Selective Targeting of Met-Kinase by RP1400 Attenuates Tumor Progression in Mouse Models of Gastric Cancer, Glioblastoma, and Hepatocellular Carcinoma

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Background: *c-Met* is a proto-oncogene that encodes the protein Met with intrinsic tyrosine kinase activity. Aberrant Met kinase activity triggers a series of unwarranted phosphorylation events and signalling processes that ultimately lead to the development of cancer. Alteration of the Met kinase signalling cascade represents an attractive approach aimed at blocking invasion and metastasis of cancer cells. Herein, we describe the biological activity and pharmacokinetic properties of RP1400, a novel, selective, and potent Met kinase inhibitor with scope to be developed as a clinical candidate for cancers mediated by dysregulated Met kinase activity.

Methods: Met Kinase activity of RP1400 was determined using an HTRF® KinEASE assay kit (Cisbio, Bedford, MA) with modifications. Met-dependent anti-proliferative effect was determined in a host of Met amplified cell lines representative of various cancers. Inhibition of constitutive Met kinase phosphorylation in MKN-45 and NCI-H441 cells was measured in an ELISA assay. Subsequently, effect of the compound on Akt and Stat-5a phosphorylation, downstream markers in the Met signalling cascade, was determined. *In vivo* efficacy of RP1400 was evaluated in subcutaneous MKN-45 (gastric cancer), U87MG (glioblastoma), and MHCC97H (hepatocellular carcinoma) xenografts using SCID or nude mice. Pharmacokinetic behaviour of the compound in plasma after single dose oral administration or IV injection was determined in Balb/c mice.

Results: RP1400 demonstrated remarkable potency against the purified Met kinase enzyme (***8.9 nM***) with ***>50-fold*** selectivity against other kinases in a 451-kinase panel. Inhibition of Met kinase activity was accompanied by a significant reduction in constitutive Met phosphorylation in MKN-45 (***28.6 nM***) and NCI-H441 (***1.8 nM***) cells. As a consequence, Akt and Stat-5a phosphorylation were inhibited half-maximally in MKN-45 cells at ***16.2 nM*** and ***11.2 nM*** respectively. RP1400 caused a significant inhibition in proliferation of Met amplified cell lines including MKN-45, EBC-1, SNU-5 and MHCC97H with IC_{50} values ranging from ***3-80 nM***. Compounded with a favourable pharmacokinetic profile, *in vitro* potency of RP1400 translated into excellent in *vivo* efficacy with ***>80%*** reduction in tumor growth noticed in MKN-45, U87MG, and MHCC97H xenografts at 100 mg/kg/BID/PO dose.

Conclusions: Our findings demonstrate the potency of RP1400, a novel and selective small-molecule inhibitor of Met kinase with efficacy values comparable or superior to existing Met kinase inhibitors in development. On lines with selective inhibitors, the compound displayed anti-proliferative effect only in cell types harbouring amplification of the Met kinase gene. RP1400 is currently undergoing extensive toxicological evaluation with clinical trials anticipated in H1 2014.

Introduction

Receptor tyrosine kinases (RTK) represent a class of high affinity cell surface receptors that play a critical role in the development and progression of several types of cancers. Among the several RTK currently under evaluation as druggable targets for cancer, Mesenchymal epithelial transition factor (c-Met), stands out due to its immense potential in regulating downstream events including cell proliferation, metastases, survival, and apoptosis. *c-Met* is a proto-oncogene that encodes the protein Met with intrinsic tyrosine kinase activity. Aberrant Met kinase activity triggers a series of unwarranted phosphorylation events and signalling processes that ultimately lead to the development of cancer. Abnormal activation of MET due to gain-of-function mutations or excessive stimulation by hepatocyte growth factor, an endogenous ligand of MET, is implicated in the progression of various tumours. Met being on the cell surface regulates several key oncogenic signalling pathways including ras/raf/MEK/ERK/PI3K thus controlling downstream events such as proliferation, metastases, motility, and cell death.

The current study describes the preclinical profile of RP1400, a potent, novel, and selective inhibitor of Met kinase with immense potential in the treatment of solid cancers.

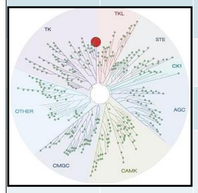
Enzyme Activity		Cellular		Apoptosis
Assay	IC_{50} (nM)	Assay	EC_{50} (nM)	Assay
Met Kinase (WT)	8.9	MKN-45 proliferation	29.4	Dose-dependent induction in MKN-45 cells
Met Kinase M1250T	100.0	pMet in MKN-45 cells	28.6	
Met Kinase F1200I	960.0	pMet in NCI-H441 cells	1.8	
		pAKT in MKN-45 cells	16.2	
		pAKT in NCI-H441 cells	4.2	
		pSTAT-5a in MKN-45 cells	11.2	
		HGF-induced pMet in MDA-MB-231 cells	9.4	

Table 1. *In Vitro* profile of RP1400

	c-Met			RP1400
Cell line	Gene Amplification	Protein Overexpression	Constitutive Activation	GI_{50} (μM)
SNU-16	-	Low	-	43.9
MKN-45	+	+	+	0.029
EBC-1	+	+	+	0.003
SNU-5	+	+	+	0.020
H441	-	+	+	>50

