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Remibrutinib, a novel Bruton tyrosine kinase inhibitor, exhibits improved target selectivity and potency *in vitro*

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Introduction: Bruton's tyrosine kinase (BTK) is a key signaling node in B cell receptor and Fc receptor signaling. BTK inhibitors (BTKi) are an emerging oral treatment option for patients suffering from multiple sclerosis (MS). Several covalent and reversible BTKi are in clinical development for MS. For covalent enzyme inhibitors, *in vitro* assays are influenced by experimental conditions and are time dependent.
Objectives: To assess the potency and selectivity of BTKi under comparable experimental conditions.
Methods: In human blood, *in vitro* binding of covalent inhibitors to BTK was assessed over time and concentration. The *in vitro* inhibition of human blood B cells and basophils for the covalent and the reversible BTKi was assessed, as well as the impact of drug washout on *in vitro* B cell inhibition of the reversible compared to a covalent BTKi. Kinase selectivity of BTKi was assessed in a binding assay to allow direct comparison of covalent and reversible BTKi. Selectivity was first screened kinome-wide, followed by Kd (dissociation constant) determinations on selected kinases.
Results: Covalent inhibitors showed time- and concentration-dependent BTK binding *in vitro* in human blood with IC_{50} at 1 hour of 21 nM for remibrutinib, 508 nM for evobrutinib, 165 nM for tolebrutinib and 427 nM for orelabrutinib. These values correlated well with the *in vitro* B cell inhibition with IC_{50} of 18 nM for remibrutinib, 320 nM for evobrutinib, 74 nM for tolebrutinib, 185 nM for orelabrutinib, and 15 nM for the reversible fenebrutinib. Comparable potency was found for basophil inhibition. B cell inhibition *in vitro* by the covalent BTKi remibrutinib was not sensitive to washout in contrast to the reversible BTKi fenebrutinib. Kinome selectivity screening at 1 µM showed the following ranking: remibrutinib, fenebrutinib, evobrutinib, orelabrutinib and tolebrutinib (from least to most off-target kinase binding). The same pattern was confirmed in a quantitative assessment of binding constants to a subset of kinases.
Conclusions: BTKi currently in clinical development for MS exhibit a varying degree of selectivity across the human kinome with the highest selectivity seen for remibrutinib. Such a distinction may translate into differences in safety and clinical efficacy.
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