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Activity of cancer metabolism inhibitor MPC-9528 in xenograft models: comparison of different dosing schedules

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Background

MPC‑9528 and MPI-0487316 are potent, selective, orally bioavailable inhibitors of the enzyme nicotinamide phosphoribosyltransferase (Nampt)[1] representing two structurally distinct lead classes. Nampt catalyzes the rate-limiting step in production of NAD from nicotinamide in the salvage pathway for NAD biosynthesis. Nampt inhibition results in NAD depletion, inhibition of ATP synthesis and cell death. Cancer cells are more sensitive to Nampt inhibition and consequent NAD depletion because of increased glycolysis and elevated activity of NAD consuming enzymes such as sirtuins and poly (ADP-ribose) polymerases (Parp). Nicotinic acid phosphoribosyltransferase (Naprt) catalyzes the first step in an alternate pathway for NAD synthesis from nicotinic acid (NA). Many cancer cell lines and primary tumors, unlike most normal tissues are deficient in Naprt activity. Administration of NA could thus prevent NAD depletion in normal tissues that results from Nampt inhibition, but not in Naprt-deficient tumors, leading to greater therapeutic index and efficacy.

Introduction

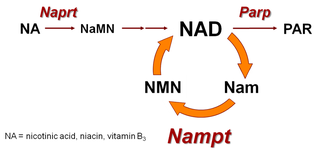
MPC‑9528 inhibited Nampt activity *in vitro* with an IC_{50} of <50 pM and suppressed NAD levels in tumor cells with a potency of 170 pM.[2] It has a median tumoricidal potency of 2.8 nM with a range of 100 pM to 62 nM in a screen of 93 cancer cell lines of diverse origin. NA prevented cell death induced by MPC-9528 in cell lines expressing Naprt, demonstrating mechanism-based activity. In contrast, NA did not prevent MPC-9528-induced cell death in 44 out of 153 cancer cell lines surveyed, which correlated with low to undetectable Naprt expression.[3] MPC-9528 showed synergistic tumoricidal activity *in vitro* with chemotherapeutics that activate Parp leading to NAD depletion, such as the alkylating agents temozolomide and the thymidylate synthetase inhibitor, 5-fluorouracil.[4]

MPC-9528 induced regressions in xenograft models and NA administration blocked anti-tumor activity in xenografts expressing Naprt, signifying mechanism-based anti-tumor activity.[5] In Naprt-deficient xenografts, NA co-administration allowed tolerance to higher doses of MPC-9528 and a substantially increased anti-tumor response relative to lower doses.[5] NA also blocked mortality in mice induced by high doses of MPC-9528, indicating mechanism-based toxicity. In a 14-day study in rats, once daily oral administration of MPC-9528 resulted in a reduction in leukocyte counts by 52% to 77% and thymic atrophy, and co-administration of NA prevented or reduced the severity of these adverse effects. [6]

Methods

For cellular studies, NAD was measured in cell lysates using a coupled enzyme system and cell viability was based upon ATP measurements using CellTiter Glo®.[1] For xenograft studies, HT1080 human fibrosarcoma cells were implanted subcutaneously into athymic mice (nu/nu). MPC-9528 and MPI-0487316 were dosed orally once or twice daily for one to three weeks as indicated in cohorts of ten for all dose groups. Plasma concentrations of MPC-9528 and MPI-0487316 were determined by LC-MS/MS.

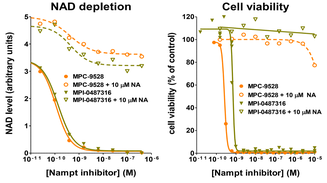
Nampt inhibition and NAD metabolism



NA = nicotinic acid, niacin, vitamin B_3

- Nampt catalyzes the rate-limiting step in salvage of NAD from nicotinamide (Nam).
- Nampt inhibition results in NAD depletion, ATP reduction and cell death.
- Cancer cells are more sensitive to Nampt inhibition due to increased metabolic demands and elevated activity of NAD consuming enzymes such as Parp and sirtuins.
- Nicotinic acid administration to Naprt-proficient cells can prevent NAD depletion caused by Nampt inhibition.
- ~30% of cancer cell lines surveyed are Naprt-deficient and cannot be rescued from Nampt inhibition by NA.

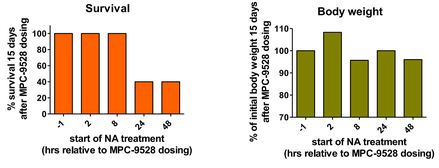
Effect of MPC-9528, MPI-0487316 on cellular NAD and viability



HCT-116 colon carcinoma cells were exposed to various concentrations of MPC-9528 or MPI-0487316 in the presence or absence of 10 μM NA and effects on cellular NAD and viability were determined. Cellular NAD was measured after 24 h (left panel). Cell viability was determined after 72 h (right panel).

