Remibrutinib exhibits improved target selectivity and potency *in vitro*

Short title: Remibrutinib *in vitro* selectivity and potency

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INTRODUCTION

Bruton's tyrosine kinase inhibitors (BTKi) are an emerging oral treatment option for patients with 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 was assessed for the covalent and the reversible BTKi, along with impact of drug washout on *in vitro* B cell inhibition. Kinase selectivity was assessed in a binding assay to directly compare covalent and reversible BTKi. Selectivity was screened kinome-wide, followed by Kd (dissociation constant) determinations on selected kinases.

RESULTS

Covalent inhibitors showed time- and concentration-dependent BTK binding in human blood with IC_{50} at 1 hour of 21 nM for remibrutinib, 508 nM for evobrutinib, 161 nM for tolebrutinib and 427 nM for orelabrutinib. These values correlated well with *in vitro* B cell inhibition with IC_{50} of 18, 320, 74, 185 and 15 nM for remibrutinib, evobrutinib, tolebrutinib, orelabrutinib, and the reversible fenebrutinib, respectively. Comparable potency was found for basophil inhibition. B cell inhibition *in vitro* by remibrutinib was not sensitive to washout, contrary to 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.

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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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Disclosure of conflict of interest

Marina Ziehn, Robert Pulz, Daniela Angst, Denis Eichlisberger, Bruno Cenni are employees of Novartis.

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