Remibrutinib, a Novel Bruton's Tyrosine Kinase Inhibitor, Exhibits Improved Target Selectivity and Potency *In Vitro*

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Introduction

- Bruton's tyrosine kinase (BTK), a cytoplasmic tyrosine kinase and a member of the TEC kinase family, is a key signaling node in B cell receptor and Fc receptor signaling[1,2]
- Inhibition of BTK offers an attractive mechanism to reduce chronic autoimmunity and neuroinflammation via inhibition of B cells and myeloid cells with an oral drug[1,2]
- Remibrutinib (LOU064) is a potent, highly selective covalent BTK inhibitor (BTKi) with a promising preclinical and clinical profile for the treatment of multiple sclerosis (MS)[3-5]
- Covalent enzyme inhibitors show time-dependent *in vitro* target binding; hence experimental data are influenced by assay conditions and comparisons should be run under identical experimental conditions[3]
- The efficacy of remibrutinib in experimental autoimmune encephalomyelitis (EAE) mouse models for MS is being presented at ECTRIMS 2022 (P134)

Objective

- To compare the potency and selectivity of covalent and reversible BTKis *in vitro* under comparable relevant experimental conditions

Methods

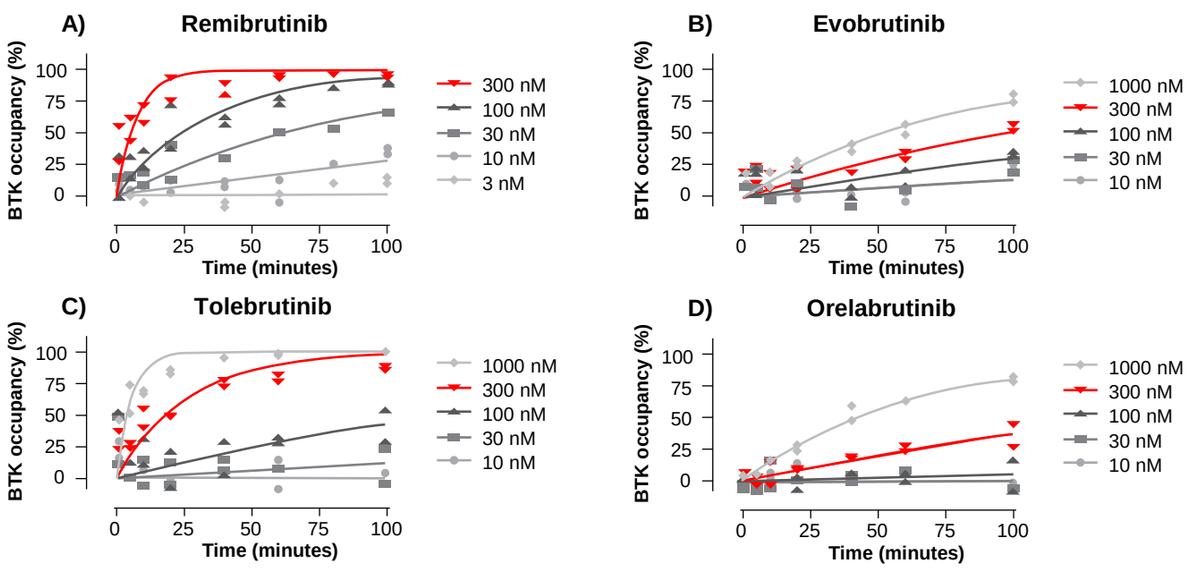
- In human blood, *in vitro* binding of covalent inhibitors to BTK was assessed over time and concentration using immunoassays detecting free and compound-bound BTK protein[3]
- The *in vitro* inhibition of human B cells and basophils was assessed in human blood for covalent and reversible BTKis.[3] The durability of B cell inhibition was compared between the covalent BTKi remibrutinib and the reversible BTKi fenebrutinib after drug washout *in vitro* with human peripheral blood mononuclear cells (PBMC)
- Selectivity across more than 456 human kinases was determined for all BTKis at 1 µM in a KINOMEscan® screen to allow for direct comparison of covalent and reversible BTKis.[3] For a panel of selected kinases, dissociation constants (Kd) were determined[3]

