4669

Differential Preclinical Activity of Bruton Tyrosine Kinase Inhibitors Against *BTK* Resistance–Associated Mutations

Matthew S. Davids, MD, MMSc,[1] Inhye E. Ahn, MD,[1] Jacob J. Riehm,[2] Melih Acar, PhD,[2] Vincent Girardi, MA,[2] Sima Patel, PhD,[2] Elizabeth L. Noey, PhD,[2] Tarikere Gururaja, PhD,[2] Jennifer Woyach, MD,[3] Lloyd T. Lam, PhD,[2] Edith Szafer-Glusman, PhD[2]

[1]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA; [2]AbbVie, North Chicago, IL, USA; [3]The Ohio State University Comprehensive Cancer Center, Columbus, OH, USA.

OBJECTIVE

To describe preclinical activity of Bruton tyrosine kinase (BTK) inhibitors (ibrutinib, acalabrutinib, zanubrutinib, and pirtobrutinib) against multiple clinically relevant *BTK* mutations and report on the clinical impact of *BTK* C481S in patients treated with ibrutinib

CONCLUSIONS

- This study confirms differential activities of BTK inhibitors against *BTK* resistance mutations
- Ibrutinib demonstrated cell killing against *BTK* variants that are associated with resistance to covalent and noncovalent BTK inhibitors
- Presence of double mutations in *BTK* conferred super resistance to both covalent and noncovalent BTK inhibitors, demonstrating that emergence of these double mutants may result in high unmet medical need
- Clinical data show that patients who develop *BTK* C481S mutations continue to derive benefit from ibrutinib for several months before progressive disease
- Together, these data demonstrate a continued role for ibrutinib in the treatment of patients with a broad spectrum of *BTK* variants

https://www.congresshub.com/Oncology/AACR2024/Ibrutinib/Davids

The QR code is intended to provide scientific information for individual reference, and the information should not be altered or reproduced in any way.

AbbVie and the authors thank the patients who participated in the study and their supportive families, as well as the investigators, study coordinators, study team, and nurses who cared for the patients. AbbVie funded this study and participated in the study design, research, analysis, data collection, interpretation of data, and the review and approval of the publication. All authors had access to relevant data and participated in the drafting, review, and approval of this publication. No honoraria or payments were made for authorship. Medical writing support was provided by Melanie Sweetlove, MSc, and funded by AbbVie.

MSD: consulting/advisory role for AbbVie, Adaptive Biosciences, Aptitude Health, Ascentage Pharma, AstraZeneca, BeiGene, Bristol Myers Squibb, Curio Science, Eli Lilly, Genentech, Genmab, Janssen, MEI Pharma, Merck, Mingsight Pharmaceuticals, Nuvalent, Research to Practice, Secura Bio, TG Therapeutics, and Takeda; research funding from AbbVie, Ascentage Pharma, Genentech, TG Therapeutics, Novartis, Surface Oncology, and MEI Pharma. IEA: nothing to disclose. JJR, MA, SP, LTL, and ES-G: employment and stock or other ownership with AbbVie. VG: employment with Everest Clinical Research; other relationship with AbbVie. TG: employment, leadership role, and stock or other ownership with AbbVie; research funding from AbbVie. JW: consulting/advisory role for AbbVie, InCyte, AstraZeneca, BeiGene, Genentech, Janssen, Loxo, Newave, and Pharmacyclics LLC, an AbbVie Company; research funding from AbbVie, Kalyopharm, Loxo, MorphoSys, Schrodinger, and Verastem.

Poster presented at the American Association for Cancer Research Annual Meeting 2024; April 5–10, 2024; San Diego, CA

INTRODUCTION

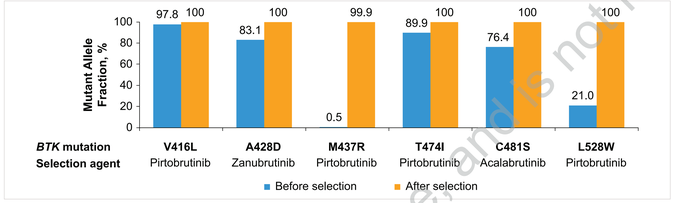
- Clinical resistance to Bruton tyrosine kinase (BTK) inhibitors is associated with mutations in *BTK*, but emerging data indicate different resistance mutation profiles across BTK inhibitors[1-9]
  - C481S is the most frequent mutation at progressive disease (PD) with ibrutinib, acalabrutinib, and zanubrutinib[1-7]
  - T474I is enriched in patients with *BTK* mutations at PD, reported in 29% of patients treated with acalabrutinib and 0% of patients treated with ibrutinib[6]
  - L528W is enriched in patients with *BTK* mutations at PD, reported in 54% of patients treated with zanubrutinib and 4% of patients treated with ibrutinib[8]
  - Non-C481 mutations within the kinase domain (including V416L, A428D, M437R, T474I, and L528W) have been reported at PD in 78% of patients treated with pirtobrutinib[9]
- Differences in profiles of acquired mutations of resistance to BTK inhibitors might have implications for treatment sequencing

