Fenebrutinib, a Potent, Highly Selective, Noncovalent BTK Inhibitor for the Treatment of Multiple Sclerosis

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Presented at MSVirtual2020, the 8th Joint ACTRIMS-ECTRIMS Meeting,
September 11-13, 2020

Presentation Number P0338

Disclosures

A Johnson is employed by Genentech, Inc., a Member of the Roche Group.

C Harp is employed by Genentech, Inc., a Member of the Roche Group.

J Yu is employed by Genentech, Inc., a Member of the Roche Group.

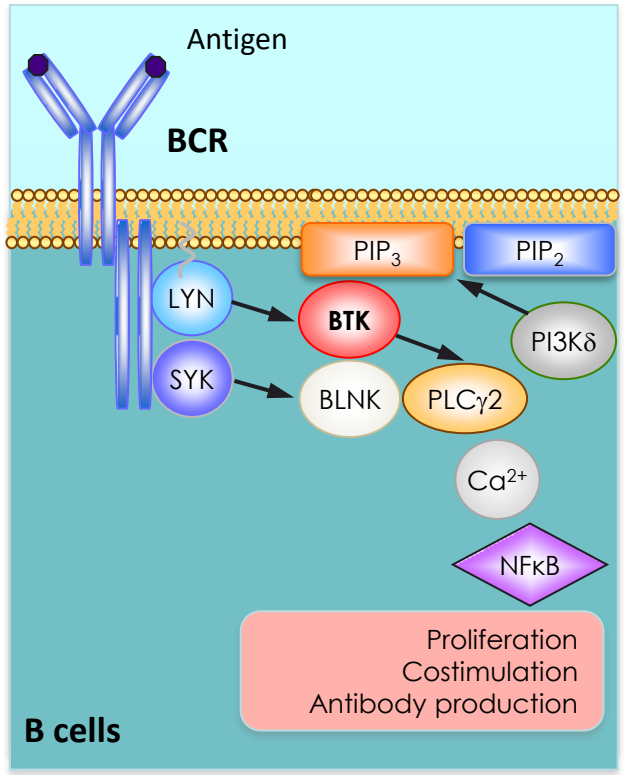
A Goodyear is employed by Genentech, Inc., a Member of the Roche Group.

J Crawford is employed by Genentech, Inc., a Member of the Roche Group.

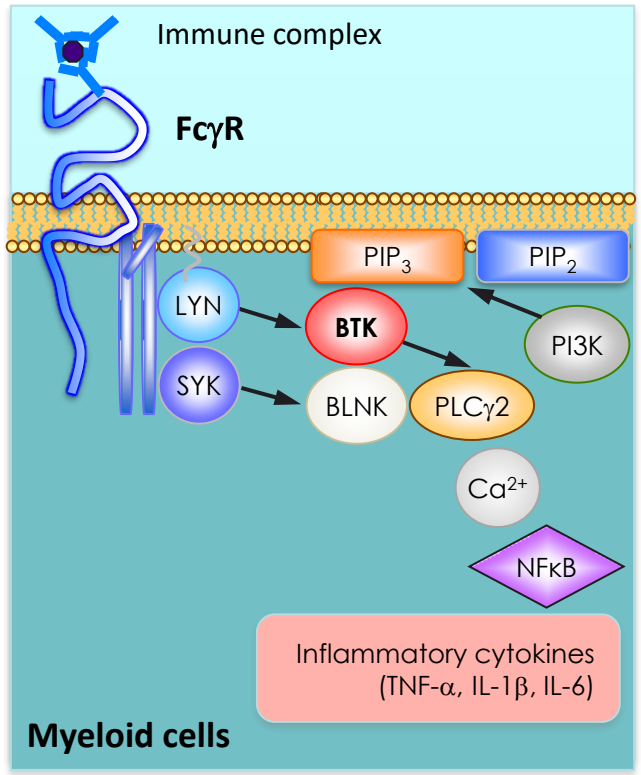
This study was sponsored by Genentech, Inc. Writing and editorial assistance for this presentation was provided by Health Interactions, USA, and funded by Genentech, Inc.

