Inhibition of PI3Kδ kinase by a selective, small molecule inhibitor suppresses B-cell proliferation and leukemic cell growth

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Abstract

Background: Phosphoinositide-3 kinase (PI3K) belongs to a class of intracellular lipid kinases that phosphorylate the 3 position hydroxyl group of the inositol ring of phosphotidylinositol. The PI3K pathway is frequently activated in human cancers and thus represents an attractive target for small molecule inhibitors. Pan-PI3K inhibitors currently in development have been associated with adverse side-effects such as insulin resistance, thus necessitating the need to develop isoform specific inhibitors of PI3K. Herein, we describe the biological and pharmacokinetic properties of RP5264, a small molecule PI3Kδ inhibitors with scope to be further developed as a clinical candidate for hematological malignancies mediated by B-cells.

Methods: Activity of RP5264 on individual PI3K isoforms was determined by a Homogenous Time Resolved Fluorescence assay (Millipore, Billerica, MA) with modifications. Cell based selectivity assays against α, β,or γ isoforms was assessed by testing the effect of the compound on PDGF, LPA, or c5a induced Akt phosphorylation in NIH-3T3 or RAW cells. Similarly, inhibition of cellular PI3Kδ activity was determined in an IgM induced human B-cell proliferation as well as LPS-induced CD19 assays. Ability to arrest cell growth and induce apoptosis was also tested. Viability assays was conducted to determine the growth inhibitory effect of the compounds in leukemic cells. Pharmacokinetic behaviour of compounds in plasma after single dose oral administration was determined in female Balb/c mice.

Results: RP5264 inhibited PI3Kδ activity in enzyme and cell based assays with IC_{50} and EC_{50} values of 22.2 & 24.3 nM respectively. The compound displayed a high degree of selectivity over the alpha (>1000 fold), beta (>30-50 fold), and gamma (>15-50 fold) isoforms. Additionally, the compound caused a half-maximal inhibition of human whole blood CD19 cell proliferation between 100-300 nM. Treatment of PBMC with RP5264 resulted initially in a G2/M arrest followed by subsequent increase in the number of Sub G0 cells. Viability assays demonstrated that the compound caused a significant inhibition in growth as well as Akt phosphorylation of immortalized and primary leukemic cells. Further, the compound exhibited good oral absorption with favourable pharmacokinetic properties in rodents.

Conclusions: Results demonstrate the PI3K delta selective nature of RP5264 along with an ability to suppress proliferation and Akt phosphorylation in cancer cells. *In vitro* selectivity and potency data indicate the therapeutic potential of the compound in hematological cancers without the deleterious effects commonly associated with the Pan PI3K inhibitors. RP5264 is poised to enter clinical development in 2012.

Introduction

Phosphoinositide-3 kinase (PI3K) belongs to a class of intracellular lipid kinases that phosphorylate the 3 position hydroxyl group of the inositol ring of phosphotidylinositol. While α and β isoforms are ubiquitous in their distribution, expression of δ and γ is restricted to cells of the hematopoietic system. Together with AKT and mTOR, PI3K regulates the hallmarks of cancer that include cell growth, proliferation, differentiation, motility, and survival. Inhibition of α and β isoforms of PI3K have been associated with an increased incidence of insulin resistance. The adverse affects observed with α and β or pan-PI3K inhibitors thus necessitate the need to develop δ selective or dual δ and γ inhibitors that would specifically target only a particular lineage of cells without affecting other organs. The current study describes the pharmacological and pharmacokinetic properties of a novel, potent, and isoform selective PI3Kδ kinase inhibitor RP5264 with immense potential in the treatment of haematological malignancies

	IC_{50}/EC_{50} (nM)	Fold-Selectivity		
	PI3Kδ	PI3Kα	PI3Kβ	PI3Kγ
Enzyme	22.23	>10000	>50	>48
Cell-based	24.27	>10000	>34	>17

Table 1. Enzyme assay for inhibition of PI3Kδ and fold-selectivity over other isoforms. Enzyme activity was determined using an PI3K HTRF Assay Kit (Millipore, Billerica, MA) with modifications.

