Abstract #LB-204

Novel Anti-Tumor Agents

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MPI-0479605: A Novel Small Molecule Inhibitor of the Mitotic Kinase TTK with Anti-Tumor Activity in Pre-Clinical Models

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Abstract

Background: TTK is a dual-specificity protein kinase that is essential for the proper attachment of chromosomes to the mitotic spindle and for maintaining the spindle assembly checkpoint. TTK is expressed at high levels during mitosis and is overexpressed in cancer cells. Disruption of TTK function results in mitotic aberrations and apoptosis. In an ongoing drug discovery program, we have developed a potent and selective inhibitor of TTK with favorable ADME/PK properties that inhibits the growth of cancer cells in culture and demonstrates anti-tumor activity in a mouse xenograft model.

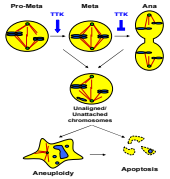
Materials and Methods: Kinase activity was measured in biochemical assays by monitoring the incorporation of ^{33}P into protein substrate. Phosphorylated Smad2 was detected by Western blot or ELISA using phospho-specific antibodies. Inhibition of the spindle assembly checkpoint was determined by image analysis of cells stained with anti-phospho-histone H3 antibody. Viability was determined by measuring cellular ATP levels or by WST1 assay. Apoptosis was assessed by measuring caspase-3/7 induction. Anti-tumor activity was evaluated in an HCT-116 xenograft in nu/nu mice.

Results: MPI-0479605 inhibited TTK kinase activity *in vitro* with an IC_{50} = 4 nM. This compound had moderate activity towards JNK (IC_{50} = 110 nM) and FER (IC_{50} = 590 nM) kinases and exhibited little or no activity against a panel of 32 other kinases, suggesting good selectivity for TTK. MPI-0479605 induced apoptotic cell death in a dose-dependent manner in HCT116 cells and demonstrated cytotoxicity against a panel of other tumor cell lines. Furthermore, nocodazole-induced activation of the spindle assembly checkpoint and phosphorylation of Smad2 were overcome by treatment with MPI-0479605. In pharmacokinetic studies, intraperitoneal administration of MPI-0479605 in Swiss Webster mice resulted in plasma concentrations high enough to support *in vivo* anti-tumor studies. Finally, in HCT-116 tumor-bearing mice, daily treatment with 30 mg/kg MPI-0479605 resulted in a 49% tumor growth inhibition, whereas treatment with 150 mg/kg (q4d x 21 days) caused a 75% tumor growth inhibition relative to vehicle-treated animals. The latter effect was comparable to treatment with 5-fluorouracil (100 mg/kg, weekly). Animals dosed with MPI-0479605 had less than a 15% reduction in body weight over the course of the study.

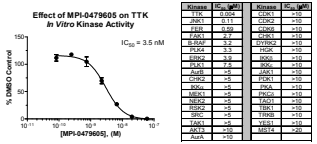
Conclusions: The small molecule MPI-0479605 potently inhibits TTK activity *in vitro* and demonstrates anti-tumor activity in a xenograft model.

Background

TTK is a dual specificity kinase required for mitosis. It facilitates the proper amphitelic attachment of chromosomes to the mitotic spindle (1). Furthermore, its kinase activity is required for maintaining the spindle assembly checkpoint to prevent premature onset of anaphase until all chromosomes are properly attached (2, 3). This preserves the integrity of the genome during cell division. Inactivation of TTK results in chromosome segregation defects, aneuploidy and cell death.



Activity and Selectivity

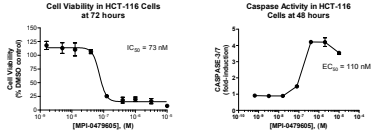


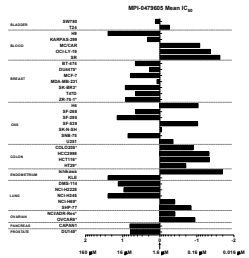
Kinase	IC_{50} (μM)	Kinase	IC_{50} (μM)
TTK	0.004	CDK1	>10
JNK1	0.11	CDK2	>10
FER	0.59	CDK4	>10
FAK1	2.7	CDK5	>10
B-RAF	3.2	DYRK2	>10
PLK4	3.3	HGK	>10
ERK2	3.8	IKK2	>10
PLK1	7.2	IKKa	>10
AurB	>5	JAK1	>10
CHK2	>5	PDK1	>10
IKKa	>5	PKA	>10
MEK1	>5	PKCt	>10
NEK2	>5	TAK1	>10
RSK2	>5	TBK1	>10
SRC	>5	TYRO	>10
TAK1	>5	YES1	>10
AKT1	>10	MET1	>20
AurA	>10		

Inhibition of enzymatic activity was determined with a ^{33}P filter plate assay. For each enzyme reaction, ATP was used at a concentration equivalent to 2-fold the experimentally determined K_m for ATP.