Bruton's tyrosine kinase (BTK) is a target in activated immune cells in MS

Adaptive immunity



Innate immunity



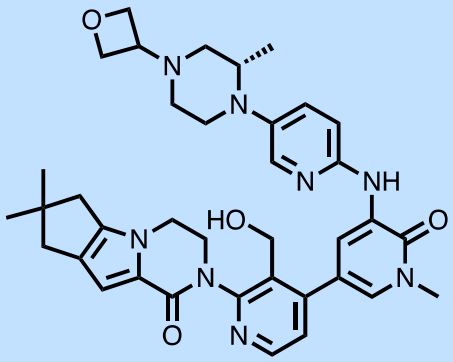
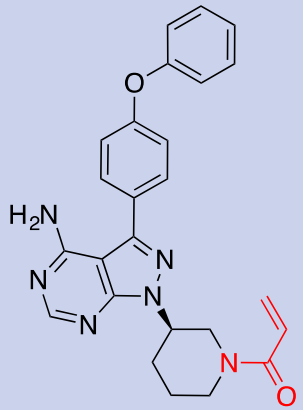
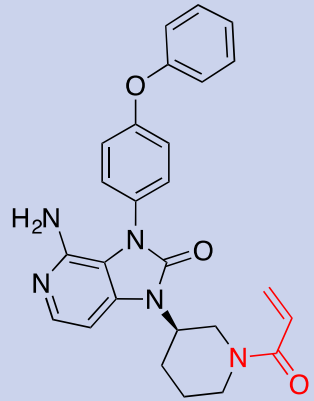
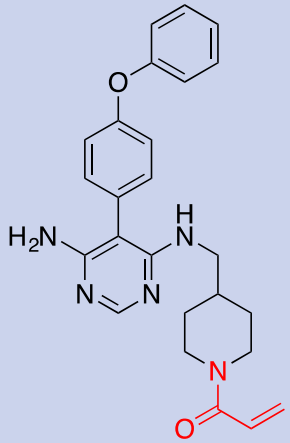
BTK

- Non–receptor tyrosine kinase[1]
- In B cells and myeloid cells (macrophages, microglia)[2,3]
- Key role in BCR and F_CR signaling[1-4]
- Adaptive and innate immunity[4]
- Target in MS (activated B and myeloid cells)[5]

BCR, B-cell antigen receptor; FcγR, Fcγ receptor; IL, interleukin; MS, multiple sclerosis; PI3K, phosphoinositide 3 kinase; TNF, tumor necrosis factor;