Cell based specificity against PI3K isoforms for select compounds. Compound specificity towards PI3Kδ was determined in an IgM-induced B cell proliferation assay. For selectivity against PI3K α, β, or γ isoforms, NIH-3T3 or RAW macrophages were seeded in a 6-well tissue culture plate and incubated with compounds at the desired concentrations followed by 20 ng/ml PDGF, 5 µM LPA, or 50 ng/ml c5a. Cells were lysed and AKT phosphorylation was determined by Western Blotting. Intensity of the bands was determined using ImageJ 1.42q (NIH, USA) and normalized to Actin (loading control).

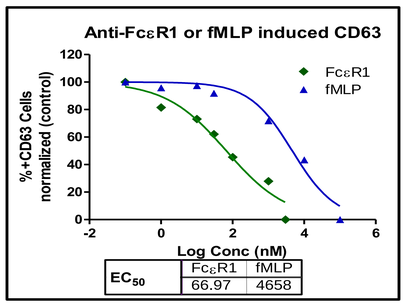


Fig. 1. Inhibition of anti-FceR1 (n=6) or fMLP (n=3) induced CD63 expression in Human Whole Blood basophils. Induction of CD63 surface expression on human whole blood basophils was measured using a Flow2CAST kit (Buhlmann Laboratories, Switzerland). Cells were stained with FITC or PE tagged CD63 and CCR3. CD63 positive cells were determined using flow cytometry and normalized to vehicle control.

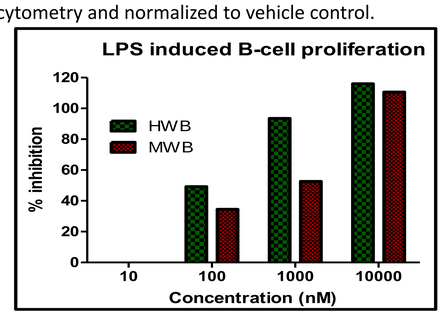


Fig. 2. Inhibition of CD19+ or CD45R+ cell proliferation in human (H) or mouse (M) whole blood (WB). Positive cells were gated from CD45+ cell and estimated by Flow Cytometry

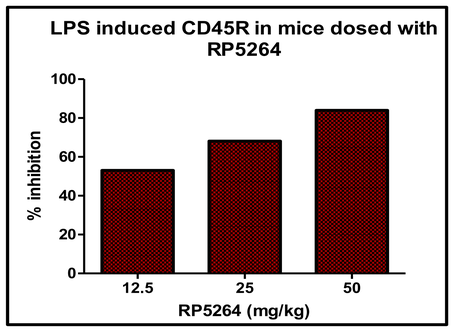
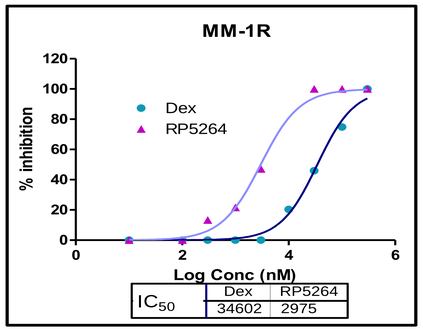


Fig. 3. Inhibition of LPS induced CD45R in Mouse Whole Blood (Dosing followed by *ex vivo* induction). Balb/c mice were dosed with RP5264 rally. Blood was collected at 30 min, diluted with RPMI medium, induced with LPS, and incubated for 72 h. Samples were analyzed by flow cytometry for CD45R expression.



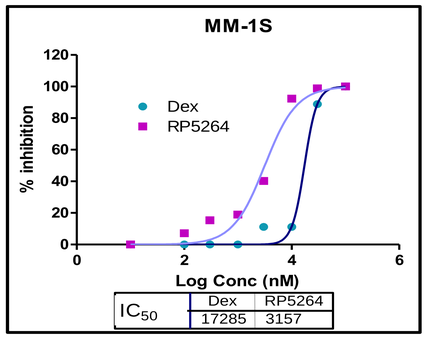


Fig. 4. Inhibition of Multiple Myeloma Cell growth. Multiple Myeloma resistant (MM-1R) or sensitive (MM-1S) cells were incubated with desired concentrations of RP5264. Growth was assessed after 96 h by a MTT assay. Results demonstrate a therapeutic potential for RP5264 in steroid resistant multiple myeloma

