Antitumor Activity of MPC-2130 in Human Hematopoietic Cell Lines and Ovarian and Prostate Tumor Xenografts in Athymic Nude Mice

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ABSTRACT

MPC-2130 was discovered at Myriad Pharmaceuticals, Inc. as a result of an extensive medicinal chemistry effort. The original lead compound, from which MPC-2130 was derived, was discovered in a yeast-based high throughput screen. Tumor cells treated with MPC-2130 display multiple indicators of apoptotic cell death, that include phosphatidylserine flipping to the outer cell membrane, release of cytochrome C from the mitochondria, caspase activation, cell condensation and DNA fragmentation. The preclinical proapoptotic activity of MPC-2130 has been studied in tissue culture models using multiple solid and hematopoietic derived cell lines. Solid tumor lines responsive to MPC-2130 include OVCAR-3 (ovarian), LNCaP and PC-3 (prostate). Responsive bone marrow derived tumors include T cell lines (H9, Molt-4, and Jurkat) as well as Burkitt's lymphomas (Daudi and Ramos). Finally, MPC-2130 was found to be equipotent for induction of apoptosis in cancer cell lines, regardless of the expression levels for the multidrug resistance ABC transporters MDR-1 (Pgp-1), MRP-1, and BCRP-1.

These studies were designed to assess the ability of MPC-2130 to inhibit the growth of OVCAR-3 and LNCaP tumor lines subcutaneously implanted into athymic nude mice. In OVCAR-3 xenografts, MPC-2130 (15 mg/kg; QD x 5 x 3; i.v.) significantly increased the time to 1500 mm³ tumor volume (survival) at day 30 when compared to vehicle (Wilcoxon and Log Rank; $p = 0.014$). Significant inhibition of the growth of OVCAR-3 xenografts was observed in animals treated with MPC-2130 (15mg/kg; QD x 5 x 3; i.v.; ANOVA; $p = 0.004$) when compared to vehicle treatment alone on Day 19. Also, MPC-2130 (15mg/kg; QD x 5; i.v.) significantly inhibited the growth of the LNCaP xenografts on day 9 when compared to animals treated with vehicle alone (ANOVA; $p = 0.0123$). Pharmacokinetic parameters were estimated in male Nu/+ mice after a single 10 mg/kg intravenous injection. The exposure, as estimated by AUC(0-inf), was 17833 hr*ng/mL and terminal elimination half-life was approximately 6.0 hours. These results suggest that MPC-2130 may be an effective therapy against multiple tumor types in humans.

MATERIALS AND METHODS

Cytotoxicity of MPC2130 in hematopoietic and solid tumors

Daudi and Ramos cells were plated at a density of 40,000 cells per well in a 96 well plate and 16 hours later incubated with varying concentrations of MPC-2130 for 48 hours. LNCaP and OVCAR-3 cells were treated similarly except for a plating density of 5,000 cells per well and a 72 hour treatment period. At the end of the dosing period, cell viability was determine using the WST-1 reagent.

MPC-2130 induced phosphatidylserine externalization

Jurkat, Ramos and Daudi cells were treated for 12 hours with 40 µM MPC-2130. OVCAR-3 cells were treated with 40 µM MPC-2130 for 72 hours. Cells were then stained with Annexin V and propidium iodide as per manufacturer recommended protocol (R&D Systems). Cells were then run on a Becton Dickinson FACSCAN flow cytometer and analyzed using CellQuest software.

Pharmacokinetics of MPC-2130 after intravenous dosing in mice

The PK parameters in mice were determined after a single IV bolus injection of MPC-2130 at a dose of 10 mg/kg dissolved in 5% Cremophor® EL, 5% Ethanol, 90% sterile 5% dextrose in water (D5W). Blood samples and whole brains were collected from 5 mice at each of 9 collection time points. Sample preparation and LC/MS/MS analysis was carried out by procedures validated for recovery along with an internal standard

OVCAR-3 Xenograft Model

Female Crl:Nu/Nu-nuBR mice were implanted subcutaneously with OVCAR-3 cells. When the average tumor volume had reached approximately 100 mm3, the mice were randomized into three test groups (N = 10). One group received vehicle (25% Cremophor® EL: 25% ethanol: 50% of 5% dextrose in water), one group was treated with MPC-2130 dosed at 15 mg/kg intravenously on Days 1-5, 8-12 and 15-19. Another group was treated with carboplatin (Paraplatin®), dosed at 20 mg/kg intraperitoneally on Days 1-5, 8-12 and 15-19. Tumor volumes were measured from Days 1 to 50. When the first animal in any of the groups achieved a tumor volume equal to or greater than 1500 mm3, the comparison of tumor volume between groups was halted. Tumor volume measurements were continued until Day 50 of the study for determining the percentage of animals with tumors greater than 1500 mm3. Body weight measurements were halted on the last day of dosing (Day 19).

