Unlocking the Potential of OPM-383: A Novel LRRK2 Inhibitor in Cancer Therapy

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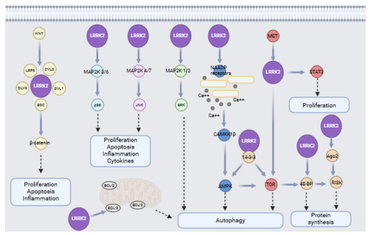
Introduction

Leucine-rich repeat kinase 2 (LRRK2) plays a pivotal role in regulating various cellular processes, such as cell proliferation, survival, and inflammation. LRRK2 exhibits dual functionality as a serine-threonine kinase and as a GTPase. It is involved in the modulation of multiple signaling pathways, including WNT, MAPK, NF-kB and mTOR. Germline mutations in LRRK2 are associated with an increased risk of cancer, particularly hormone-related and colorectal cancers. LRRK2 also promotes tumor cell growth and survival in papillary renal and thyroid carcinomas, DLBCL and cholangiocarcinoma cells.

OPM has designed and developed a novel oral LRRK2 inhibitor, OPM-383, using its proprietary Nanocyclix® technology. In this study, we have evaluated the pharmacokinetic properties, efficacy and tolerability of OPM-383 in a colon carcinoma model and in a panel of patient-derived organoids



Multiple signalling pathways have been associated with LRRK2 function in physiology and/or disease.

Adapted from Wallings R, et al. FEBS J. 2015. Created with BioRender.com

Materials & Methods

Cellular LRRK2 kinase activity was measured using LanthaScreen technology from Invitrogen. SH-SY5Y neuroblastoma cells are transfected with hG2019S or hWt LRRK2. LRRK2 pS935/total LRRK2 ratios were measured in mouse fibroblast 3T3 cell line to evaluate LRRK2 kinase inhibition. Cellular IC50 values (nM) are reported for OPM-383. A radiometric protein kinase assay (PanQinase® Activity Assay) was used for measuring the kinase activity of a selected protein kinases panel. OPM-383 was dissolved in the appropriate matrix with 1% DMSO. The seven main cytochrome P450 isoforms (CYP1A, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) were investigated in the Cytochrome P450 Inhibition assay. OPM-383 was dissolved in 1% tween 80 and 1% HPMC in water and administered by oral route. Rodents were sacrified at different times after administration and blood and tissues were collected. OPM-383 was quantified using LC/MS-MS method. OPM-383 (5 µM) protein binding in brain and plasma was analyzed after 4h of incubation using UPLVC/MS-MS. In vitro metabolism, permeability and protein binding were evaluated at Cyprotex, UK. hERG studies were conducted at Cerep; France.

OPM-383 was dissolved in 1% tween 80, and 1% HPMC in water and administered by oral route into CD1 at 50 mg/kg. Ninety minutes after administration, mice were sacrified and blood, brain and kidney were rapidly dissected and snap-frozen in liquid nitrogen. For immunoblot procedures, antibodies against pS935 and total LRRK2 were used. Western blot detection and quantification were used and LRRK2 pS935/total LRRK2 ratios were calculated to compare LRRK2 kinase inhibitor-dosed groups respect to vehicle group.

MC-38 cells were inoculated into C57BL/6 mice. When tumor masses reached 75 mm³, mice were randomized to receive OPM-383 (50 and 100 mg/kg, orally, bidaily), anti-PD1 antibody (10 mg/kg, ip, twice weekly), or their combination. Treatments with OPM-383 were administered by oral gavage (PO) via a gastric tube. The administration volume was 10 mL/kg adjusted to the most recent individual body weight. Anti-PD-1 treatment was injected into the peritoneal cavity (IP). Animals were treated for 35 days.

OPM-383 was evaluated in patient-derived organoids using the SEngine-Paris® platform. The cells were treated on the first day using acoustic liquid-handling robots, with different concentrations ranging from 0.32 to 10 µM. On the sixth day, cell viability in each well is determined as a percentage relative to vehicle-treated wells. To assess drug sensitivities, the AUC data from drug response curves were subjected to hierarchical clustering. Thus, SEngine determined a threshold value (SPM) to define the molecule's activity in organoids. If SPM > 9, organoids are considered sensitive to the drug, while SPM < 9 indicates resistance.