Results

BTK occupancy of covalent BTKis in human blood *in vitro*

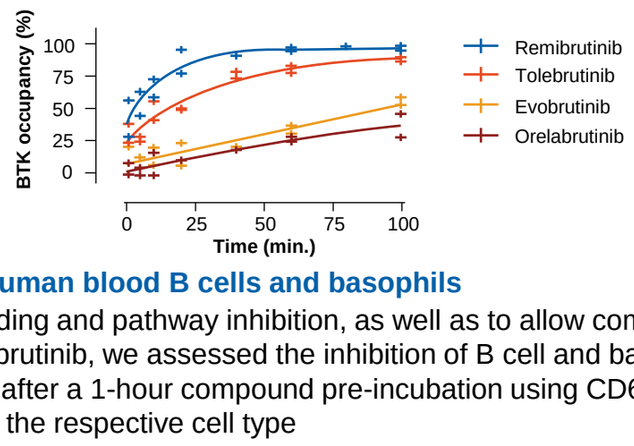
- In human blood, covalent inhibitors showed time- and concentration-dependent BTK binding *in vitro* (**Figure 1;** data from two independent donors and experiments). The 50% inhibitory concentration (IC_{50}) at 1 hour was 21 nM for remibrutinib, 508 nM for evobrutinib, 165 nM for tolebrutinib and 427 nM for orelabrutinib

Figure 1. Occupancy of covalent BTKis in human blood



- Remibrutinib showed a very rapid and complete covalent binding to BTK at concentrations as low as 300 nM, which is well within the range of blood levels reached in humans[4] **(Figure 2)**

Figure 2. BTK occupancy comparison at 300 nM



In vitro inhibition of human blood B cells and basophils

- To correlate BTK binding and pathway inhibition, as well as to allow comparison with the reversible BTKi fenebrutinib, we assessed the inhibition of B cell and basophil activation in human blood *in vitro* after a 1-hour compound pre-incubation using CD69 and CD63 as activation markers in the respective cell type
- Human blood IC_{50} values for B cell inhibition were consistent with BTK occupancy values at the1-hour timepoint with a similar pattern for basophil inhibition, suggesting that remibrutinib and fenebrutinib show more potent *in vitro* BTK pathway inhibition in human blood **(Table 1)**

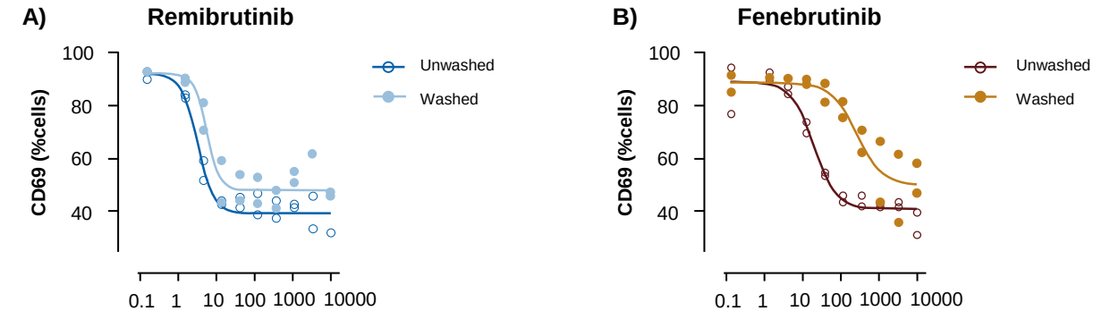
Table 1. B cell and basophil inhibition *in vitro*

Blood B cell CD69 IC_{50}, nM					
	Remibrutinib (n=6)	Fenebrutinib (n=8)	Evobrutinib (n=8)	Tolebrutinib (n=2)	Orelabrutinib (n=2)
Average (SD)	18 (5)	15 (15)	320 (74)	74 (30)	185 (21)
Blood basophil CD63 IC_{50}, nM					
	Remibrutinib (n=6)	Fenebrutinib (n=2)	Evobrutinib (n=4)	Tolebrutinib (n=2)	Orelabrutinib (n=2)
Average (SD)	67 (54)	22 (2)	1182 (819)	228 (58)	537 (20)

Effect of washout on B cell inhibition

- *In vitro* B cell inhibition by remibrutinib was not sensitive to drug washout; this was in contrast to the reversible BTKi fenebrutinib, which showed an approximate ten-fold loss of inhibition after brief washing of PBMC **(Figure 3)**