LNCaP Xenograft Model

Crl:Nu/Nu-nuBR mice were implanted subcutaneously with LNCaP cells, which were allowed to grow to an average volume of 50 mm3. Mice were randomized into two test groups (N = 15). One group received vehicle (25% Cremophor® EL: 25% ethanol: 50% of D5W), and the other group was treated with MPC-2130 dosed at 15 mg/kg intravenously on Days 1-5. The mice were observed daily for mortality and signs of toxicity. Tumors and body weights were measured from Days 1 to 9.

RESULTS

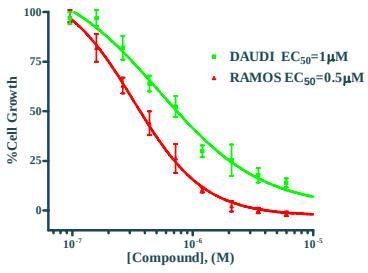
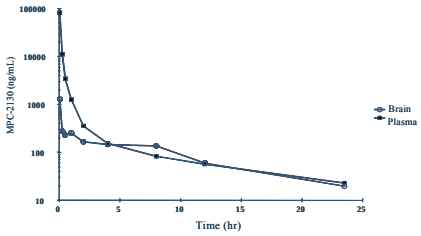


Figure 1. MPC-2130 inhibits the growth of Burkitt's Lymphoma lines



Tissue	t 1/2 (hr)	T_{max} (hr)	C_{max} (µg/mL)	C_{max} (µM)	$AUC_{(0-\infty)}$ (hr*ng/mL)	CL (mL/hr/kg)
Plasma	6.02	0.05	82.5	168	17833	561
Brain	6.46	0.05	1.29	2.63	2439	N/A

Figure 3. Pharmacokinetics of MPC-2130 after intravenous dosing in mice

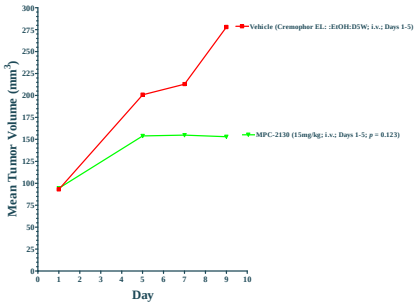


Figure 5. MPC-2130 inhibits the growth of LNCaP tumors

A.

Cell Line	Tumor Type	IC_{50} (µM)
LNCaP	Prostate Carcinoma	1.6
OVCAR-3	Ovarian Adenocarcinoma	0.8

B.

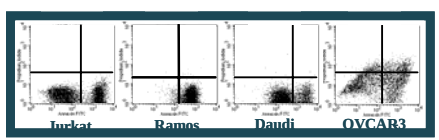


Figure 2 (A) Determination of MPC-2130 cytotoxicity IC50 values LNCAP and (B) MPC-2130 induced phosphatidylserine externalization

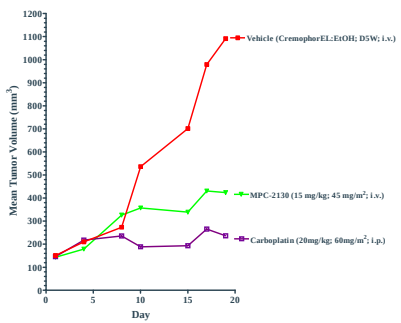


Figure 4. MPC-2130 decrease the growth of OVCAR-3 tumors

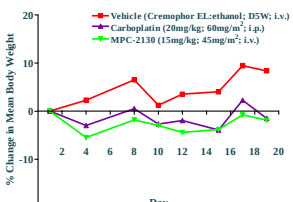


Figure 6. Treatment with MPC-2130 does not cause significant body weight loss in mice with OVCAR-3 xenografts

CONCLUSIONS

- MPC-2130 is kills hematopoietic derived and solid tumor lines through an apoptotic mechanism
- MPC-2130 shows good pharmacokinetic parameters with high brain penetration
- MPC-2130 significantly inhibits OVCAR-3 xenograft growth
- MPC-2130 shows a trend toward inhibition of LNCaP xenograft growth
- Animals dosed on a QD x 5 regime do not lose significant body weight

MPC-2130 is currently in Phase 1 clinical development for the treatment of leukemias, lymphomas and solid tumors.

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