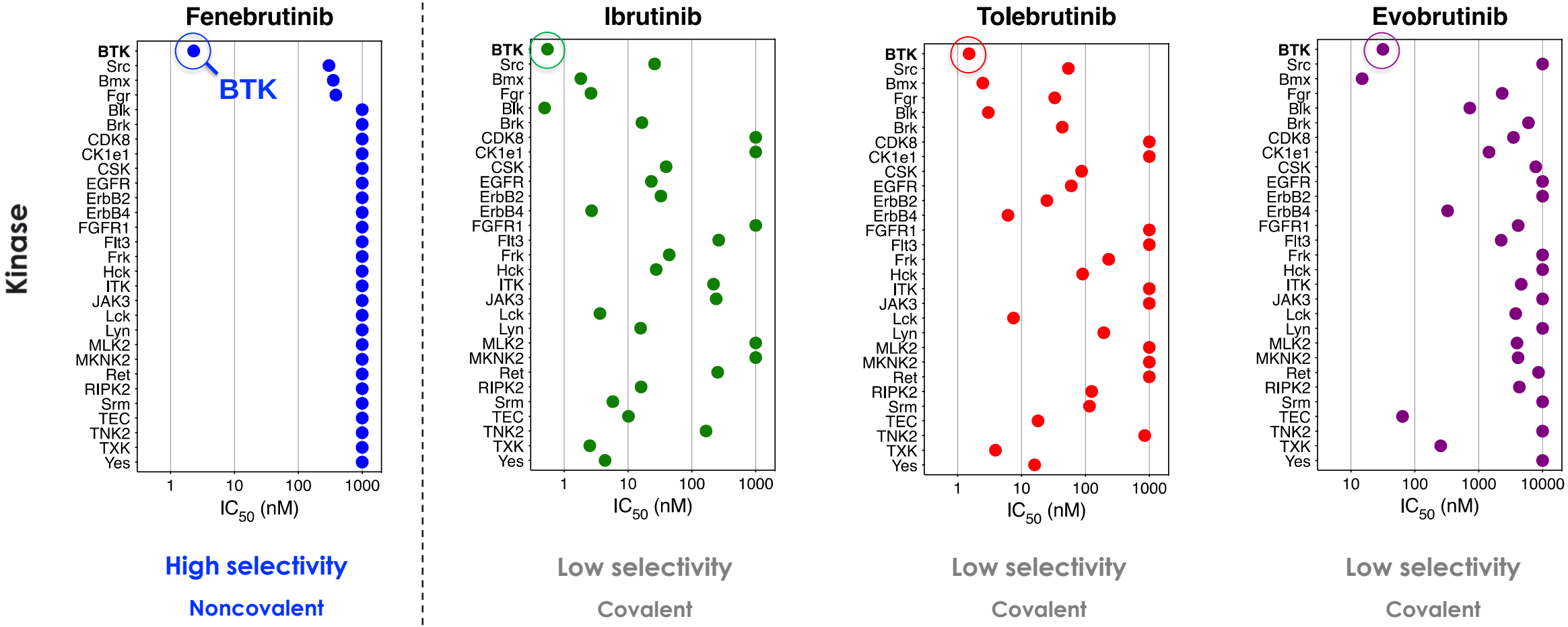
1. Satterthwaite AB, *et al*. *Semin Immunol* 1998;10:309–316; 2. Khan WN. *Immunol Res* 2001;23:147–156; 3. Schmidt U, *et al*. *Int Arch Allergy Immunol* 2004;134:65–78;
4. Brunner C, *et al*. *Histol Histopathol* 2005;20:945–955; 5. Torke S, Weber MS. *Expert Opin Investig Drug* 2020. doi: 10.1080/13543784.2020.1807934.

Fenebrutinib is a potent, highly selective, noncovalent BTK inhibitor for MS

Fenebrutinib (GDC-0853)	**Ibrutinib** (PCI-32765)	**Tolebrutinib** (PRN-2246, SAR442168)	**Evobrutinib** (M2951, MSC 2364447)
Phase 3	Launched	Phase 3	Phase 3
MS	Oncology	MS	MS
			
Noncovalent, reversible	Covalent, irreversible	Covalent, irreversible	Covalent, irreversible
BTK IC_{50}, 2 nM	BTK IC_{50}, 1 nM	BTK IC_{50}, 1 nM	BTK IC_{50}, 32 nM
High selectivity	Low selectivity	Low selectivity	Low selectivity

BTK IC_{50} values determined *in vitro* using a kinase activity assay that monitors phosphorylation of a peptide substrate with ATP at its apparent K_m. BTK, Bruton's tyrosine kinase; IC_{50}, half maximal inhibitory concentration; MS, multiple sclerosis.

Fenebrutinib is highly selective for BTK over other kinases[a,b]

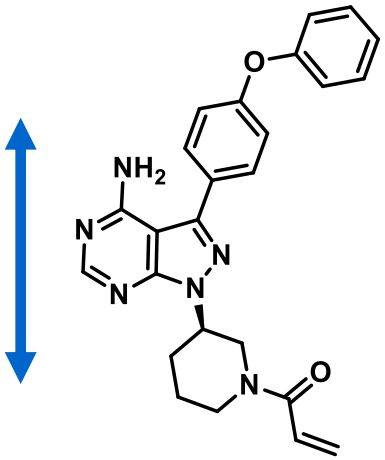


Fenebrutinib clinical safety data thus far, relative to other BTKi, appear to confirm the importance of high selectivity.