Figure 3. Durability of *in vitro* B cell inhibition after drug washout



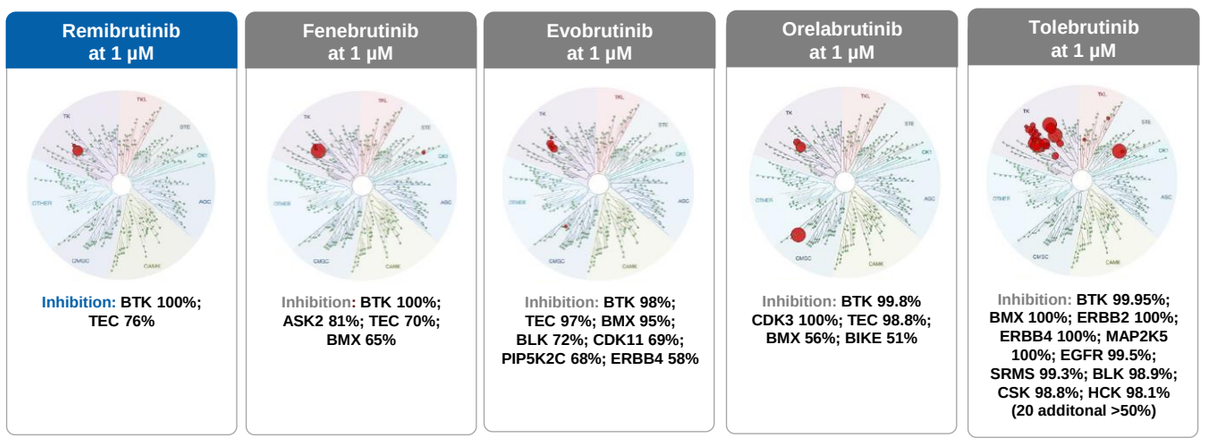
IC_{50} (nM)	Unwashed	Washed
	3.2	5.6

IC_{50} (nM)	Unwashed	Washed
	21.5	277

BTKi kinase selectivity

- Remibrutinib showed competition of probe binding to the lowest number of non-BTK kinases at 1 µM followed by fenebrutinib, evobrutinib, orelabrutinib, and tolebrutinib **(Figure 4)**
- A quantitative assessment of binding constants to a subset of kinases confirmed the very high potency and selectivity of remibrutinib, followed by fenebrutinib, tolebrutinib, orelabrutinib and evobrutinib **(Table 2)**

Figure 4. Kinase selectivity screen*



*KINOMEscan® screen: Dot size indicates extent of kinase binding (percent remaining probe binding vs control). The small green dots indicate inhibition below assay-relevant 35% threshold. Image generated using TREEspot™. Software Tool and reprinted with permission from KINOMEscan®, a division of DiscoveRx Corporation.

Table 2. Binding affinity to selected kinases

K_d (nM), Fold selectivity*	BTK	TEC	BMX	ITK	EGFR	ERBB2	ERBB4	JAK3
Remibrutinib	0.63	110 175x	540 857x	>10000 >15873x	>10000 >15873x	>10000 >15873x	>10000 >15873x	>10000 >15873x
Fenebrutinib	0.96	390 406x	210 219x	410 427x	>10000 >10417x	>10000 >10417x	>10000 >10417x	>10000 >10417x
Tolebrutinib	3.4	800 235x	3.8 1.1x	880 259x	20 5.9x	2.4 0.7x	5.1 1.5x	980 288x
Orelabrutinib	5.1	35 6.9x	450 88x	>10000 >1961x	2600 510x	7600 1490x	3000 588x	>10000 >1961x
Evobrutinib	16	4.5 0.3x	31 1.9x	3700 231x	7100 443x	>10000 625x	5600 350x	>10000 625x

*The higher the value, the better the selectivity between BTK affinity and off-target affinity. Binding affinities are based on KINOMEScan® Kd determinations, Eurofins/DiscoverX.

Conclusions

- *In vitro* target binding of covalent BTKis in human blood was time- and concentration-dependent. Remibrutinib showed the fastest and complete binding to BTK at low concentrations compared to other BTKis
- BTK binding in blood correlated well with the inhibition of BTK-dependent pathways under comparable *in vitro* conditions; as the potency for B cell and basophil inhibition showed a similar compound ranking with remibrutinib and fenebrutinib being more potent than the other BTKis
- Kinome-wide screening, as well as dedicated off-target binding quantifications showed that remibrutinib is highly selective than fenebrutinib, evobrutinib, orelabrutinib, and tolebrutinib
- Remibrutinib showed a highly favorable *in vitro* selectivity and potency profile which could translate to a best-in-class clinical profile for patients with MS

References

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Disclosures

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