Characterization of an orally bioavailable homolog of MPC-6827 (MPI-0443803) that maintains high brain penetration

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Abstract

MPI-0443803 was developed in an extensive medicinal chemistry effort to produce an orally bioavailable homolog of MPC-6827 (Azixa™, Myriad Pharmaceuticals, Inc.). Like MPC-6827, MPI-04438303 is a potent inducer of apoptosis that binds at or near the colchicine binding site on b-tubulin and prevents the polymerization of tubulin into microtubules. MPC-6827 is an investigational new drug that has shown anti-cancer activity in two completed phase I studies and is currently being studied in the Phase Ib/II setting for the treatment of patients with recurrent high grade gliomas and in the Phase Ib setting for the treatment of patients with metastatic melanoma to the brain. Here we report that MPI-0443803 displays pro-apoptotic activity, with potency at low nanomolar concentrations in multiple cancer types including pancreatic, breast, colorectal, non-small cell lung, melanoma, ovarian and leukemia. MPI-0443803 was 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. Unlike the vinca alkaloids (e.g., vinblastine, vincristine and vinorelbine), MPI-0443803 has excellent oral bioavailability that approaches 100% at a 60 mg/kg dose in mice when formulated in 5% dextrose:water with a half life ($t_{1/2}$) of approximately 10 hours. MPI-0443803 crosses the blood brain barrier and distributes rapidly into the CNS with exposure in the brain approximately 7 times higher than in plasma after oral or intravenous dosing. Statistically significant ($r < 0.00005$) inhibition of tumor growth was observed in melanoma (B16-F0) allografts in nude mice dosed with oral MPI-0443803 relative to vehicle. These studies demonstrate that MPI-0443803 has potent and broad spectrum *in vitro* and *in vivo* antitumor activity with high oral bioavailability. Therefore, MPI-0443803 is a promising candidate for development as an oral alternative to MPC-6827.

Methods

Cytotoxicity of MPI-0443803 in tumor cell lines and in MDR-1, MRP-1 and BCRP-1 over-expressing cell-lines

Human colon, NSCLC, ovarian and pancreatic and mouse lymphoma and melanoma cell lines were exposed to various final concentrations of MPI-0443803. The MCF-7 human breast cancer cells, NCI/ADR-RES (over-express MDR-1), MCF-7/MX (over-express BCRP) and MCF-7/VX (over-express MRP-1) and mouse lymphoma P388 and P388/ADR cells were similarly treated with MPI-0443803 and various chemotherapeutics know to be substrates for ABC transporters. The inhibition of cell growth due to exposure to the drug was determined using the ATP-Lite assay.

Pharmacokinetics of MPI-0443803 in male Nu/+ mice

Animals were dosed with approximately 2.5 mg/kg *i.v.* in the tail vein or 5, 10, 30 or 60 mg/kg *p.o.* in fed and 60 mg/kg *p.o* in a fasted state. Blood samples and whole brains were collected from five mice at each of the nine collection time points (approximately 0.05, 0.25, 1.0, 2.0, 4.0, 8.0, 12.0 and 24.0 hours) after administration of the dose. Plasma was collected from blood samples, and whole brain sample were homogenized. Both tissues were analyzed for concentration of MPI-0443803. Pharmacokinetic parameters were estimated by non-compartmental analysis using WinNonlin. These studies conformed to the recommendations set forth in the U.S. Public Health Service *Policy on Humane Care and Use of Laboratory Animals.*[1]

Disruption of microtubules in intact cells

A549 cells were treated with saline or 10nM MPC-6827 on cover-slips. After one or three hours, the cover slips were removed from media, fixed in formaldehyde and permeabilized with 0.1% Triton-X 100. Following blocking, cover slips were stained for tubulin, actin and nuclei.

B16-F0 xenografts

Female Crl:Nu/Nu-nuBR mice (Charles River Labs, Wilmington, MA) were implanted subcutaneously with 10^6 cells in the right flank. Animals were housed by groups in Positive Individual Ventilation cages in flat-bottom cages with no more than ten mice per cage. Environmental controls were set to maintain a temperature between 65 and 75 °F with a relative humidity of 30-70% in a 12:12 hour light:dark cycle. Animals were fed and watered *ad libitum*. Tumors were allowed to grow to approximately 100 mm^3 and then mice were placed into test groups (N = 10). Animals were treated with vehicle, 50 mg/kg MPI-0443803 qd x 5 *p.o.* or 200 mg/kg MPI-0443803 qwk x 2 *p.o.* The mice were observed daily for mortality and signs of toxicity. Tumor growth was monitored externally using a caliper and volumes calculated using the formula [p/6 (width^2 x length)], in which width represents the smaller tumor diameter. Studies were completed when the first animal achieved a tumor volume > 1500 mm^3. Statistical analysis of variance with unadjusted pair wise comparison was performed using SAS software (Cary, NC).[1]

[1] The U.S. Public Health Service *Policy on Humane Care and Use of Laboratory Animals*, Office of Laboratory Animal Welfare, National Institutes of Health, Department of Health and Human Services, Bethesda, MD 20892-7982

Results

Table 1. Cytotoxicity of MPI-0443803 in multiple tumor lines

Cell Line	Tumor	72 hr Cytotoxicity (IC50, nM ± SD)
HT-29	Colorectal	29.0 ± 2.0
HCT-116	Colorectal Carcinoma	7.6 ± 1.9
P388	Lymphoma	6.1 ± 2.2
A549	Lung	54.0
OVCAR-3	Ovarian	29.0 ± 20.1
MIAPaCa-2	Pancreatic	56.0
B16F1	Melanoma	37.0 ± 0.0