Nampt inhibitors from both structural classes kill tumor cells by NAD depletion and NA can block their activity.

Nicotinic acid prevents MPC-9528 toxicity in mice



Female CD-1 mice were given a single lethal dose of MPC-9528 (300 mg/kg) which resulted in complete mortality within 5 days. Nicotinic acid (3 g/kg) was administered orally 1 h prior to the dose of MPC-9528 or 2, 4, 8, 24 or 48 h following MPC-9528 (n=5 mice per time point). Nicotinic acid dosed 1 h prior or up to 8 hours after MPC-9528 completely prevented MPC-9528-induced lethality and weight loss.

Toxicity of MPC-9528 in mice is mechanism-based.

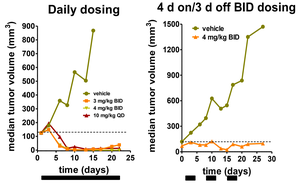
Activity of MPC-9528 and MPI-0487316 *in vitro*

Compound	Potencies (IC_{50})			
	Enzymatic assay	Cellular assays		
		Mechanism based		Cell survival
	Nampt inhibition	NAD reduction	PAR reduction	HCT116 cells
MPC-9528	< 50 pM	240 pM	100 pM	1.5 nM
MPI-0487316	170 pM	140 pM	98 pM	710 pM

Potency of Nampt inhibition by MPC-9528 and MPI-0487316 was determined by measuring IC_{50} values for inhibition of Nampt enzyme activity *in vitro* and by effects on cellular NAD and PAR levels and cell survival.[1]

Inhibitors from both structural classes potently inhibit Nampt activity *in vitro* and in cells.

Activity of MPC-9528 in HT1080 xenograft model



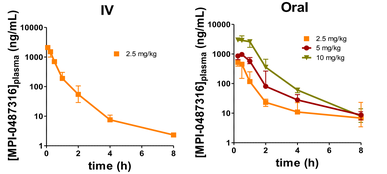
Response and cure rates for QD and BID dosing

Dose (mg/kg)		3	4	4	10
Schedule		BID X21	BID X21	4 d on/ 3 d off X3	QD X21
Response rate on Day 21(%)	Partial	10	20	20	30
	Complete	30	60	50	30
	Overall	40	80	70	60
Animals (%) with no tumors at study end (*Day 77, **Day 34)		60*	50*	40**	40*

Athymic mice (nu/nu) bearing HT1080 human fibrosarcoma xenografts were dosed once daily (left panel) or with a 4 days on/3 days off, BID schedule (middle panel) and tumor volume was measured over time. The table on the right lists response rates on Day 21 and the percentage of animals with no detectable tumors at the end of the study.

MPC-9528 is potent and efficacious in a xenograft model with low doses administered orally QD or BID, inducing profound regressions and cures at high rates.

Pharmacokinetics of MPI-0487316 in mice

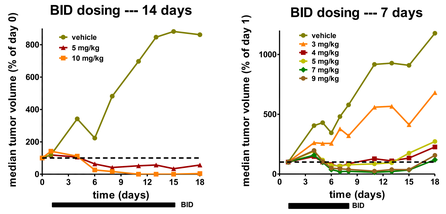


Pharmacokinetics of MPI-0487316 in mice. Cohorts of five CD-1 mice were administered various single doses of MPI-0487316 and plasma concentrations were determined by LC-MS/MS at various times as indicated. The table at the bottom summarizes the key pharmacokinetic parameters.

MPI-0487316 shows high oral bioavailability in mice similar to MPC-9528.[7]

Dose (mg/kg)	ROA	$t_{½}$ (h)	C_{max} (ng/mL)	$AUC_{(0-t)}$ (h*ng/mL)	% F_{PO}
2.5	IV	1.4	2093	1125	---
2.5	PO	3.6	517	503	42
5		1.9	962	1262	52
10		1.1	2969	4528	100

Activity of MPI-0487316 in HT1080 xenograft model



Athymic mice (nu/nu) bearing HT1080 human fibrosarcoma xenografts were administered various doses of MPI-0487316 orally BID for 14 days (left panel) or 7 days (middle panel). The graphs show change in median tumor volume with time.

MPI-0487316 is potent and efficacious in a xenograft model and shows a dose-dependent reduction in median tumor volume with oral BID dosing.

Conclusions

- **MPC-9528 and MPI-0487316 are potent inhibitors of Nampt that kill tumor cells by reducing cellular NAD.**
- **Nicotinic acid can block the activity of MPC-9528 and MPI-0487316, demonstrating on-target activity *in vitro* and *in vivo*.**
- **MPI-0487316 shows high oral bioavailability in mice.**
- **Oral administration with MPC-9528 and MPI-0487316 results in xenograft tumor regression and high response rates with a variety of regimens, based upon once or twice daily dosing.**
- **MPC-9528 and MPI-0487316 represent leads in two distinct chemical classes with potential for development as therapeutics for a wide variety of cancers.**

References

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