kg)	30,00
C0 (ng/mL)	440,54	Cmax (ng/mL)	3431,94
Vd (L/kg)	51,92	Tmax (h)	2,00
Cl (mL/min/kg)	87,53	T1/2 (h)	NC
T1/2 (h)	8,01	AUC0-last (ng·h/mL)	13523,62
AUC0-last (ng·h/mL)	123,41	AUC0-inf (ng·h/mL)	NC
AUC0-inf (ng·h/mL)	208,44	AUCExtra(%)	NC
AUCExtra(%)	37,12	MRT0-last (h)	3,05
MRT0-last (h)	2,16	Rsq	NC
Rsq	0,8854	%F	365,27

Figure 1. A) Mean (±SD) CPT-2036 plasma concentrations following IV (1.0 mg/kg) and IP administration (30 mg/kg). **B)** Table summarizing basic PK parameters of CPT-2036 after IV and IP administration.

CPT-2036 SHOWS POTENT AND CRBN DEPENDENT CYTOTOXIC ACTIVITY IN HEMATOLOGICAL CANCER CELL LINES

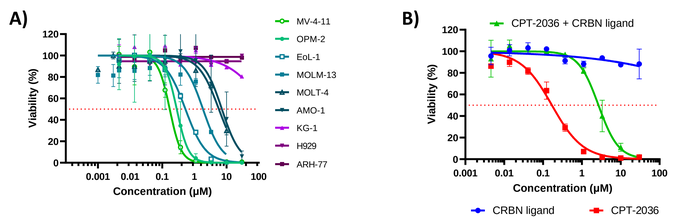


Figure 2. A) Cytotoxicity of the CPT-2036 in panel of hematological cancer cell lines using a 72-hour CellTiter-Glo® Assay. As controls, MCL-1-dependent (MV4-11 - acute myeloid leukemia) and MCL-1-independent (ARH-77 - plasma cell leukemia) cell lines were used. **B)** The effect of the CRBN ligand presence on the CPT-2036 cytotoxic activity in MV4-11 cells.

CPT-2036 DEMONSTRATES SYNERGISTIC EFFECT IN COMBINATION WITH BCL-2 INHIBITOR IN MV4-11 CELLS

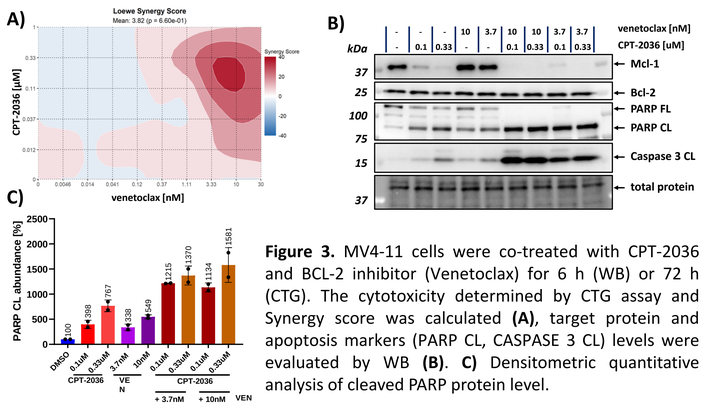


Figure 3. MV4-11 cells were co-treated with CPT-2036 and BCL-2 inhibitor (Venetoclax) for 6 h (WB) or 72 h (CTG). The cytotoxicity determined by CTG assay and Synergy score was calculated **(A)**, target protein and apoptosis markers (PARP CL, CASPASE 3 CL) levels were evaluated by WB **(B)**. **C)** Densitometric quantitative analysis of cleaved PARP protein level.

CPT-2036 DRIVEN MCL-1 DEGRADATION IS PROTEASOME AND CRBN DEPENDENT

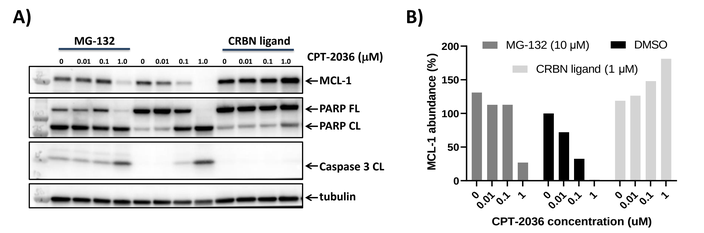
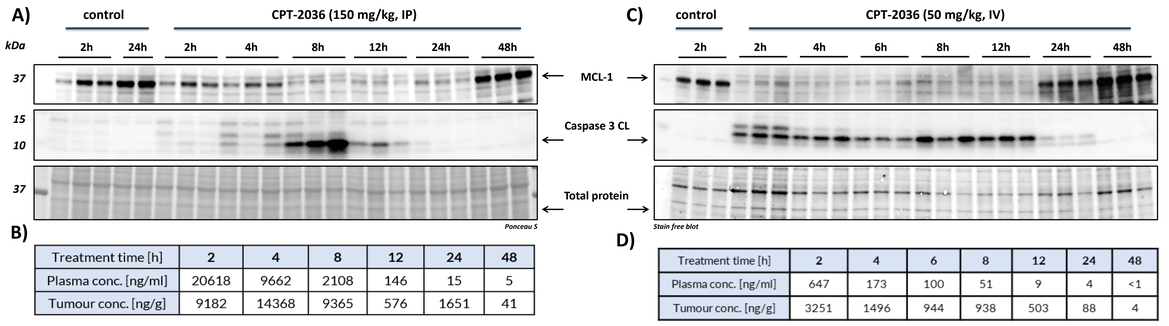


Figure 4. OPM-2 cells were pretreated for 1 hour with a proteasome inhibitor, MG-132 (10μM) or CRBN ligand (1μM) and then treated with CPT-2036 for 6 hours. **A)** Cell lysates were analyzed for MCL-1 degradation and apoptosis induction (cleaved PARP or caspase-3) using WB. **B)** Densitometric quantitative analysis of the level of MCL-1 protein degradation.