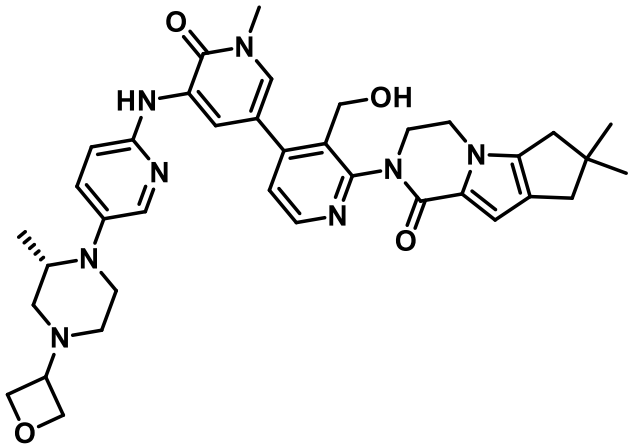
[a]To identify off targets, inhibitors were screened at 1 μM against 219 kinases in the SelectScreen panel (ThermoFisher); evobrutinib was also tested at 10 μM due to its weaker BTK IC_{50}. Of 218 off-target kinases tested, the following number of kinases were inhibited by >50%: fenebrutinib, 3; ibrutinib, 31; tolebrutinib, 19; evobrutinib, 18 (10 μM), 3 (1 μM); [b]To determine potencies, IC_{50} values were compared against those of kinases that were inhibited by >50% in the initial large panel screen. BTK, Bruton's tyrosine kinase; BTKi, Bruton's tyrosine kinase inhibitor; IC_{50}, half maximal inhibitory concentration.

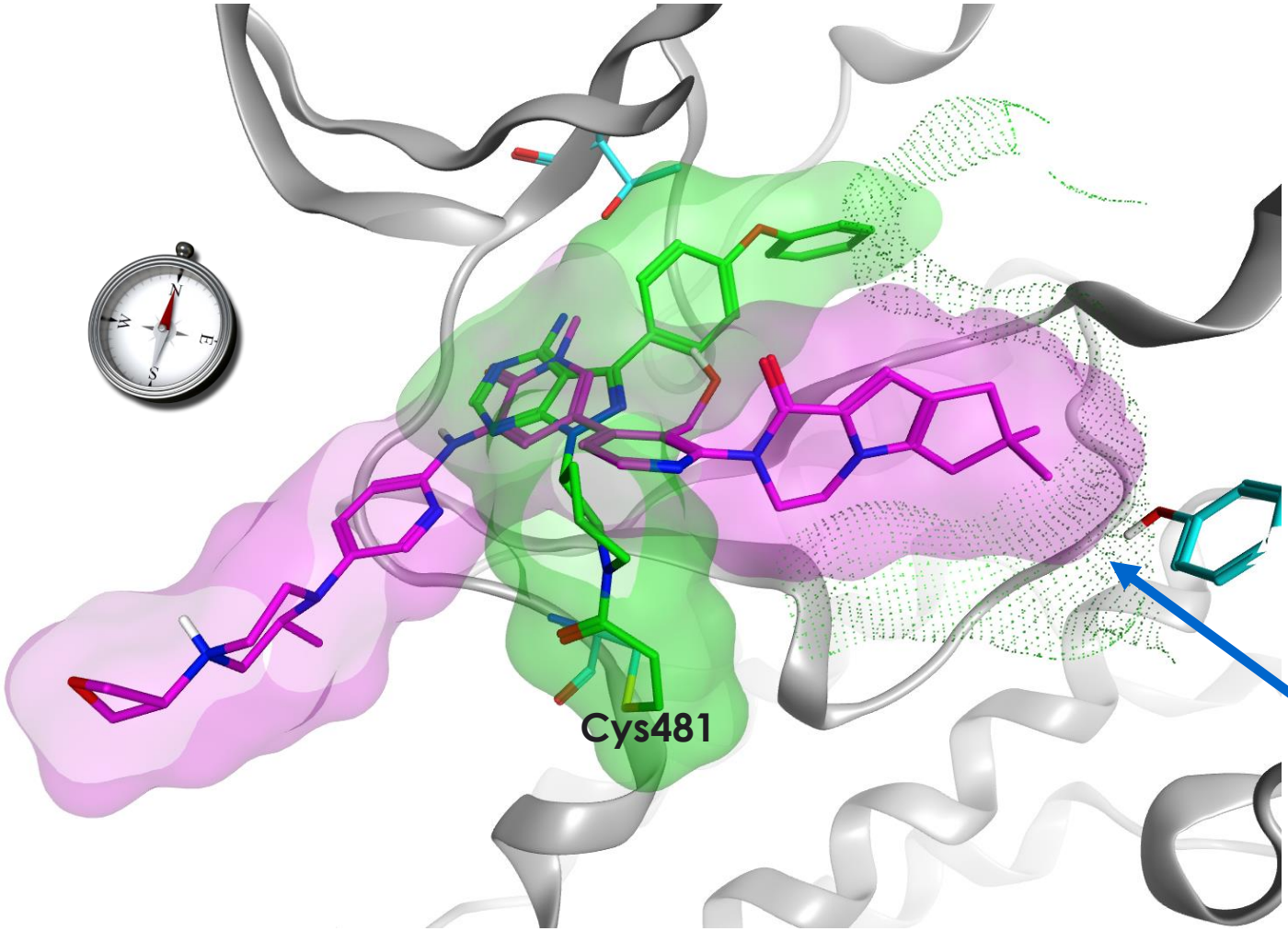
Fenebrutinib's high selectivity for BTK is due to its unique binding mode

