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

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SUMMARY

1 **The potency and selectivity of covalent (remibrutinib, evobrutinib, tolebrutinig and orelabrutinib) and reversible (fenebrutinib) Bruton's tyrosine kinase inhibitors (BTKis) were assessed *in vitro* under comparable relevant experimental conditions**

2 ***In vitro* target binding of the covalent BTKis was time and concentration dependent, and remibrutinib showed the fastest and most complete binding to BTK at low concentrations compared with other BTKis; BTK binding in blood correlated well with the inhibition of BTK-dependent pathways**

3 **Compared with the other BTKis assessed in this analysis, remibrutinib and fenebrutinib demonstrated more potent BTK inhibition. Additionally, remibrutinib demonstrated higher selectivity and also a highly favorable *in vitro* selectivity and potency profile, which could translate to a best-in-class clinical profile for patients with MS**

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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; thus, 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 mouse models for MS is being presented at AAN 2023 (P013)

OBJECTIVE

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

METHODS

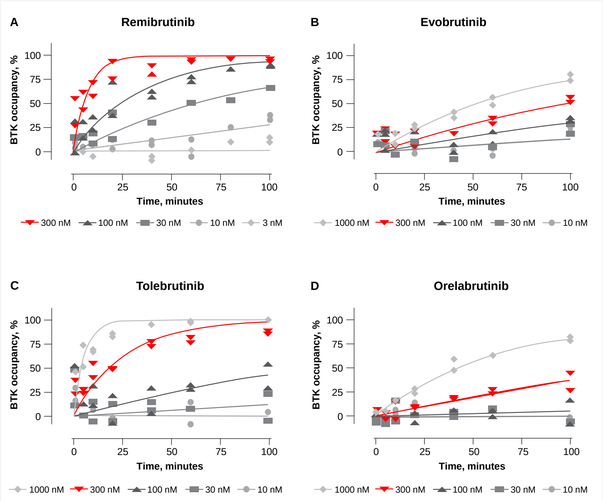
- In human blood, *in vitro* binding of covalent inhibitors to BTK was assessed over time and concentration, using immunoassays detecting free and compound-bound BTK protein[3]
- The *in vitro* inhibition of human B cells and basophils was assessed in human blood for covalent and reversible BTKis.[3] 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 (PBMCs)
- Selectivity across >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 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 2 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

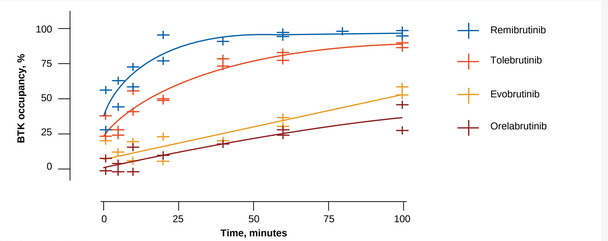
Figure 1. Occupancy of Covalent BTKis in Human Blood



BTK, Bruton's tyrosine kinase; BTKi, Bruton's tyrosine kinase inhibitor

- 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



BTK, Bruton's tyrosine kinase

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 preincubation 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 time point, 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

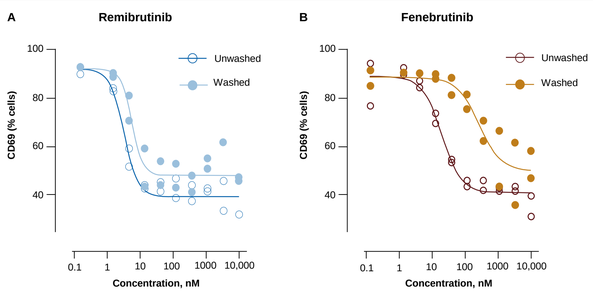
Blood B-cell CD69 IC_{50}, nM					
	Remibrutinib (n=6)	Fenebrutinib (n=8)	Evobrutinib (n=8)	Tolebrutinib (n=2)	Orelabrutinib (n=2)
Average (SD)	18 (5)	15 (15)	320 (74)	74 (30)	185 (21)
Blood basophil CD63 IC_{50}, nM					
	Remibrutinib (n=6)	Fenebrutinib (n=2)	Evobrutinib (n=4)	Tolebrutinib (n=2)	Orelabrutinib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

IC_{50}, 50% inhibitory concentration; SD, standard deviation

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 ~10-fold loss of inhibition after brief washing of PBMC (**Figure 3**)

Figure 3. Durability of *In Vitro* B-Cell Inhibition After Drug Washout



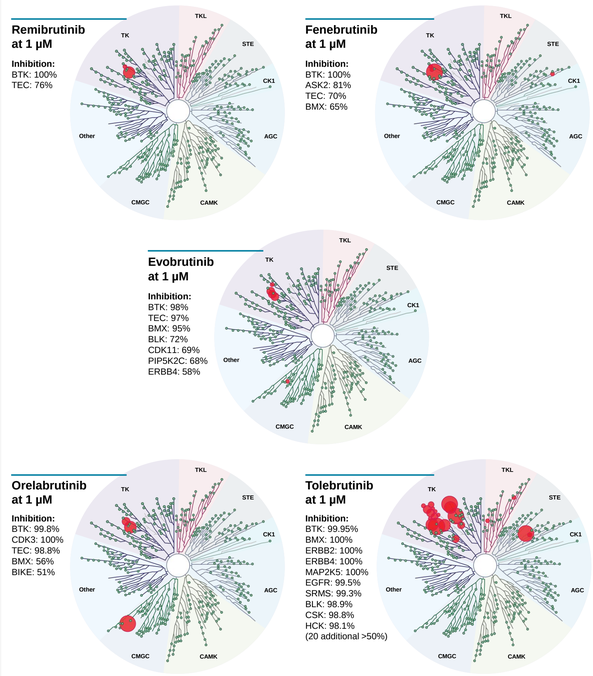
IC_{50}, nM	Unwashed	Washed
Remibrutinib	3.2	5.6
Fenebrutinib	21.5	277

IC_{50}, 50% inhibitory concentration

BTK/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*



BTK, Bruton's tyrosine kinase
*KINOMEscan® screen: Dot size indicates extent of kinase binding (percent remaining probe binding vs control). The small green dots indicate inhibition below the assay-relevant 35% threshold. Image generated using the TREEspot™ software tool and reprinted with permission from KINOMEscan®, a division of DiscoverX Corporation

Table 2. Binding Affinity to Selected Kinases

Kd, nM Fold selectivity*	BTK	TEC	BMX	ITK	EGFR	ERBB2	ERBB4	JAK3
Remibrutinib	0.63	110 175×	540 857×	>10,000 >15,873×	>10,000 >15,873×	>10,000 >15,873×	>10,000 >15,873×	>10,000 >15,873×
Fenebrutinib	0.96	390 406×	210 219×	410 427×	>10,000 >10,417×	>10,000 >10,417×	>10,000 >10,417×	>10,000 >10,417×
Tolebrutinib	3.4	800 235×	3.8 1.1×	880 259×	20 5.9×	2.4 0.7×	5.1 1.5×	980 288×
Orelabrutinib	5.1	35 6.9×	450 88×	>10,000 >1961×	2600 510×	7600 1490×	3000 588×	>10,000 >1961×
Evobrutinib	16	4.5 0.3×	31 1.9×	3700 231×	7100 443×	>10,000 625×	5600 350×	>10,000 625×

BTK, Bruton's tyrosine kinase; Kd, dissociation contstant
*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)

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