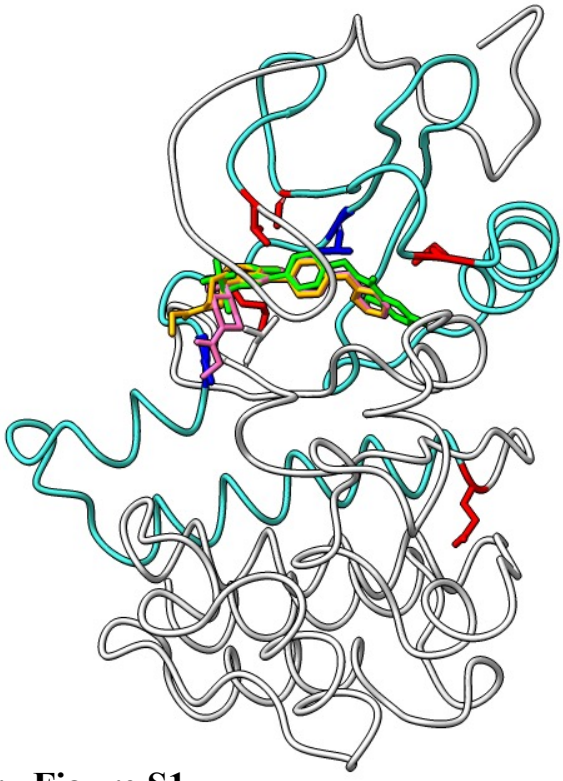
Evaluating ibrutinib, pirtobrutinib, remibrutinib, rilzabrutinib, and zanubrutinib in 34 BTK-variants at 7 inhibitor resistance sites

Abdulrahman Hamasy, Qing Wang, Brisejda Rustemi, Eleni Afentaki, Alex Villa, Md Enamul Haque, Anna Berglöf, Mauno Vihinen, C.I. Edvard Smith, Rula Zain

Supplementary table 1. The BTK codons and the corresponding nucleotide alterations in the respective plasmids

V416A	GTG > GCG
V416E	GTG > GAG
V416G	GTG > GGG
V416L	GTG > CTG
V416M	GTG > ATG
A428D	GCC > GAC
A428G	GCC > GGC
A428P	GCC > CCC
A428S	GCC > TCC
A428T	GCC > ACC
A428V	GCC > GTC
M437I	ATG > ATC
M437K	ATG > AAG
M437R	ATG > AGG
M437T	ATG > ACG
M437V	ATG > GTG
M477I	ATG > ATC
M477K	ATG > AAG
M477R	ATG > AGG
M477T	ATG > ACG
M477V	ATG > GTG

E513G	GAG > GGG
E513A	GAG > GCG
L528F	TTG > TTC
L528M	TTG > ATG
L528S	TTG > TCG
L528V	TTG > GTG
L528W	TTG > TGG
T316A	ACA > GCA
T316I	ACA > ATA
T316K	ACA > AAA
T316P	ACA > CCA
T316R	ACA > AGA
T316S	ACA > TCA



Supplementary Figure S1.

Three-dimensional structure of the BTK kinase domain including ibrutinib (PDB ID 5p9i) (Bender *et al.* 2017). The region containing the investigated amino acids (416-528) is indicated with a cyan ribbon. The structure was superimposed with complexes for zanubrutinib (6j6m) (Guo *et al.* 2019) and pirtobrutinib (8fll) (Gomez *et al.* 2023). Ibrutinib is in yellow, zanubrutinib in pink, and pirtobrutinib in green. The investigated residues are in red, and residues 474 and 481, studied earlier, are indicated in blue.

References

Bender AT, Gardberg A, Pereira A, Johnson T, Wu Y, Grenningloh R *et al*. Ability of Bruton's Tyrosine Kinase Inhibitors to Sequester Y551 and Prevent Phosphorylation Determines Potency for Inhibition of Fc Receptor but not B-Cell Receptor Signaling. Mol Pharmacol. 2017;91:208-219.

Gomez EB, Ebata K, Randeria HS, Rosendahl MS, Cedervall EP, Morales TH *et al.* Preclinical characterization of pirtobrutinib, a highly selective, noncovalent (reversible) BTK inhibitor. Blood. 2023;142:62-72.

Guo Y, Liu Y, Hu N, Yu D, Zhou C, Shi G *et al.* Discovery of Zanubrutinib (BGB-3111), a Novel, Potent, and Selective Covalent Inhibitor of Bruton's Tyrosine Kinase. J Med Chem. 2019; 62:7923-7940.