The Novel Calcium Release-Activated Calcium (CRAC) Channel Inhibitor RP4010 Exerts Potent Antitumor Effects in NOD/SCID/IL2Rg-/- Mice with Diffuse Large B Cell Lymphoma (DLBCL) Cell Line Xenografts

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Abstract
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BACKGROUND & AIM

- Calcium ions (Ca^{2+}) act as second messengers in cell signaling triggering gene transcription, secretion, cell proliferation, migration, and apoptosis.
- Store-operated Ca^{2+} entry (SOCE) is activated by Ca^{2+} release from the endoplasmic reticulum. Two genes are responsible for SOCE activity: Stromal interaction molecule 1 (STIM1), an ER Ca^{2+} sensor that detects store depletion and Orai1, the pore-forming subunit of Ca^{2+} release-activated Ca^{2+} (CRAC) channel.
- Emerging evidence points to the involvement of aberrant CRAC channel activity in human diseases including Diffuse Large B Cell Lymphoma (DLBCL)
- RP4010 is a potent inhibitor of CRAC channel activity (IC_{50}=60 nM) with demonstrated efficacy across a range of cancer cell lines *in vitro*.
- This study aimed at investigating the activity of RP4010 in preclinical DLBCL models.

REFERENCES

1. Parekh, A. B. (2010). Nat Rev Drug Discov, 9(5), 399–410.
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METHODS & RESULTS

RP4010 treatment induced:

- time and dose-dependent cell growth inhibition (down to 80%) in GCB-DLBCL (SUDHL-4, SUDHL-6, SUDHL-16, and SUDHL-10) and ABC-DLBCL (SUDHL-8, RCK-8, RIVA, and SUDHL-2) cell lines (**Fig. 1A**).
- significant levels of apoptosis in GCB-DLBCL cell lines and in two out of four ABC-DLBCL cell lines (SUDHL-8 and SUDHL-2), whereas modest or no effects were detected in the RCK-8 and RIVA ABC-DLBCL cell lines (**Fig. 1B**).
- caspase-dependent cell death (**Fig. 2**).
- no cytotoxic effects on the *in vitro* growth of hematopoietic colony-forming cells from healthy volunteers (**Fig. 3**).
- strong cell death which is highly correlated with Orai1 expression (**Fig. 4**).
- down-modulating MAPK and PI3K/Akt pathways (**Fig. 5**).
- dose-dependent TGI (range 50 to 80%) (**Figs. 6-7**).

Fig. 1 - Cell Proliferation and Apoptosis – WST and Annexin-V/PI staining

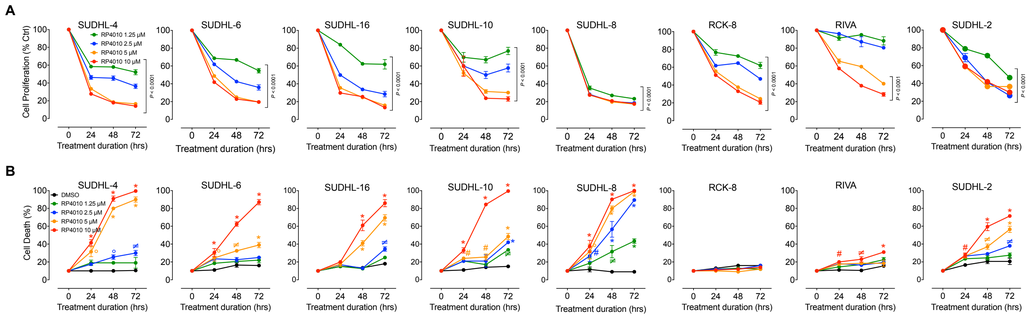


Fig. 2 - Caspase-dependent Cell Death

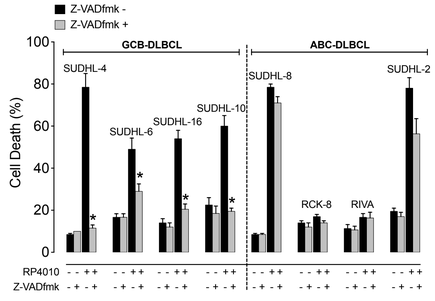


Fig. 3 - Hematopoietic Colony-forming Cells Apoptosis – Annexin-V/PI staining

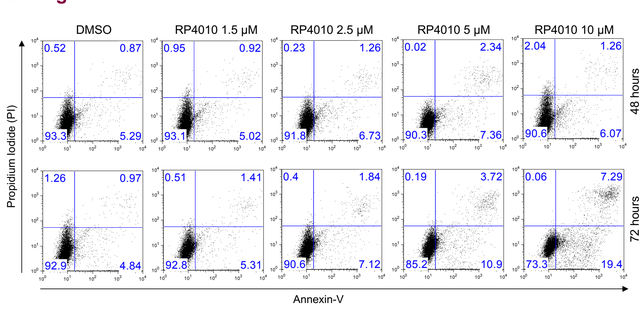


Fig. 4 – Orai1 Gene Expression Analysis

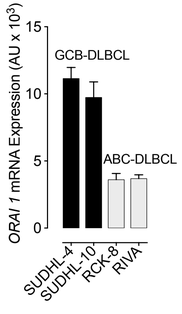


Fig. 5 - Targeting PI3K/Akt and MAPK pathways

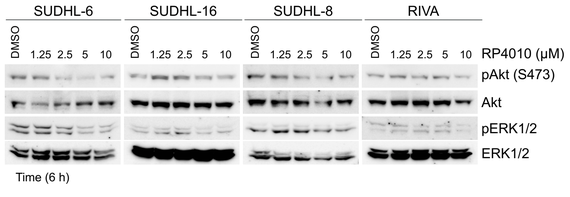


Fig. 6 - In Vivo RP4010 Treatment Schedule

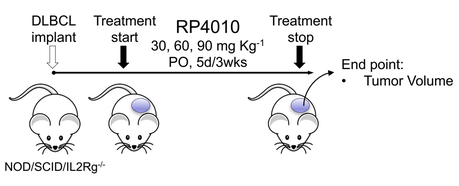
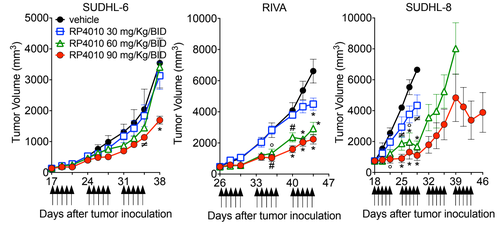


Fig. 7 - Tumor Growth Inibition (TGI)



CONCLUSIONS

In all DLBCL cell lines, treatment with RP4010 induced potent antitumor effects.

In vitro:
- Striking increase in caspase-dependent cell death, and marked reduction of cell proliferation.
- Down-regulation of MAPK and PI3K/Akt pathways.

In vivo:
- Inhibition of tumor volume.

A Phase 1/1b study in Relapsed/Refractory Non-Hodgkin Lymphoma (NHL) patients is currently ongoing in the US (ClinicalTrials.gov Identifier: NCT03119467). Our data warrant further clinical evaluation of RP4010 in DLBCL patients.

DISCLOSURES

S. Viswanadha: Employment – Incozen Therapeutics.

S. Vakkalanka: Equity & Employment – Rhizen Pharmaceuticals