Ibrutinib orients similarly to ATP and covalently modifies Cys481



Fenebrutinib binds orthogonally

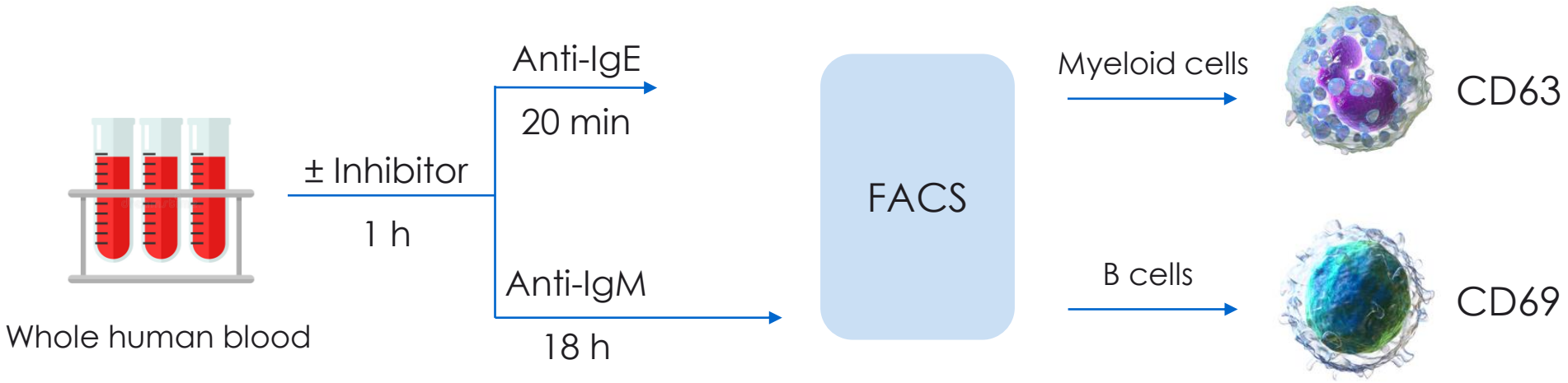




BTK, Bruton's tyrosine kinase; Cys, cystein.

Crawford JJ, *et al*. *J Med Chem* 2018;61:2227–2245.

Fenebrutinib potently inhibits myeloid and B-cell activation in whole blood



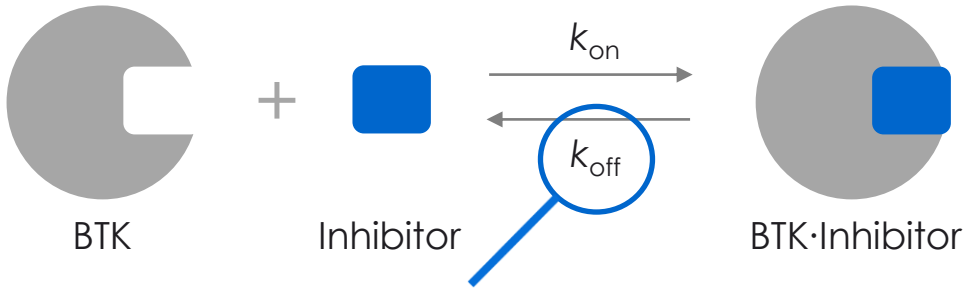
Whole human blood assay	Fenebrutinib[1]	Ibrutinib[1]	Tolebrutinib[2]	Evobrutinib[3]
Myeloid cell **CD63 IC_{50}, nM**	31	171	166	1660
B cell **CD69 IC_{50}, nM**	8	12	10	84

FACS, fluorescence-activated cell sorting; IC_{50}, half maximal inhibitory concentration; Ig, immunoglobulin.

