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Drug-resistance mutations in BTK occur in distinct enzymatic classes and are overcome by BTK degradation

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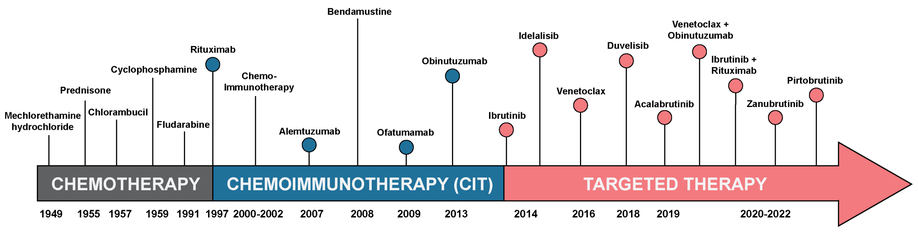
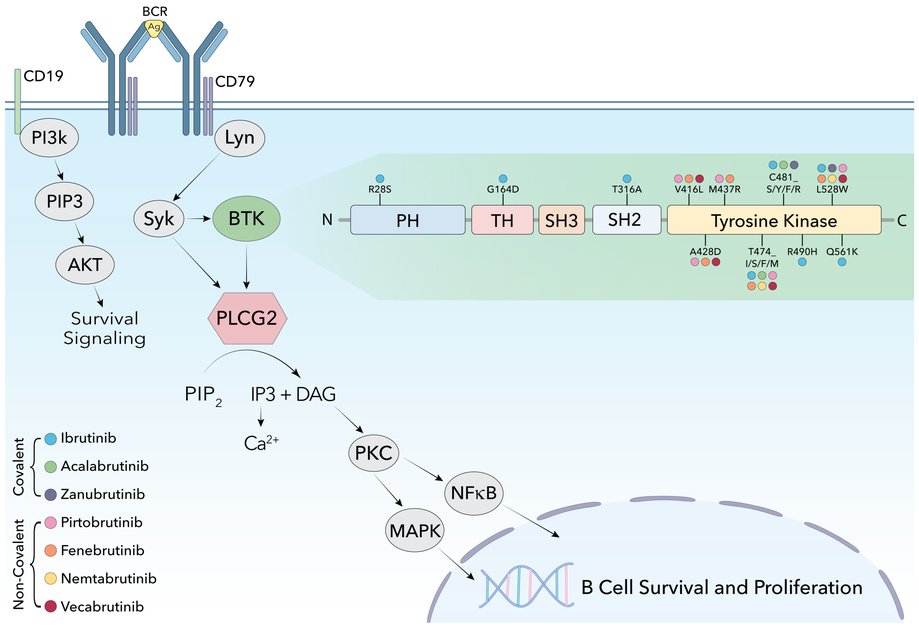
Background

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.

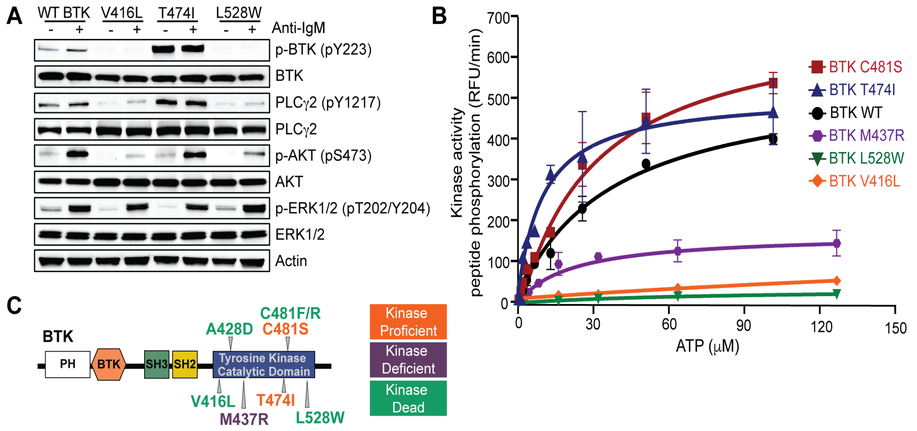


Increasing use of covalent Bruton tyrosine kinase (BTK) inhibitors ibrutinib, acalabrutinib, and zanubrutinib as well as noncovalent inhibitors nemtabrutinib and pirtobrutinib, have elucidated a series of acquired BTK mutations, some of which can confer cross-resistance to other BTK inhibitors in patients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

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



BTK mutants lack enzymatic function but sustain downstream BCR signaling



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).

