**Background**

Bruton’s tyrosine kinase (BTK) inhibitors have transformed the therapeutic landscape for patients with chronic lymphocytic leukemia (CLL) and Non-Hodgkin lymphomas (NHL), due to the critical role of BTK in the proliferation and survival of B-cell malignancies.

Increasing use of covalent Bruton tyrosine kinase (BTK) inhibitors ibrutinib, acalabrutinib, and zanubrutinib as well as noncovalent inhibitors nemastructibrutinib and pitrobrolbnilin, have elucidated a series of acquired BTK mutations, some of which can confer cross-resistance to other BTK inhibitors in patients with B-cell malignancies.

**BTK Mutations in CLL patients confer resistance to non-covalent BTK inhibitors**

**Results**

Figure 1: Kinase dead forms of BTK have sustained B-cell receptor (BCR) signaling through enhanced interactions with additional signaling proteins downstream of BCR. (A) IgM-mediated intracellular calcium release in BTK mutants. (B) Phosphoproteomics design schematic with differentially phosphorylated peptides of kinase active BTK mutant, T474I, and kinase dead BTK mutant, L528W (E) revealed enhanced physical interactions of kinase dead BTK with several protein kinases malign B cells (F). Interactions with HCK and ILK were further validated by western blot analysis (G).

**BTK mutants lack enzymatic function but sustain downstream BCR signaling**

Figure 2: BTK degrader NX-2127 overcomes non-covalent and covalent BTK inhibitor resistance

**Methodology**

BTK Mutant Signaling Studies: To define the signaling mechanisms of kinase dead BTK mutants, we generated CRISPR-CAS9 knockin mutant cells and utilize several orthogonal proteomic approaches in BTK-dependent human lymphoma B cells expressing WT or mutant (C481S, V416L, T474I and L528W) BTK. We performed global phosphoproteomics, kinobead assays, BTK immunoprecipitation mass spectrometry studies, and 2D differential gel electrophoresis to unbiasedly elucidate a novel scaffolding function of BTK.

BTK Degrader Studies: Given that mutations conferring resistance to BTK inhibitors lack enzymatic activity, we evaluated means to eliminate, rather than enzymatically inhibit, mutant BTK proteins. Nurix generated NX-2127, a heterobifunctional degrader molecule which brings BTK into close proximity with cereblon, leading to ubiquitylation and proteasomal degradation of BTK (as demonstrated by biophysical and structural data).

**Conclusion**

These data reveal a distinct oncogenic scaffolding function of kinase dead BTK which confers resistance across FDA-approved BTK inhibitors. Importantly, regardless of enzymatic group, the BTK mutants evaluated in this study are susceptible to BTK degradation both preclinically and in patients currently being treated in a first-in-human Phase 1b dose expansion trial of NX-2127.

**Acknowledgements**

Skye Montoya is supported by the Ruth L. Kirschstein National Research Service Award for Individual Predoctoral Fellows (F31) under the award number 1F31CA275378-01. JT is supported by the Doris Duke Charitable Foundation and the Sylvester Comprehensive Cancer Center.