Store-operated calcium signaling is an effective therapeutic target in Acute Myeloid Leukemia (AML).

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Background

- Acute myeloid leukemia (AML) is the most prevalent adult leukemia characterized by genetic or mutational disruption of myeloid differentiation, growth arrest, and apoptosis.
- Standard "7 + 3" chemotherapy consisting of cytarabine in combination with an anthracycline such as daunorubicin has been used for more than four decades. However this course only results in 10 year disease free survival in 15% of patients age <60 years and 2% among those > 60 years.
- The discovery of novel agents applicable to a broader patient population is a critical unmet need in this disease.
- Intracellular calcium is a common signaling molecule used in a variety of cellular processes, including those relevant to tumor cell growth such as proliferation and cell cycle progression.
- One of the major pathways which regulates calcium entry is store operated calcium entry (SOCE) through calcium release-activated Ca2+ (CRAC) channels, primarily via interaction of ORAI1 (expressed on the plasma membrane) and STIM1 (expressed on the endoplasmic reticulum).
- Leukemic cells in particular often rely on the influx of calcium through CRAC for proliferation, therefore targeting these channels are a promising therapeutic option.
- In the current study, we explore the preclinical use of RP4010, a novel inhibitor of ORAI1 developed by Rhizen Pharmaceuticals, in AML

RP4010 inhibits ORAI1 mediated SOCE

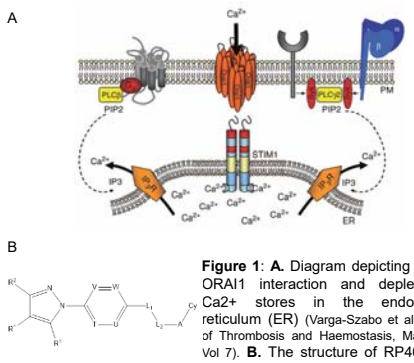

Figure 1: **A.** Diagram depicting STIM1-ORAI1 interaction and depletion of Ca2+ stores in the endoplasmic reticulum (ER) (Varga-Szabo et al, Journal of Thrombosis and Haemostasis, May 2009, Vol 7). **B.** The structure of RP4010 (as defined in PCT/IB2010/002539).

Results – *In Vitro* Studies

RP4010 inhibits proliferation of AML cell lines and primary AML cells

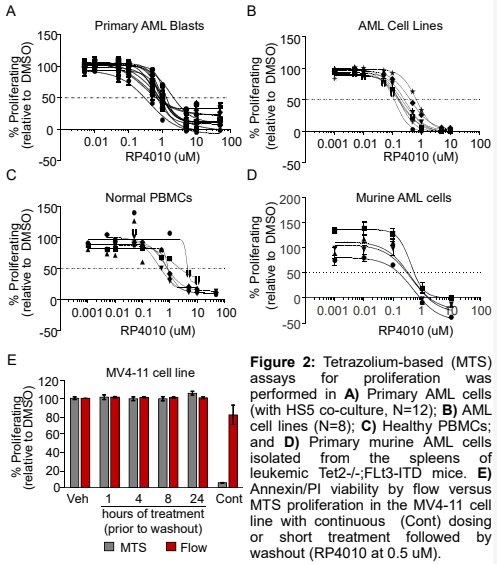

Figure 2: Tetrazolium-based (MTS) assays for proliferation was performed in **A)** Primary AML cells (with HS5 co-culture, N=12); **B)** AML cell lines (N=8); **C)** Healthy PBMCs; and **D)** Primary murine AML cells isolated from the spleens of leukemic Tet2-/-;Flt3-ITD mice. **E)** Annexin/PI viability by flow versus MTS proliferation in the MV4-11 cell line with continuous (Cont) dosing or short treatment followed by washout (RP4010 at 0.5 uM).

RP4010 has a variable effect on colony formation in primary human AML cells

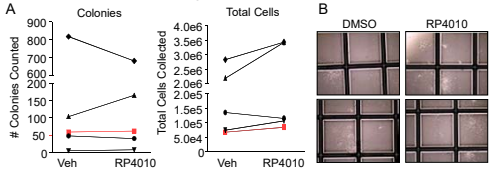

Figure 3: Leukemic cells from primary AML patients (N=5) treated with 1uM RP4010 or vehicle control were plated in methocult for colony forming assays (CFU). Results show the total number of colonies determined (left) and the total number of cells collected (right) at day 14. **B)** Representative images of the colonies formed in each condition. Images are from the sample indicated in red in Figure 3A.