METHODS

Preclinical Data

- Inhibition of kinase activity (IC_{50}; half-maximal inhibitory concentration) by BTK inhibitors was evaluated in a cell-free kinase activity assay (Nanosyn)
- *BTK* mutations were generated in TMD8 (diffuse large B-cell lymphoma) cells using CRISPR/Cas9 (Synthego)
  - *BTK*-mutant cell lines were enriched for the mutant allele by eliminating wild type (WT) cells in 10-day culture using a BTK inhibitor that is ineffective against the selected mutation
  - Droplet digital polymerase chain reaction (ddPCR) was used to determine the mutant allele fraction before and after selection

BTK Mutant Knock-In TMD8 Cells Were Enriched for the Mutant Allele After Selection



V416L, T474I, and L528W mutations were selected using pirtobrutinib 1 μM for 10 days; A428D was selected using zanubrutinib 1 μM for 10 days; M437R was selected using pirtobrutinib 100 nM for 10 days; C481S was selected using acalabrutinib 1 μM for 10 days.

- Cell-killing activity (EC_{50}; half-maximal effective concentration) on *BTK* mutant knock-in TMD8 cells was assessed after BTK inhibitor treatment for 72 hours based on cell viability measured using CellTiter-Glo (Promega)
- Downstream phosphorylation of AKT and ERK was measured using AlphaLISA (Revvity) in lysates from cells treated with ibrutinib for 24 hours
- Schrödinger's Protein free-energy perturbation method[10] was used to compute the noncovalent contribution of relative free energies of binding and therefore the relative potencies of ibrutinib to WT and mutant *BTK*

Clinical Data

- *BTK* mutations were assessed in 419 patients with chronic lymphocytic leukemia (CLL) treated with ibrutinib using pooled data from 5 clinical trials

Clinical Trials

Ibrutinib-Treated Patients (N=419)				
Previously untreated (n=247)			Relapsed/refractory (n=172)	
RESONATE-2[a] n=111	iLLUMINATE n=113	NHLBI Phase 2 n=23	RESONATE n=107	RESONATE-17 n=65

NHLBI, National Heart, Lung, and Blood Institute.
[a]PCYC-1115 and PCYC-1116.

- Next-generation sequencing (Ion Torrent) of *BTK* coding regions was performed on CD19+–enriched peripheral blood mononuclear cell (PBMC) samples at the time of PD or on the latest sample available prior to PD
  - The variant allele fraction (VAF) cutoff for *BTK* C481S was 0.5%
- ddPCR was performed on CD19+–enriched PBMC samples collected at earlier time points (3- to 6-month intervals) to identify the first incidence of *BTK* C481S
  - The VAF cutoff for *BTK* C481S was 0.1%
- Time from first detection of mutation to PD was evaluated in patients who had the *BTK* C481S variant and PD on ibrutinib treatment

RESULTS

In Vitro Kinase Activity Against C481S and T474I Was Highest for Ibrutinib Compared With Acalabrutinib or Zanubrutinib

IC_{50} (nM)	*BTK*		
	WT	C481S	T474I
Ibrutinib	0.52	26.3	1.17
Zanubrutinib	0.35	84.8	4.37
Acalabrutinib	3.14	338.0	43.6

Ibrutinib Maintained Cell-Killing Activity Against a Broad Range of *BTK* Mutants

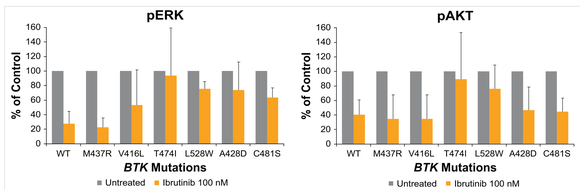
EC_{50}(nM)	*BTK*									
	WT	C481S	M437R	T474I	L528W	V416L	A428D	C481S/ T474I[a,b]	C481S/ L528W[c]	C481S/ A428D
Ibrutinib	0.6	414	0.2	2	181	0.5	205	2376	2559	1532
Acalabrutinib	4	2974	2	43	4	106	≥3000	≥3000	≥3000	≥3000
Zanubrutinib	0.7	2322	0.3	12	≥3000	0.6	1726	≥3000	≥3000	≥3000
Pirtobrutinib	8	13	50	2728	≥3000	1898	≥3000	≥3000	≥3000	≥3000

Most potent cell-killing activity

Least potent cell-killing activity

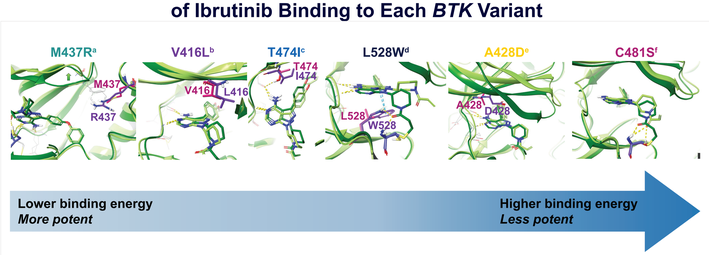
[a]Confers "super resistance" to irreversible BTK inhibitors.[11]
[b]Reported frequency among patients with *BTK* mutations at PD: 26% with acalabrutinib, 0% with ibrutinib.[6]
[c]Reported frequency among patients with *BTK* mutations at PD: 38% with zanubrutinib, 4% with ibrutinib.[8]