Table 2. Met expression dependent anti-proliferative activity

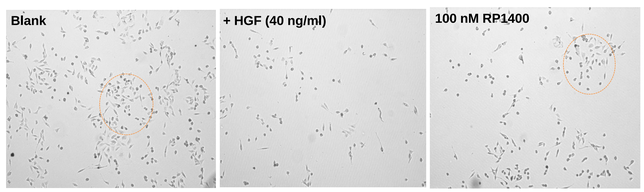


Fig. 1. Inhibition of HGF stimulated scatter in MKN-45 cells

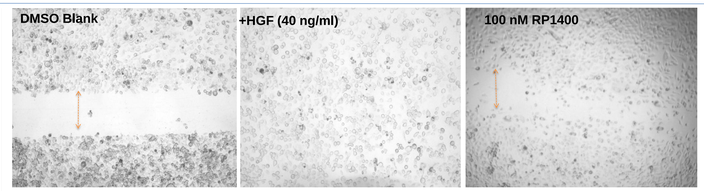


Fig. 2. Scratch wound assay in PC3 cells

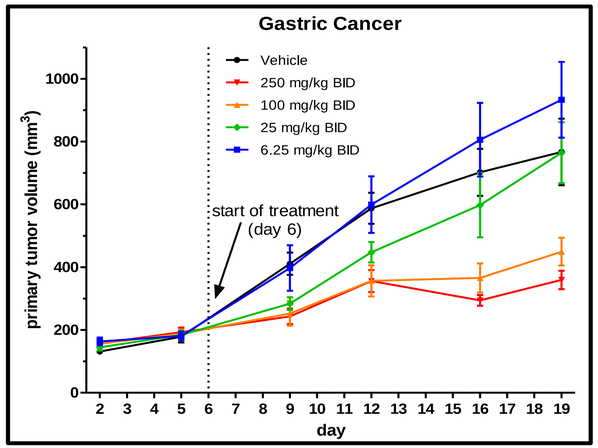


Fig. 3. Inhibition of tumor growth in female NMRI nude mouse subcutaneous MKN-45 xenograft model (*Study conducted at ProQinase, Germany*)

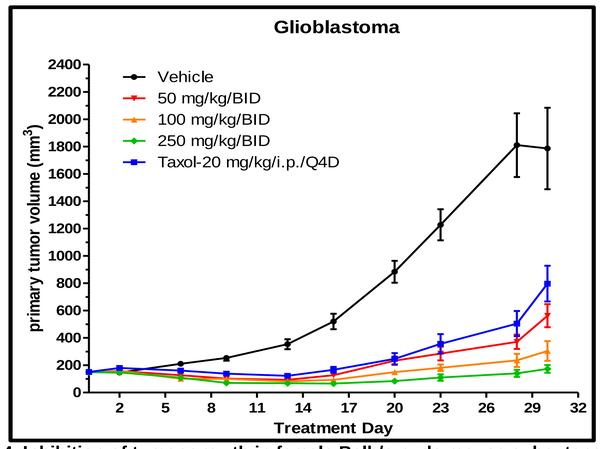


Fig. 4. Inhibition of tumor growth in female Balb/c nude mouse subcutaneous U87-MG xenograft model (*Study conducted at Crownbio, China*)

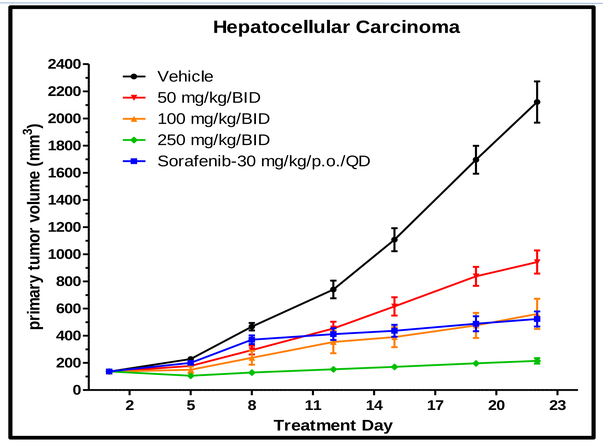


Fig. 5. Inhibition of tumor growth in female Balb/c nude mouse subcutaneous MHCC97H xenograft model (*Study conducted at Crownbio, China*)

		RP1400		
Parameter	Units	Rat	Mice	Dog
Dose	mg/kg	30	50	50
N		4M	3M*	1
C_{max}	μM	18.41	20.48	13.95
AUC_{0-t}	μM.hr	24.04	25.25	17.91
AUC_{0-inf}	μM.hr	24.21	25.27	17.99
T_{max}	hr	0.50	0.5	0.25
$t_{1/2}$	hr	3.10	0.99	1.35
V_d	L/kg	0.92	1.95	2.45
CL_z	mL/min/kg	25.59	61.68	35.62
f	%	56.02	85.76	35.67

Table 3. Single dose oral pharmacokinetic profile of RP1400

Summary

- RP1400 identified as lead based on *in vitro* potency and cellular activity.
- Additionally, RP1400 demonstrated significant inhibition of tumor growth in models representative of gastric carcer, glioblastoma, and hepatocellular carcinoma.

Current status and future direction

Toxicological evaluation of RP1400 with Phase-1 clinical trials planned in 2014

Acknowledgements

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