MPI-0479605 demonstrates potent activity towards TTK and is selective against 34 other kinases tested.

Cytotoxicity Profile



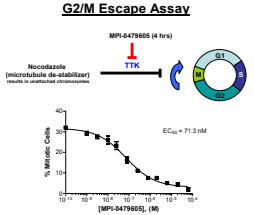


Viability was determined with either the WST1 or Cell Titer Glo assay. Caspase-3/7 activity was determined with the Caspase-Glo 3/7 assay. Data are normalized to DMSO treated controls. In the lower graph, data are represented as the log deviation from the mean IC_{50} for all cell lines tested.

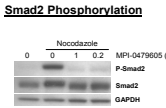
MPI-0479605 demonstrates cytotoxic activity against tumor cell lines derived from various tissues, including colon.

Mechanism-Based Cellular Assays

G2/M Escape Assay



Smad2 Phosphorylation

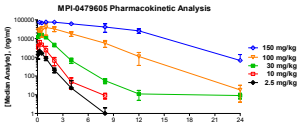


HeLa cells were treated with 250 ng/ml nocodazole for 17 hours to induce unattached chromosomes and activation of the spindle checkpoint. In the G2/M escape assay, MPI-0479605 was added for an additional 4 hours and cells were fixed and stained with anti-phospho-histone H3 antibody and Hoechst dye. The percentage of cells in mitosis for each sample was determined. Data were normalized to the percentage of mitotic cells treated with nocodazole alone.

In the Smad2 phosphorylation assay, MPI-0479605 was added to nocodazole - treated HeLa cells for an additional 2 hours and cell lysates were analyzed by Western blot with antibodies for phospho-Smad2 (Ser245/Ser250/Ser255), Smad2 and GAPDH.

MPI-0479605 promotes the transition from metaphase to anaphase in the presence of unattached chromosomes and potently inhibits Smad2 phosphorylation.

Pharmacokinetic Characterization



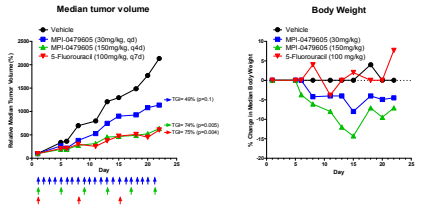
Dose (mg/kg)	$t_{1/2}$ (hr)	Cmax (ng/mL)	AUC (0-last) (hr*ng/mL)
2.5	0.5	2002	2063
10	1	6405	6457
30	6.1	19229	30068
100	1.9	36418	170366
150	2.1	39509	196371

Swiss Webster mice were injected IP with a single dose of MPI-0479605 at the indicated concentrations. Plasma concentrations of MPI-0479605 were determined from serum samples taken at the various time points.

IP administration of MPI-0479605 gives rise to plasma concentrations high enough to support *in vivo* anti-tumor studies.

Animal Model

HCT-116 Colon Cancer Xenograft



Nude mice with a median tumor volume of ~100 mm^3 were dosed IP with MPI-0479605 or 5-fluorouracil as indicated. Tumor growth inhibition (TGI) for each treatment was calculated at day 22. One animal died at day 11 in the 150 mg/kg MPI-0479605 cohort and one died at day 15 in the 30 mg/kg MPI-0479605 cohort.

MPI-0479605 induces significant tumor growth inhibition in the HCT-116 colorectal xenograft model.

Summary and Conclusions

- MPI-0479695 potently and selectively inhibits TTK activity and induces apoptosis.
- Inhibition of TTK activity disrupts the spindle assembly checkpoint and impairs Smad2 phosphorylation.
- MPI-0479605 exhibits good pharmacokinetic properties and demonstrates anti-tumor activity in a colon cancer xenograft model.

These data support continued effort to identify TTK inhibitors for clinical development.

References

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