Inhibition of Downstream Phosphorylation by Ibrutinib Correlated With *In Vitro* Activity



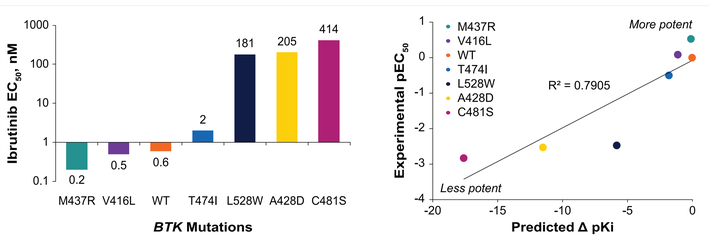
pAKT, phosphorylated AKT; pERK, phosphorylated extracellular-regulated kinase.

BTK Mutations Alter the Binding Pocket and the Strength of Ibrutinib Binding to Each *BTK* Variant



pEC_{50}, -log(EC_{50}); pKi, -log(Ki).
Lower predicted binding energy (higher pKi) correlates with more favorable relative EC_{50}.
Schrödinger's protein free-energy perturbation method was used to compute the noncovalent contribution of relative free energies of binding and therefore the relative potencies of ibrutinib to WT and mutant *BTK*.[10]
Quantum mechanical calculations (B3LYP-D3/6-31g**//PBF(water)) were used to estimate the covalent contribution to potency in the C481S mutation.

Ibrutinib Cell-Killing Activity Against BTK Mutants Correlated With Predicted Potency



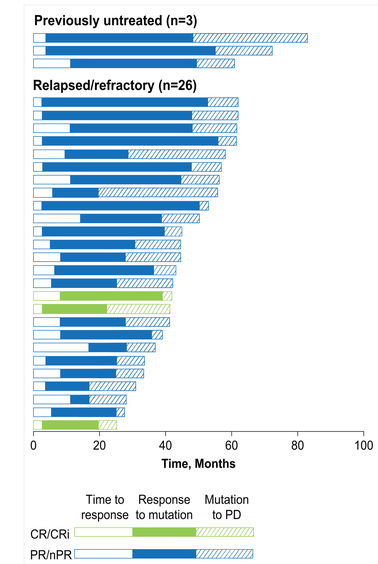
[a]The glycine-rich loop must move to accommodate the M437R mutation; binding of ibrutinib is not greatly affected. [b]The V416L mutation does not significantly alter the binding of ibrutinib. [c]The T474I mutation perturbs the hinge binding of ibrutinib, weakening these H-bond contacts. [d]The L528W mutation displaces the electrophile of ibrutinib. [e]Asp 428 places an acid over the pyrazolo-pyrimidinamine ring of ibrutinib (>4 A), which is electrostatically unfavorable; here, the beta strands above ibrutinib are moved due to this mutation, and the binding of ibrutinib is greatly perturbed. [f]The C481S mutation makes the ligand a noncovalent binder.

Clinical Data

- In pooled clinical data, C481S was detected prior to clinical PD in 3 patients (1%) with previously untreated CLL and in 26 patients (15%) with relapsed/refractory CLL

	Previously Untreated (n=3)	Relapsed/Refractory (n=26)
Median Time from first detection of C481S to PD (range), months	17.0 (11.2-34.3)	10.0 (2.6-35.7)

Clinical PD Occurred Several Months After Emergence of C481S in Most Patients With Mutations Detected Prior to Clinical PD



CR, complete response; CRi, complete response with incomplete bone marrow recovery; nPR, nodular partial response; PR partial response.
Each bar represents 1 patient.

References

1. Woyach JA et al. *N Engl J Med*. 2014;370:2286–2294.
2. Woyach JA et al. *J Clin Oncol*. 2017;35:1437–1443.
3. Ahn IE et al. *Blood*. 2017;129:1469–1479.
4. Ahn IE et al. Presented at: International Workshop on CLL; October 6–9, 2023; Boston, MA. Abstract 1549556.
5. Sun C et al. Presented at: 65th ASH Annual Meeting and Exposition; December 9–12, 2023; San Diego, CA. Abstract 1891.
6. Woyach JA et al. Presented at: International Conference on Malignant Lymphoma; June 13–17, 2023; Lugano, Switzerland. Poster 163.
7. Brown JR et al. Presented at: 65th ASH Annual Meeting and Exposition; December 9-12, 2023; San Diego, CA. Abstract 1890.
8. Blombery P et al. *Blood Adv*. 2022;6:5589–5592.
9. Wang E et al. *N Engl J Med*. 2022;386:735–743.
10. "FEP+ | Schrödinger". www.schrodinger.com.
11. Estupiñán H et al. *Leukemia*. 2021;35:1317–1329.