PK/PD RELATIONSHIP OF CPT-2036 IN MV4-11 XENOGRAFT MICE



B)

Treatment time [h]	2	4	8	12	24	48
Plasma conc. [ng/ml]	20618	9662	2108	146	15	5
Tumour conc. [ng/g]	9182	14368	9365	576	1651	41

D)

Treatment time [h]	2	4	6	8	12	24	48
Plasma conc. [ng/ml]	647	173	100	51	9	4	<1
Tumour conc. [ng/g]	3251	1496	944	938	503	88	4

Figure 5. CB-17 SCID mice with 350 mm^3 MV4-11 tumors were treated with 150 mg/kg dose of CPT-2036 administered IP or with 50 mg/kg dose administered IV; at the indicated timepoints mice were sacrificed, plasma and tumor samples were collected. Tumor samples collected from mice dosed IP (**A**) and IV (**C**) were analyzed for MCL-1 degradation and apoptosis induction using WB. **(B&D)** Compound concentration in plasma and tumor samples.

CPT-2036 TREATMENT SHOWS POTENT ANTI-TUMOR ACTIVITY IN MV4-11 XENOGRAFT MODEL

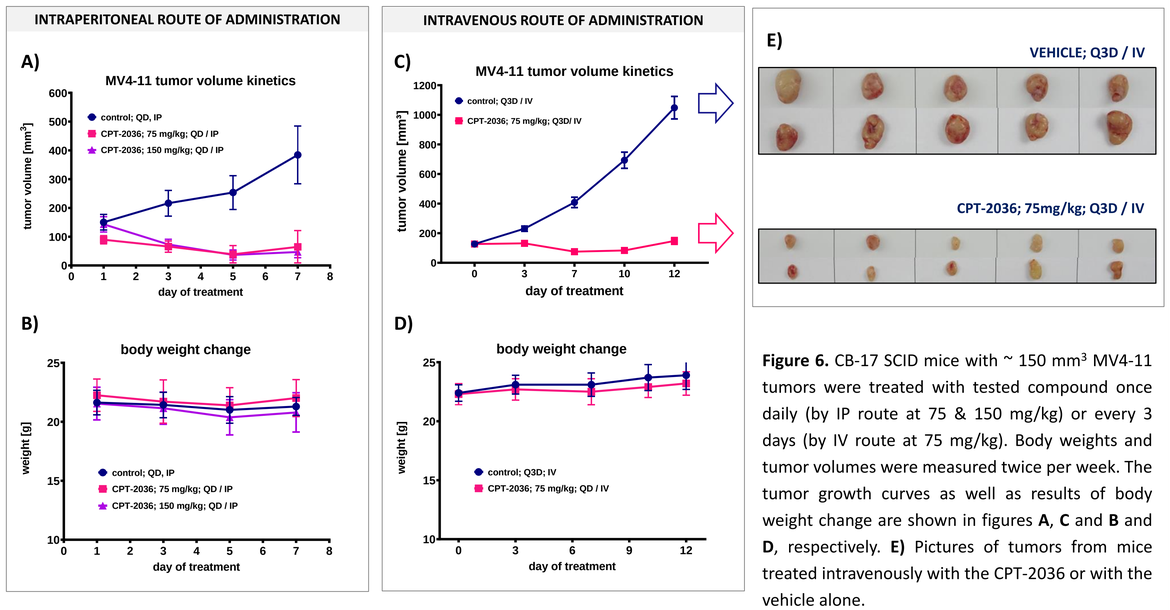


Figure 6. CB-17 SCID mice with ~ 150 mm^3 MV4-11 tumors were treated with tested compound once daily (by IP route at 75 & 150 mg/kg) or every 3 days (by IV route at 75 mg/kg). Body weights and tumor volumes were measured twice per week. The tumor growth curves as well as results of body weight change are shown in figures **A**, **C** and **B** and **D**, respectively. **E)** Pictures of tumors from mice treated intravenously with the CPT-2036 or with the vehicle alone.

RESULTS SUMMARY

The reported compound binds both E3 Ligase and target protein MCL-1 with high affinity and forms a ternary complex *in vitro*. In cancer cells, it induces the degradation of MCL-1 which results in apoptosis induction and cell death. The compound shows a desirable PK and PD profile, as well as causes *in vivo* tumor growth inhibition in human AML MV4-11 xenograft mouse model.

CONCLUSIONS

Presented results indicate that targeting MCL-1 protein by induction of its degradation could represent a new and effective strategy for cancer treatment.

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