Antitumor activity of RP4010, a novel small-molecule inhibitor of the calcium release-activated calcium (CRAC) channel pathway

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Introduction
Store operated calcium channels namely calcium release activated calcium (CRAC) channels contribute to calcium influx in non-excitatory cells. Elevation of cytosolic calcium through activation of CRAC channels mediates an array of cellular responses including metabolism and gene expression, cell growth, and proliferation. Aberrant CRAC activity has been linked to various autoimmune disorders and certain cancers via the NFAT pathway. Herein, we describe the preclinical profile of RP4010, a novel small molecule inhibitor of the CRAC channel pathway.

Targeted Activity

Inhibition of thapsigargin induced calcium influx in Jurkat cells

RP4010 (nM) 10 100 1000 10000 1 % Inhibition

Figure 1. Thapsigargin-induced calcium influx in Jurkat cells. Flu-8 loaded cells were treated with desired concentration of inhibitors for 10 min. Release of endoplastic reticulum calcium was induced by addition of 1 μM thapsigargin and calcium influx into cells was determined fluorometrically.

A. Induction of Caspase-3 activity in Lung Cancer Cell lines

B. Effect of migration in A549 cells: A scratch was made to a serum-starved monolayer of A549 cells followed by washing and incubation with desired concentrations of RP4010 in media with 10% FBS for 10 h. The distance between the two edges of the wound was measured and its inhibition was calculated with respect to control. RP4010 caused a dose-dependent reduction in FBS induced migration of A549 cells thereby implicating a role for this compound in the attenuation of metastasis.

Figure 2. A. Induction of Caspase-3 activity in NSCLC cell lines; induction of Caspase-3 by RP4010 was measured fluorometrically. Cells were incubated with desired concentrations of the compound for 4 h. An equal number of cells per well (0.3 × 10^5 cells) were used. Increase in apoptosis was manifested by an elevation in caspase-3 levels as determined using a Caspase-3 kit from Millipore. A dose-dependent increase in caspase-3 was observed with RP4010.

Figure 3. Anti-proliferative effect of RP4010 across cell lines. Cell lines were plated in media at a pre-determined density in 96-well plates and incubated with RP4010. After 72 h, cell viability was determined by MTT assay and IC50 was calculated using Graphpad prism. Majority of the cell lines tested were sensitive to RP4010 with GI50 ranging between 0.3 – 3 μM. Interestingly, RP4010 did not effect growth of the normal lung line, WI-38 indicating specificity towards cancer cells.

Table 1. Apoptosis in cell lines treated with RP4010. An antibody to activated caspase-3 was used to label cells from early to late stage apoptosis. The concentration of test compound that caused a 2-fold induction in the caspase-3 signal is reported, indicating a significant apoptosis induction. (Eurofins OncolScan Panel).

SUMMARY & CONCLUSIONS
RP4010 is a potent inhibitor of CRAC channel function with subsequent inhibition of downstream NFAT activity.
- Demonstrated activity in several cell lines representative of solid tumors and hematological malignancies.
- Marked inhibition of oncogenic markers downstream with favourable pharmacokinetics in vivo.
- A Phase-1 trial (NCT03119467) in patients with relapsed or refractory Non-Hodgkin Lymphoma is currently ongoing in USA.

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