Results

Kinase Dead BTK mutants sustain downstream BCR signaling utilizing novel signaling interactions

Figure 1

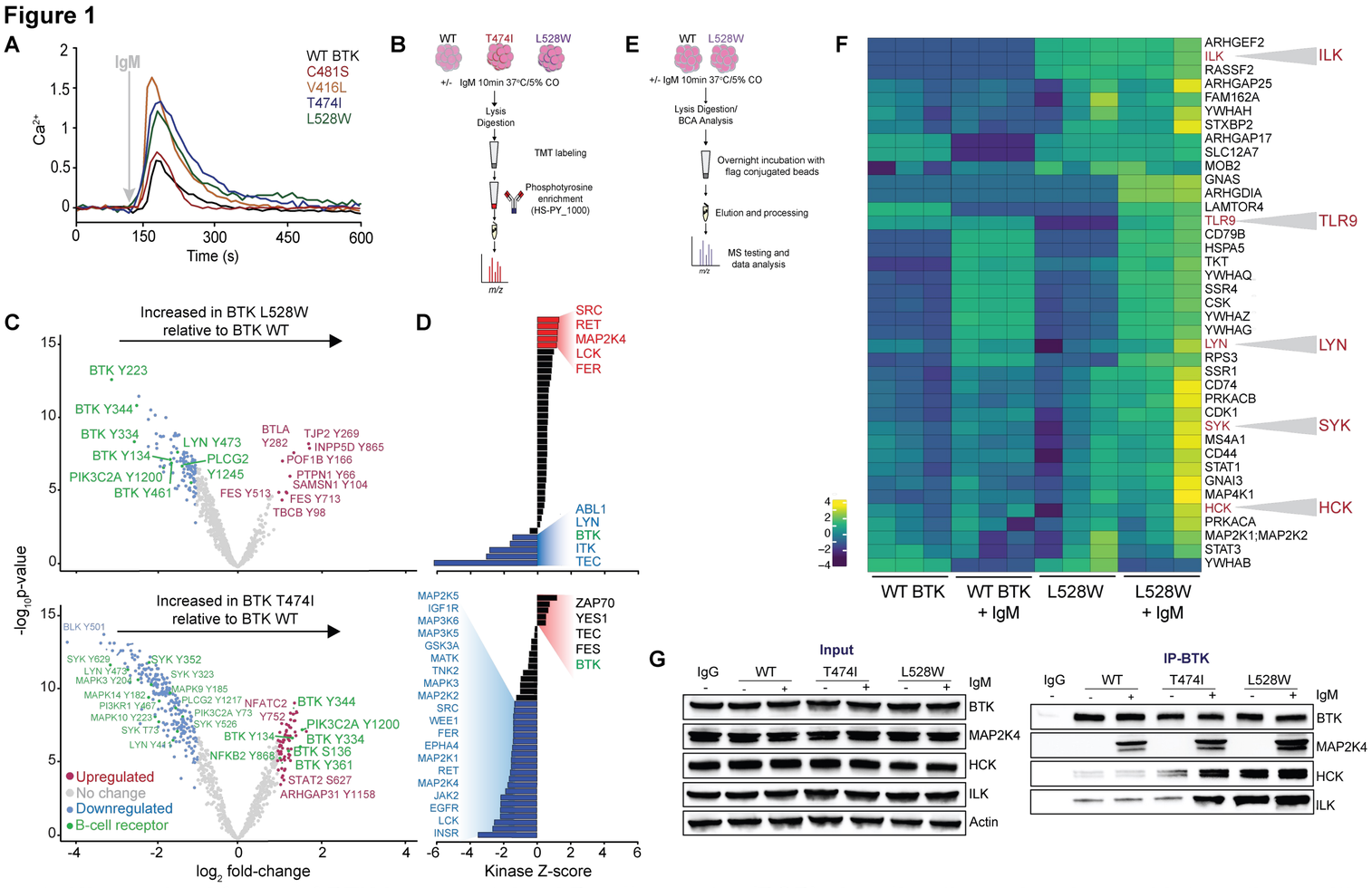
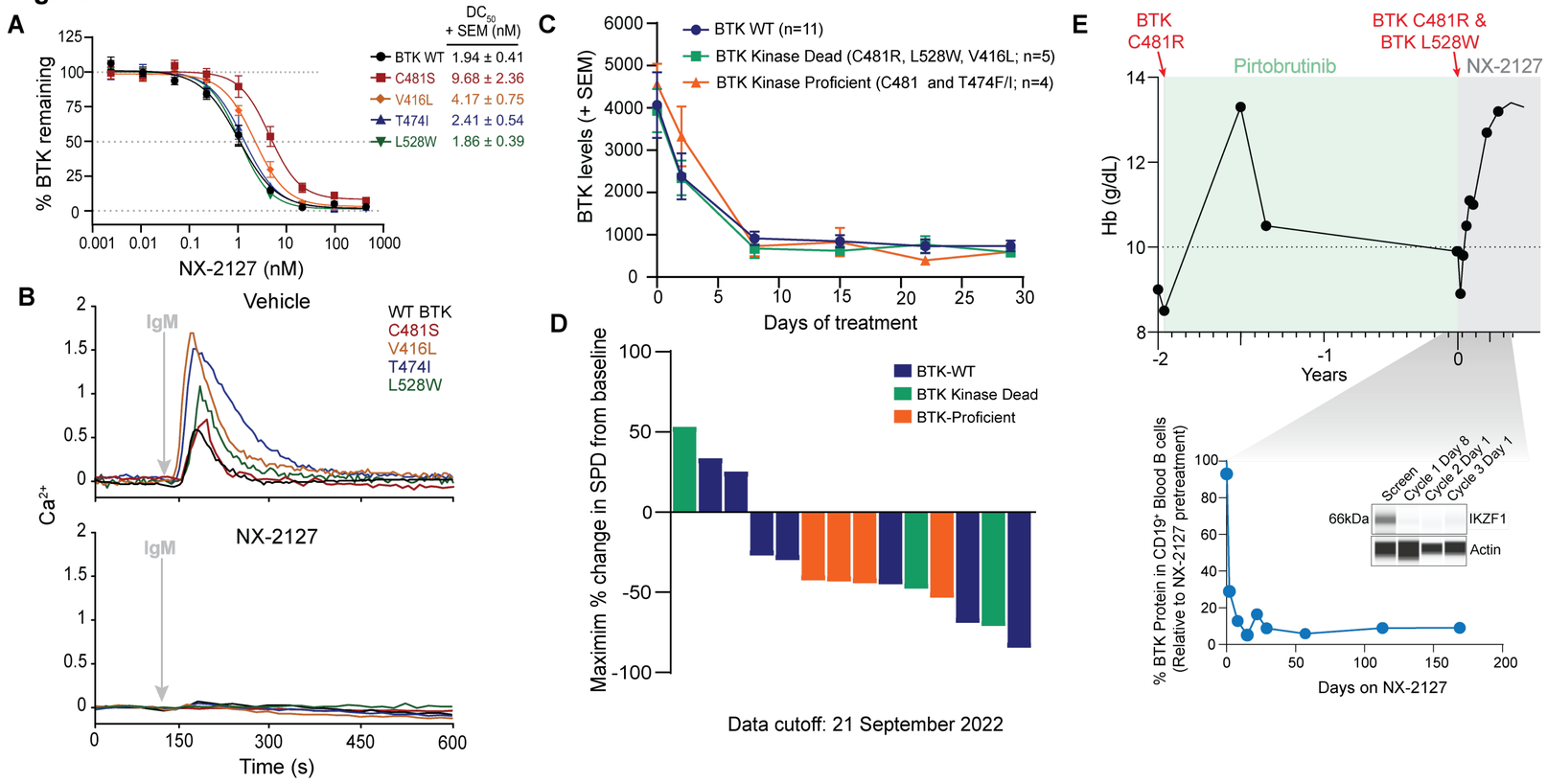


Figure 1: Kinase dead forms of BTK have sustained B-cell receptor (BCR) signaling through enhanced interactions with additional signaling proteins downstream of BCR. **(A)** IgM-mediated intracellular calcium release in BTK mutants. **(B)** Phosphoproteomics design schematic with differentially phosphorylated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

BTK degrader NX-2127 overcomes non-covalent and covalent BTK inhibitor resistance

Figure 2



NX-2127 degrades BTK drug resistant mutant proteins and abrogates BCR signaling. **(A)** Dose curves to determine degradation concentration (DC_{50}) of BTK for WT and mutant BTK treated with NX-2127 for 24 hours. **(B)** IgM-mediated intra-cellular calcium release in BTK mutants treated with either vehicle (DMSO) or NX-2127. **(C-D)** Patient data from Phase 1b dose expansion trial of NX-2127 with one exemplary patient highlighted in **(E)**.

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 (clinicaltrials.gov NCT04830137).

Acknowledgements

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