The Bruton’s Tyrosine Kinase (BTK) Inhibitor ARQ 531 Effectively Inhibits Wild Type and C481S Mutant BTK and Is Superior to Ibrutinib in a Mouse Model of Chronic Lymphocytic Leukemia

Sean Reiff, Rose Mantle, Lisa Smith, Samantha McWhorter, Virginia Goettl, Amy Johnson, Sudharshan Eathiraj, Giovanni Abbadesse, Brian Schwartz, John C. Byrd, and Jennifer A. Woyach
1 The Ohio State University College of Medicine, Columbus, OH; 2 Division of Hematology; The Ohio State University, Columbus, OH; 3 ArQule Inc., Burlington, MA.

Introduction
The Bruton’s tyrosine kinase inhibitor ibrutinib improves survival in chronic lymphocytic leukemia (CLL) compared to standard chemotherapy or immune therapy. In a subset of patients, somatic mutation (C481S) of its BTK binding site results in acquired resistance to ibrutinib therapy with poor clinical outcome. ARQ 531 is an ATP competitive, orally bioavailable, potent inhibitor of BTK (biochemical IC50 is 0.85nM for WT BTK, and 0.39nM for C481S BTK) and other relevant kinases including Src and Tec family members. Herein we present preclinical data with ARQ 531 in CLL including C481S mutated BTK and its efficacy versus ibrutinib in the TCLA1 mouse model of CLL.

Methods
Potency of ARQ 531 and its binding kinetics were measured in enzymatic and Surface Plasmon Resonance (SPR) binding assays. Primary CLL B cells were negatively selected using RosetteSep isolation followed by density gradient centrifugation. B cell receptor signaling was investigated by immunoblot following a 1 hour drug incubation. CLL cells migrating towards CXCL12 and CXCL13 after 4 hours across a 5.0 micron transwell insert were counted by flow cytometry. Annexin V and propidium iodide flow cytometry was used to measure CLL viability over a range of drug concentrations and time. CpG mediated CLL cell activation was measured by CD40 and CD86 expression by flow cytometry. In vivo investigation utilized B6 mice engrafted with 1E7 CD5+/CD19+ TCL1 lymphocytes via tail vein injection. Mice were randomized to treatment with vehicle, ARQ 531, or ibrutinib following the establishment of CD5+/CD19+ population >10% in peripheral blood.

ARQ 531 Displays Long Residence Time with WT and C481S BTK

ARQ 531 Is Cytotoxic to CLL Cells with WT and C481S BTK

ARQ 531 Inhibits TLR9 Mediated Chemokine Mediated CLL Migration

ARQ 531 Promotes Neutrophil Development In Vivo

Conclusions
• ARQ 531 is a potent inhibitor of BTK with promising activity both in vitro and in vivo.
• Multi-targeted inhibition of cytokine, chemokine, and BCR pathways by ARQ 531 decreases activation, migration, and viability of CLL cells.
• Unlike ibrutinib, ARQ 531 inhibits activation of C481S mutated BTK variants and maintains cytotoxicity in ibrutinib resistant clones.
• ARQ 531 demonstrates remarkable efficacy in an in vivo TCLA1 adoptive transfer model, improving survival to a greater extent than ibrutinib and restoring granulocyte production.
• These data warrant transition of ARQ 531 into clinical trials.

Acknowledgements & References

Funding provided by NIH CA138944, NIH RO1CA20563, ARS-CIAT006, and REI: CA187070. Drug and xenograft mice provided by ArQule Inc., Eathiraj et al. 2016 Pan Pacific Lymphoma Conference, Kobe, NF, USA.