1. Crawford JJ, *et al*. *J Med Chem* 2018;61:2227–2245; 2. Francesco MR, *et al*. *ACTRIMS-ECTRIMS* 2017; Abstract 200644; 3. Haselmayer P, *et al*. *J Immunol* 2019;202:2888–2906.

Fenebrutinib dissociates slowly from BTK, which may positively influence efficacy

Inhibitor dissociation and residence time



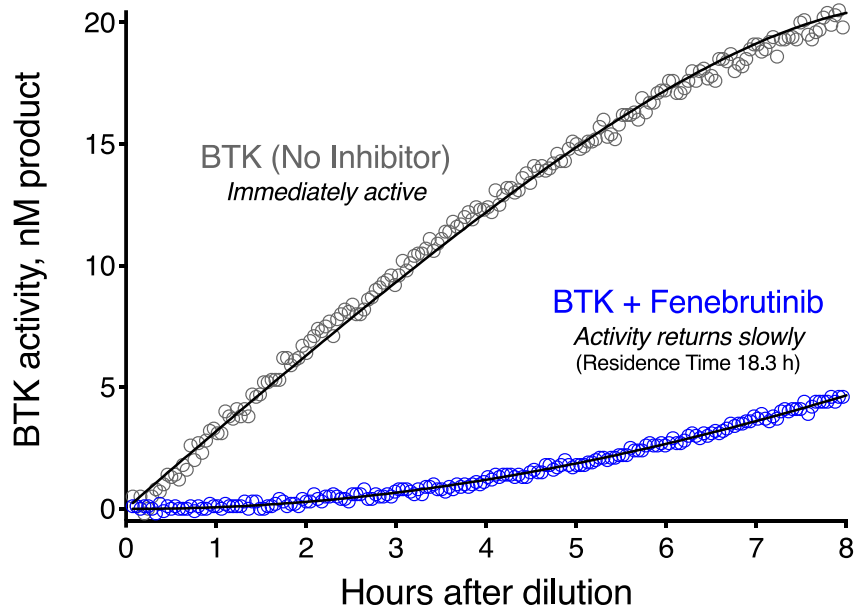
Residence time = $1/k_{off}$

Fast dissociation → large k_{off} → short residence time

Slow dissociation → small k_{off} → **long residence time**

Measuring fenebrutinib dissociation *in vitro*

Activity recovery experiment[1]
(room temperature, ATP 45 μM)



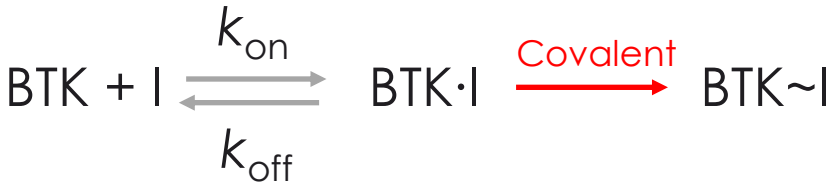
Fenebrutinib's residence time *in vivo* may be <18 h (since cells have higher ATP levels than used here), but its slow dissociation from BTK may positively influence efficacy.

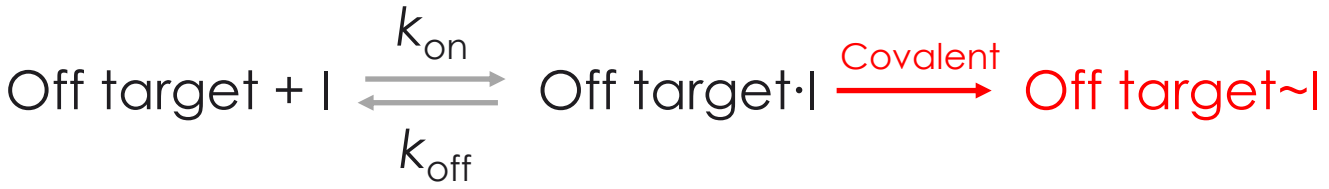
BTK, Bruton's tyrosine kinase; k_{off}, dissociation constant; k_{on}, association constant.

