Targeting Covalent and Non-Covalent BTKi-Resistant CLL Using the Irreversible/Reversible 4th Generation BTK Inhibitor Rocbrutinib

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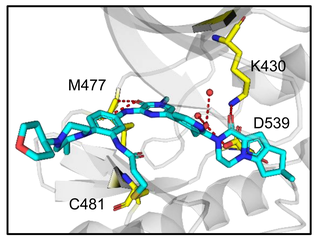
Background

- The treatment of chronic lymphocytic leukemia (CLL) has been revolutionized through usage of targeted therapies against the B-cell receptor (BCR) signaling cascade, mainly through Bruton's tyrosine kinase (BTK) inhibitors.
- Resistance to BTK inhibition has emerged in patients through the acquisition of mutations in BTK, namely the C481S mutation with Covalent BTK (cBTKi) and T474I and L528W with Non-Covalent BTKi (ncBTKi)[1,2].
- In the presence of BTKi resistance mutations, BCR signaling remains intact, suggesting targeting molecules downstream of BTK may be an effective therapeutic strategy.
- There are currently no standard of care treatment options for patients who have progressed on both cBTKi and ncBTKI.
- Agents capable of inhibiting both wild type (WT) and mutant BTK are ideal for the treatment of CLL as they would retain BTK inhibition and prevent clinical relapse through the most common resistance mechanisms.
- Rocbrutinib is a novel ultra-selective 4th generation BTKi with an active warhead capable of covalent interaction with WT BTK or non-covalent binding when a BTK C481 mutation is present.
- Therefore, we hypothesized the inhibition of BTK through Rocbrutinib could be a therapeutic strategy to overcome BTKi resistant CLL.

Rocbrutinib Binding Mechanism and Selectivity



BTK	IC_{50} (nM)
WT	4.7
C481S	49.7
T474I	13.1
L528W	80.4

C

Compound K_i (nM)	BTK Protein				
	WT	C481S	V416L	M437R	L528W
Rocbrutinib	**4**	**8**	**4**	**110**	**25**
Ibrutinib	2	60	1.4	0.6	210
Acalabrutinib	7	>10000	250	22	11
Zanubrutinib	3	>10000	1.4	1	>10000
Nemtabrutinib	33	23	1290	422	>10000
Pirtobrutinib	3	4	480	1060	>10000

Figure 1. (**A**) Crystal structure of rocbrutinib bound to WT BTK. (**B**) NanoBRET assay of rocbrutinib binding towards WT, C481S, T474I, or L528W BTK protein. (**C**) K_i (nM) values of current BTKi towards WT, C481S, V416L, M437R, and L528W mutant BTK.

References

References: [1] Woyach, JA et al. (2014). *NEJM* 370, 2286-2294
[2] Wang, E et. Al (2022). *NEJM* 386, 735-746

Rocbrutinib Inhibits BCR Dependent Signaling

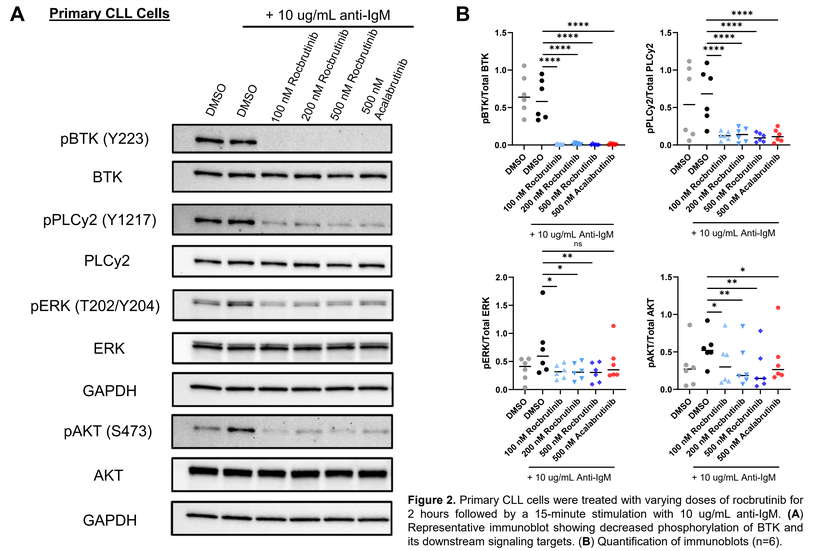


Figure 2. Primary CLL cells were treated with varying doses of rocbrutinib for 2 hours followed by a 15-minute stimulation with 10 ug/mL anti-IgM. (**A**) Representative immunoblot showing decreased phosphorylation of BTK and its downstream signaling targets. (**B**) Quantification of immunoblots (n=6).

