CG‘806, A NON-COVALENT PAN-FLT3/PAN-BTK INHIBITOR, EXHIBITS UNIQUE BINDING TO WILD TYPE AND C481S MUTANT BTK AND GREATER POTENCY THAN IBRUTINIB AGAINST MALIGNANT B CELLS

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INTRODUCTION

Bruton’s tyrosine kinase (BTK) is a validated drug target due to its role in B-cell malignancy development. Ibrutinib, an irreversible BTK inhibitor that covalently binds to cysteine residue 481 (C481) and is approved for chronic lymphocytic leukemia (CLL) and other B-cell malignancies, is limited by its adverse effects and resistance resulting from C481S or other mutations. A safe and potent inhibitor against all forms of BTK is needed for patients intolerant, refractory and resistant to ibrutinib. CG‘806 is an oral small molecule pan-FLT3/pan-BTK inhibitor, designed to solve ibrutinib’s shortcomings. It is in development for acute myeloid leukemia (AML) and B-cell malignancies.

OBJECTIVES

We compared CG‘806 and ibrutinib with respect to BTK binding mode, kinase inhibition profiles and cytotoxic activity against cultured and patient-derived malignant B-cells.

METHODS

• Crystallography: CG‘806 was co-crystallized with the kinase domain of wild type (WT) and C481S mutant forms of BTK.

• Biochemical kinase inhibition assay: CG‘806 was tested at 1 µM for biochemical inhibition of 583 kinases, and IC50s were determined on the most sensitive kinases with ATP concentration at Km and 1mM.

• The Safety44 panel (DiscoverX) was screened to identify potential off-target activities (ref #2).”

• Cytotoxicity assay: cultured malignant B-cell lines, BTK transfected Ba/F3 cells or freshly isolated mononuclear cells from patients were treated with CG‘806 or ibrutinib at the indicated concentration for 72 hr and MTS assay at the end.

• Western blotting: Vehicle or CG‘806 treated cell lysates were assayed by Western blotting for cell signaling pathways.

• NFkB activity: nuclear extract of vehicle or CG‘806 treated cells was assayed by TransAM NFkB colorimetric kit from Active Motif.

REFERENCES