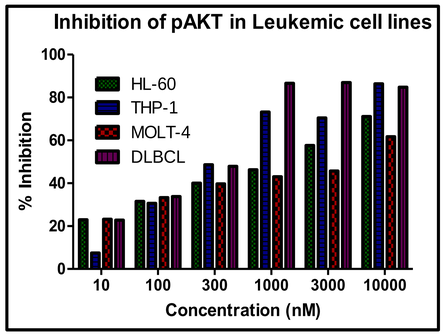
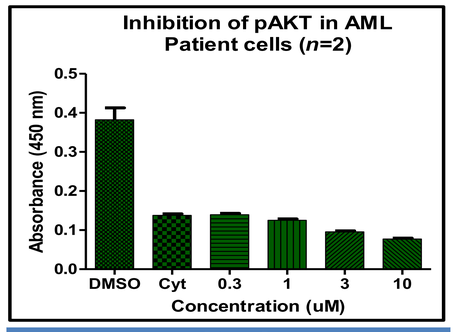


Fig 5. Inhibition of pAKT in leukemic cell lines with constitutive expression. Cells were treated with compound, lysed, and pAKT determined by Western blotting. Intensity of the bands was determined using ImageJ 1.42q (NIH, USA) and normalized to Actin (loading control).



Gender	Age	Type	Primary Bone Marrow Morphology	Therapy	Prognosis
M	48	AML-M4	hyperplasia grade II, Myeloblast 32% , primitive naïve monocyte 23%.	NA	NA
M	76	AML-M2a	extremely active hyperplasia, primitive naïve cell 21.5% with normal cell .	no therapy	poor

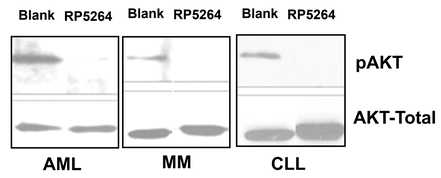
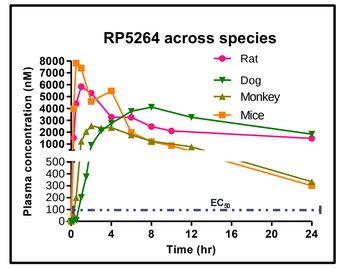


Fig. 6. Inhibition of pAKT in primary patient leukemic cells. (TOP) Cells were isolated from bone marrow of patients diagnosed with acute myeloid leukemia with extreme active hyperplasia of the bone marrow. Leukemic cells were incubated with compound for 48 h and pAKT determined by ELISA. Cytaribine (cyt) was used as a positive control.
BOTTOM: pAKT in patient cells treated with 3 µM RP5264 and analyzed by Western Blotting.

Nonclinical Pharmacokinetics – RP5264



Parameter	Units	Mice#	Rat#	Dog#	Monkey#
Dose	mg/kg	20	20	30	50
C_{max}	µM	7.81	6.08	4.21	2.68
#Clz	mL/min /kg	6.27	33.36	2.83	12.47
f	%	46.78	100.00	49.65	30.29

Table 2. Pharmacokinetic Profile of RP5264
clearance calculated based on i.v. data

Study	RP5264
hERG channel assay	Non significant inhibition (at 10 µM)
Acute oral (gavage) toxicity study in Wistar Rats	MTD: >2000 mg/kg bw LD_{50}: >2000 mg/kg bw
Acute oral (gavage) toxicity study in Balb/c mice	MTD: ≥ 1000 mg/kg bw LD_{50}: >2000 mg/kg bw
14-days repeated dose toxicity study in Wistar Rats	MTD: >500 mg/kg bw/day (higher doses not studied)

Table 3. Safety Profile of RP5264 in rodents

Summary

- RP5264 is a potent and selective inhibitor of PI3Kδ with a translational reduction in proliferation of antigen induced B-cells manifested by a reduction in CD19+ or CD45R+ cells.
- Reduction in pAKT, an effective biomarker in leukemic cell lines as well as in cells derived from leukemic patients
- In addition RP5264 exhibited desirable pharmaceutical/ADME/PK properties along with an excellent safety profile

Current status and future direction

- Phase I enabling studies in progress. To be completed by June 2012
- Expected to file Phase I in B cell lymphoma patients in Q3, 2012

For further information please contact at info@rhizen.com