Results – *In Vivo* Studies

In vivo drugging scheme

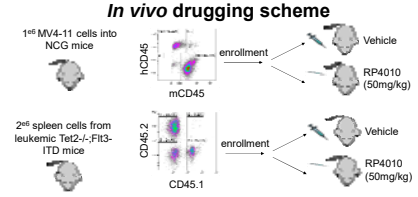

Figure 4: A) MV4-11 cells were engrafted via the tail vein into NCG mice (Charles River). Successful engraftment was determined in representative animals by flow for human CD45 in the bone marrow. Animals were dosed daily with 50 mg/kg RP4010 or vehicle until reaching early removal criteria (ERC). **B)** Spleen cells from CD45.2+ Tet2-/-;Flt-ITD were engrafted via the tail vein into CD45.1+ NCG mice. Animals were monitored weekly by examining the percent CD45.2+ cells via flow cytometry, and enrolled in two groups at 5 or 6 weeks post-engraftment (CD45.2+ ranging from 20%-70%). Animals were then dosed daily with 50 mg/kg RP4010 or vehicle until reaching endpoint (7 weeks post-treatment initiation or ERC).

RP4010 prolongs survival in the MV4-11 model

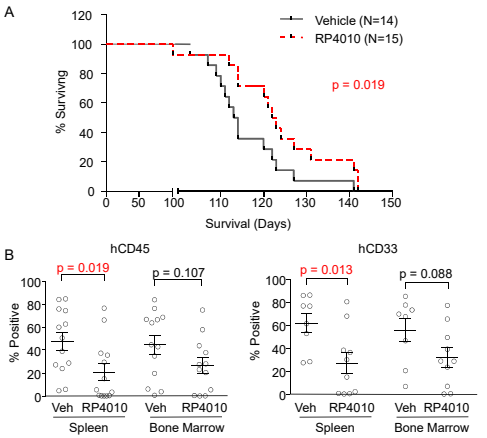

Figure 5: NCG mice were engrafted with MV4-11 cells as shown in in Figure 4A. Animals were treated daily with 50 mg/kg RP4010 (N=15) or vehicle control (Veh; N=14). Overall survival was determined **(A)** and spleen and bone marrow disease burden was determined by human CD45 and human CD33 upon euthanasia when reaching ERC **(B)**.

RP4010 inhibits *ex vivo* colony formation in the Tet2-/-;Flt3 adoptive transfer model

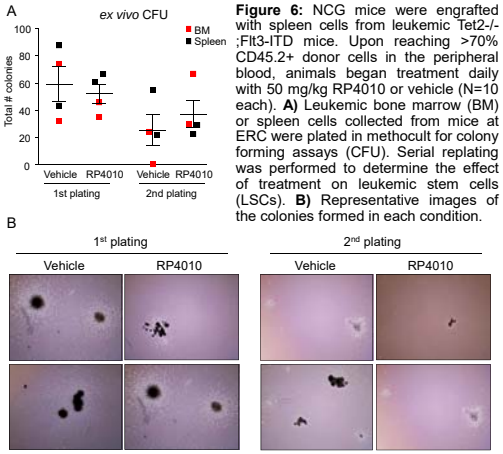

Figure 6: NCG mice were engrafted with spleen cells from leukemic Tet2-/-;Flt3-ITD mice. Upon reaching >70% CD45.2+ donor cells in the peripheral blood, animals began treatment daily with 50 mg/kg RP4010 or vehicle (N=10 each). **A)** Leukemic bone marrow (BM) or spleen cells collected from mice at ERC were plated in methocult for colony forming assays (CFU). Serial replating was performed to determine the effect of treatment on leukemic stem cells (LSCs). **B)** Representative images of the colonies formed in each condition.

Conclusions and Future Directions

- We provide here evidence that inhibition of ORAI1 with RP4010 is a promising therapeutic strategy in AML.
- RP4010 decreased cell proliferation in both AML cell lines and primary AML cells, and prolonged survival and decreased colony formation in AML mouse models.
- We are currently investigating the impact of RP4010 on AML cell migration, as knockdown of ORAI1 has previously been shown to inhibit transwell migration in AML cell lines.
- A Phase 1/1b study in R/R Non-Hodgkin Lymphoma (NHL) is currently underway (ClinicalTrials.gov: NCT03119467), and our data would support expansion to other hematological malignancies as well, particularly AML.

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