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

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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. Methods: Activity of RP5264 on individual PI3K isoforms was determined by a homogeneous Time Resolved Fluorescence assay (Billerica, MA) with modifications. Cell based selectivity assays against β, γ, δ isoforms was assessed by testing the effect of the compound on PDGF, LPA, or Fcα induced Akt phosphorylation in NIH-3T3 or RAW cells. Similarly, inhibition of cell viability PI3K activity was determined in an IgM induced human B-cell proliferation as well as LPS induced COX-2 assays. Ability to arrest cell growth and induce apoptosis was also tested. Viability assay was conducted to determine the growth inhibitory effect of the compounds in leukemic cells. Pharmacokinetic behavior of compounds was assessed after single dose oral administration was determined in female Balb/c mice.

Results: RP5264 inhibited PI3K activity in enzyme and cell based assays with IC50 and IC30 values of 21.2 & 24.3 nM respectively. The compound displayed a high degree of selectivity over the α (1000 fold), beta (150-30 fold), and gamma (15-50 fold) isoforms. Additionally, the compound caused a half-maximal inhibition of human whole blood COX-2 cell proliferation between 10-300 nM. Treatment of PBMCs with RP5264 resulted initially in a G2M 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 δteta 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 2014. 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 phosphatidylinositol. 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 hallmark of a cancer that include cell survival, 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 a β and pan-PI3K inhibitors thereby necessitate the need to develop β 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 PI3K δ kinase inhibitor RP5264 with immense potential in the treatment of haematological malignancies.