Table 2. MPI-0443803 is not a substrate for MDR-1, MRP-1 and BCRP-1 ABC transporters

	Various MDR Cell Line Cytotoxicity IC50, nM				
	MPI-0443803	Vinblastine	Docetaxel	Epirubicin	CPT11
P388	18.0	0.5	3.3		
P388/ADR (MDR-1)	27.0	8.3	200.0		
MCF-7	27.0	1.4	13.0	160.0	20.0
NCI/ADR-Res (MDR-1)	13.0	100.0	900.0		
MCF-7/MX (BCRP-1)	9.0			400.0	15.0
MCF-7/VP (MRP-1)	13.0			2,100.0	

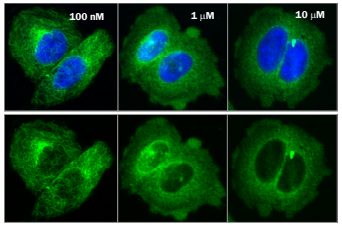


Figure 1. MPI-0443803 inhibits microtubule formation in A549 cells

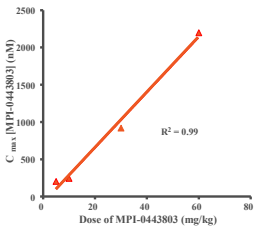
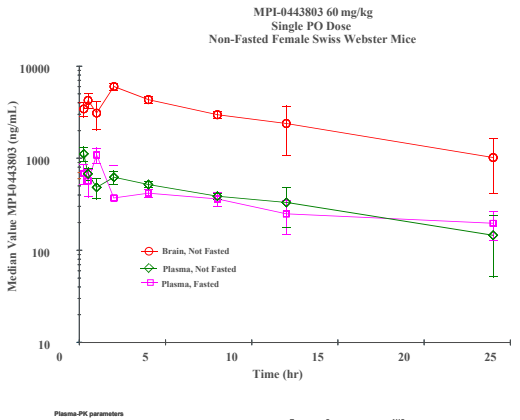


Figure 2 Oral Dose proportionality of MPI-0443803



Plasma-PK parameters

Study ID	Compound ID	Fast Status	ROA	Dose (mg/kg)	t 1/2 (hr)	T_{max} (hr)	C_{max} (ng/mL)	Cmax (uM)	AUC_{0-inf} (hr*ng/mL)	CL (mL/hr/kg)	V (mL/kg)	%F
3803PK06-PO	MPI-0443803	Not Fasted	PO	60	10.8	0.25	1120	3.8	10746	558	8914	98.0
3803PK01-IV	MPI-0443803	Not Fasted	IV	2.5	6.2	0.05	513	1.7	467	5358	47759	
3803PK05-PO	MPI-0443803	Fasted	PO	60	18.3	1.00	1090	3.7	12855	4668	123424	114.8

Brain-PK parameters

Study ID	Compound ID	Fast Status	ROA	Dose (mg/kg)	t 1/2 (hr)	T_{max} (hr)	C_{max} (ng/mL)	Cmax (uM)	AUC_{0-inf} (hr*ng/mL)	CL (mL/hr/kg)	V (mL/kg)
3803PK06-PO	MPI-0443803	Not Fasted	PO	60	9.0	2.00	6030	20.4	77290	776	10109

Figure3. Pharmacokinetics of MPI-0443803 in mice dosed *p.o.* and *i.v.*

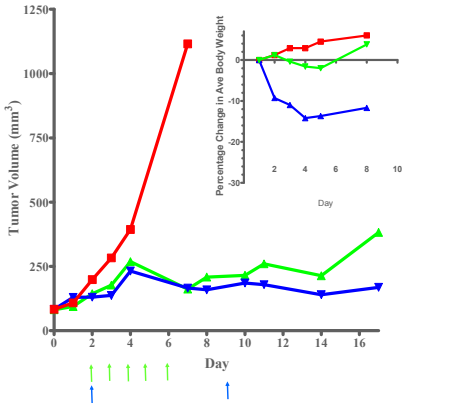


Figure 4. MPI-0443803 inhibits the growth of B16-F0 grafts

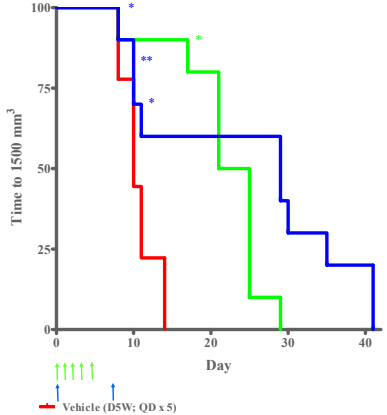


Figure 5. Time to Sacrifices in B16-F0 mice

Conclusions

- **MPI-0443803 is not a substrate for the ABC transporters MDR-1, MRP-1 and BCRP-1**
- **MPI-0443803 inhibits microtubule formation**
- **Statistically significant ($p < 0.00005$) inhibition of tumor growth was observed in melanoma (B16-F0) allografts in nude mice dosed with oral relative to vehicle**
- **Dose proportional increases in C_{max} were observed in mice dosed orally with MPI-0443803 up to 60 mg/kg**
- **No significant differences in pharmacokinetics were observed between fed and fasted animals**
- **MPI-0443803 is almost completely bioavailable after oral dosing**
- **Brain concentrations of MPI-0443803 were approximately 7-fold higher than plasma levels (AUC_{0-t})**

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