Rocbrutinib improves survival in both the Eμ-MTCP1 and the Eμ-TCL1 mouse models

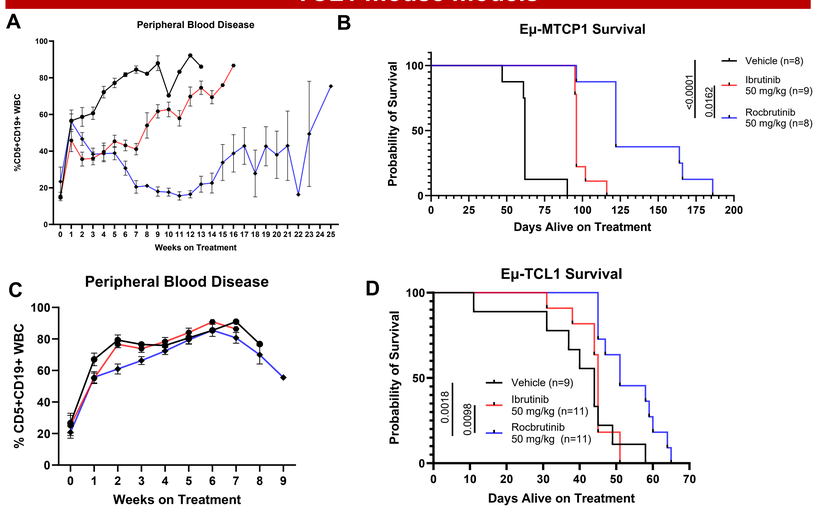
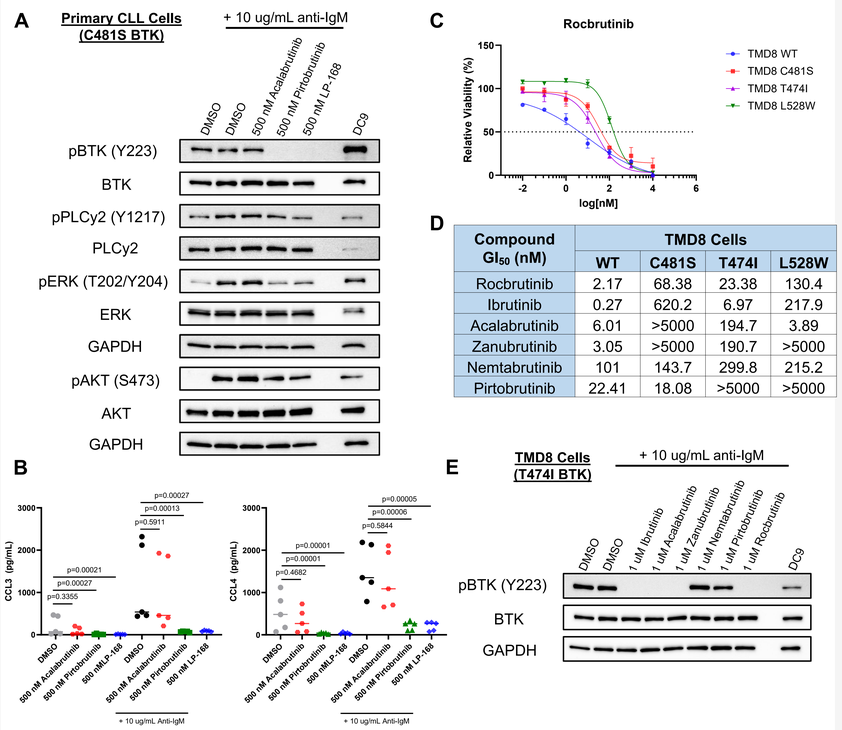


Figure 3. (**A**) Measurement of peripheral disease characterized by percent of CD45+/CD5+/CD19+ % in peripheral blood in Eμ-MTCP1 mice. (**B**) Overall survival of Eμ-MTCP1 mice. (**C**) Measurement of peripheral disease characterized by percent of CD45+/CD5+/CD19+ % in peripheral blood in Eμ-TCL1 mice. (**D**) Overall survival of Eμ-TCL1 mice.

Rocbrutinib retains efficacy in cBTKi and ncBTKi Resistant CLL



Compound GI_{50} (nM)	TMD8 Cells			
	WT	C481S	T474I	L528W
Rocbrutinib	2.17	68.38	23.38	130.4
Ibrutinib	0.27	620.2	6.97	217.9
Acalabrutinib	6.01	>5000	194.7	3.89
Zanubrutinib	3.05	>5000	190.7	>5000
Nemtabrutinib	101	143.7	299.8	215.2
Pirtobrutinib	22.41	18.08	>5000	>5000

Figure 4. (**A**) Representative immunoblot showing decrease phosphorylation of BTK and its downstream signaling targets in primary CLL cells harboring the C481S BTK mutation. (**B**) CCL3 and CCL4 cytokine production in primary CLL cells with C481S BTK mutant treated with BTKi for 24 hours. (**C**) IC_{50} curves of rocbrutinib towards TMD8 cells with either WT, C481S, T474I, and L528W (**D**) IC_{50} (nM) values of current BTKi towards WT, C481S, V416L, and L528W mutant BTK. (**E**) Representative immunoblot of T474I BTK TMD8 cells treated with various BTKi for 2-hours followed by a 15-minute anti-IgM stimulation showing inhibition of BTK phosphorylation.

Conclusions

- Rocbrutinib is a 4th generation potent and ultra-selective BTKi.
- Treatment of CLL cells with rocbrutinib results in inhibition of the BCR pathway.
- In vivo studies demonstrate superiority in survival advantage compared to ibrutinib.
- Rocbrutinib is capable of overcoming both cBTKi and ncBTKi mediated resistance mechanisms.
- Together, our results establish rocbrutinib as a potential therapeutic to treat CLL patients regardless of BTK mutation status.

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