Results

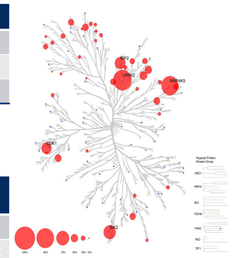
OPM-383 possess an ideal balance of LRRK2 cellular potency, narrow kinase selectivity, metabolic stability, and brain penetration properties

	Biochemical Assay IC50 (nM)	Cellular Lanthascreen IC50 (nM)		pLRRK2 in vivo CD1 mice, 50 mpk PO, % inh at 90 min			Tissue concentrations		S50 at 150 nM	hERG
	LRRK2 WT	LRRK2-WT	LRRK2-G2019S	Brain	Kidney	PBMC	Brain (ng/g)	Plasma (ng/mL)	%	IC50 (µM)
OPM-383	5	42	33	56	69	73	5302	43441	5.2	6.9

Overview of OPM-383 inhibitor properties in a panel of biochemical and functional cell-based assays

	Microsomal stability				Cyp inhibition at 1µM					
	Clint (uL/min/mg)		Half Life (min)		1A2	2D6	3A4BFC	3A4BQ	2C9	2C19
	Human	Mouse	Human	Mouse						
OPM-383	66	38.5	21	36	24	0	52*	32	7	6

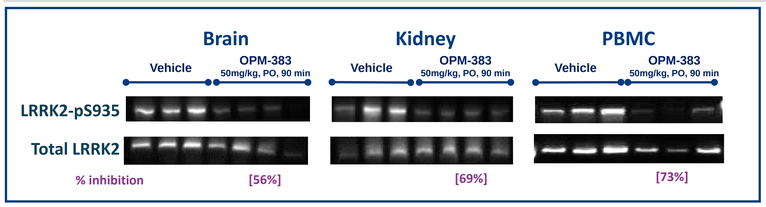
OPM-383 showed no significant inhibition of CYPs 1A2, 2D6, 3A4BQ, 2C19, and 2C9. Only moderate inhibitory activity was observed on CYP3A4BFC



	Tissue Protein Binding (Brain) (Recovery %)			Plasma Protein Binding (Recovery %)		
	Rat	Mouse	Human	Rat	Mouse	Human
OPM-383	99.41 (80)	99.35 (61)	/	99.94 (73)	99.30 (79)	99.09 (105)

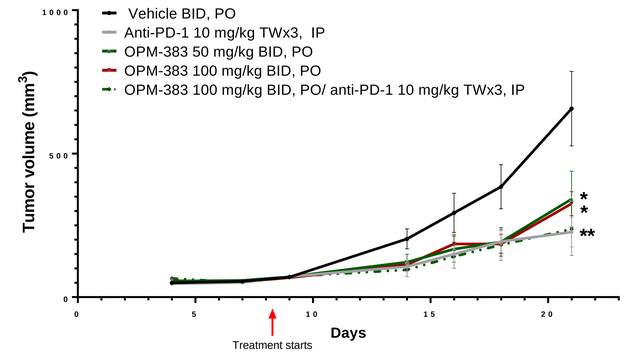
OPM-383 displayed high protein binding

Modulation of LRRK2 phosphorylation in mouse tissues



OPM-383 exhibits robust potency as an inhibitor of LRRK2 phosphorylation (Ser935) in PBMC, brain, and kidney tissues

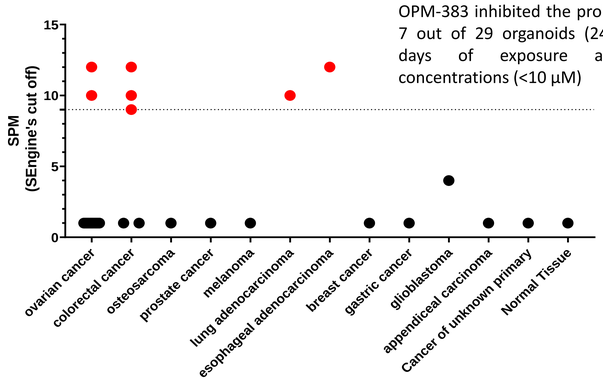
OPM-383 significantly inhibits tumor growth in mice bearing colon carcinoma MC-38 tumors



One-way ANOVA test followed by Dunnett's multiple comparisons test (* p<0.05; ** p<0.01 respect to control group)

OPM-383 inhibits the *in vitro* proliferation of cancer patient-derived organoids

OPM-383 inhibited the proliferation of 7 out of 29 organoids (24%) after 6 days of exposure at various concentrations (<10 µM)



Conclusions

This study presents the identification of a novel LRRK2 inhibitor, OPM-383, demonstrating its potency, selectivity, and antitumor efficacy in a colon carcinoma model and in a panel of tumor-patient-derived organoids.

As the lead compound of this series, OPM-383 displays good permeability, metabolic stability and capability to cross the blood-brain barrier with favorable drug-like properties.

These findings highlight OPM-383 as a promising lead scaffold, laying the foundation for the design and synthesis of a novel class of kinase inhibitors. With its potential applications in cancer therapy, OPM-383 emerges as an attractive candidate, paving the way for innovative advancements in the field.

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