1. Crawford JJ, *et al*. *J Med Chem* 2018;61:2227–2245.

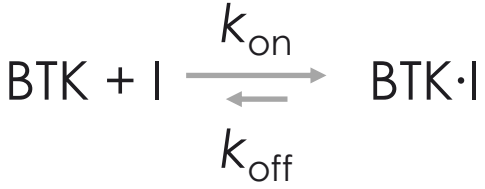
A noncovalent BTK inhibitor may be safer than a covalent BTK inhibitor

- Covalent BTK inhibitor
  - Irreversible binding to BTK and off targets
  - Irreversible binding to off targets **may impact safety**[1]





- Noncovalent BTK inhibitor
  - Reversible binding to BTK
  - Slow dissociation kinetics **may positively influence efficacy**
  - No irreversible binding to compromise safety

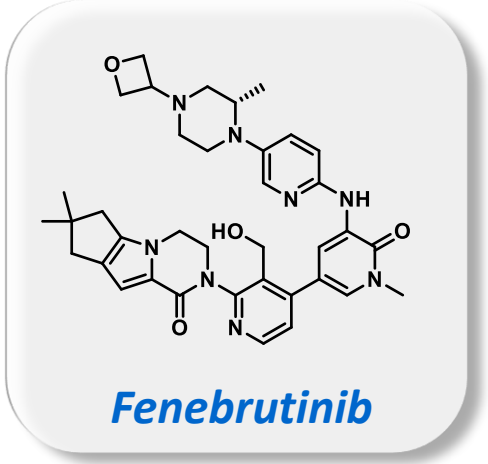


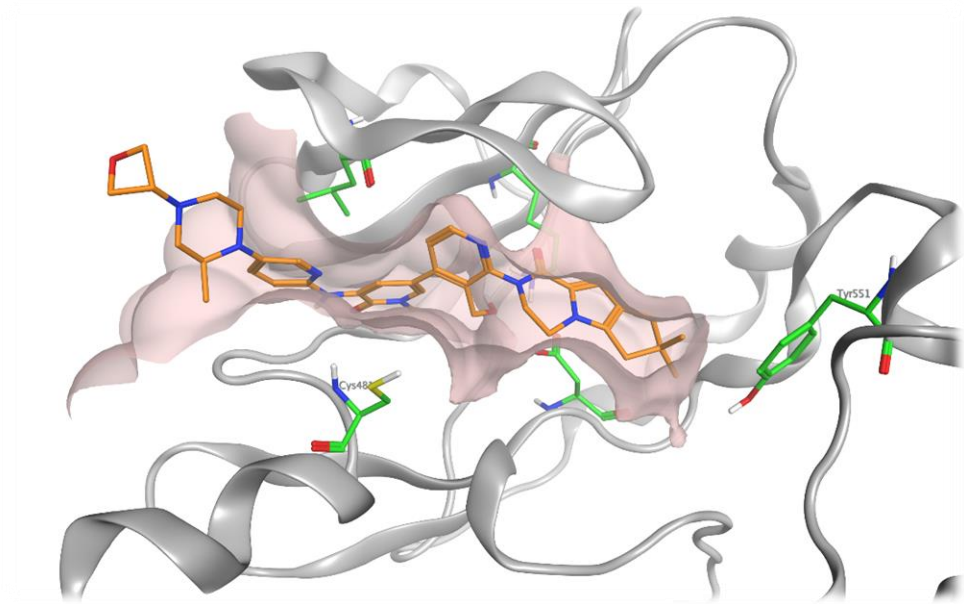
BTK, Bruton's tyrosine kinase; k_{off}, dissociation constant; k_{on}, association constant.

1. Ulrich RG. *Annu Rev Med* 2007;58:17–34.

Fenebrutinib has best-in-class potential in MS

- *Fenebrutinib is a highly selective, noncovalent BTK inhibitor*
- *Fenebrutinib may be safer than less-selective, covalent BTK inhibitors*
- ***Fenebrutinib has best-in-class potential in MS***





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