### CG-806, a First-in-Class Pan-FLT3/Pan-BTK Inhibitor, Exhibits Broad Signaling Inhibition in Chronic Lymphocytic Leukemia Cells

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**Introduction**

Bruton tyrosine kinase (BTK) inhibitors, such as ibrutinib, have fundamentally changed treatment of chronic lymphocytic leukemia (CLL). However, ibrutinib is not curative and used as a long-term therapy with associated toxicities and the risk of developing drug resistance, which are the most common reasons for treatment discontinuation. Ibrutinib resistance is related to acquired ibrutinib-resistant BTK or PLCG2 mutations. CG-806 is an oral small molecule non-pan-BTK/pant-FLT3 inhibitor. CG-806 was structurally designed to target BTK without binding to C481 residual and target other oncogenic kinases. It is being developed for treatment of CLL/SLL, non-Hodgkin’s lymphomas and myeloid malignancies.

**Methods**

Peripheral blood mononuclear cells from patients with CLL were cultured for 14 days until outgrowth of nurse-like cells (NLC). Before treatment with inhibitors, non-adherent cells were harvested and re-plated on autologous NLC at a concentration of 1x10⁴ cells/cm². MEC-1 CLL cell line was incubated with escalating doses of CG-806 or ibrutinib for 72 hours. The IC₅₀ of CG-806 and ibrutinib were measured using TAC59 XTT cell proliferation/viability assay. The percentage and absolute number of viable MEC-1 and primary CLL cells were determined by flow cytometry with counting beads after staining with DOC6/propiolactone. For immunoblotting, CLL cells were pre-treated with 806 or ibrutinib for 2 hours and then stimulated with anti-lgM for 15 minutes. CLL cells co-cultured with NLC were treated with inhibitors for 24 hours before assessing B cell receptor (BCR) signaling by immunoblot and CLL3 and CCL4 concentration in the supernatants by ELISA.

**Disclosures**

Zhang – Aptose Biosciences, Inc (Employment); Thompson – AstraZeneca; Pharmaceutical Research Funding); Amgen (Consultancy and Research Funding); Genentech, Glaxo (Consultancy and Honoraria); Jain – AbbVie, Janssen Pharmaceuticals, Inc. (Consultancy); Pharmacia (Consultancy and Research Funding); Wieland – Genzyme (Consultancy); AbbVie, Actelion, Genentech, Glaxo, GSK, Novartis, Janssen, Juno, Karyopharm, Kite, Lasso, Miragen, Oncpem; Pharmacists; Suneesia, Kenzor (Research Funding); Rice – Aptose Biosciences, Inc (Employment, Equity Ownership and Membership of an Entity’s Board of Directors or Advisory Committee)

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**CG-806 hinders microenvironmental regulation of CLL B-cell survival**

A. CG-806 dose-dependently inhibited viability of primary CLL cells in co-culture with NLC

B. CG-806 decreased adherence of patient-derived NLC

C. CG-806 dose-dependently reduced CCL3, CCL4, CXCL13 in CLL/NLC co-culture

D. CG-806 inhibited SYK, BTK, AKT/S6 and MYC signaling in primary CLL cells in co-culture with NLC

E. CG-806 inhibited BCR/SYK/BTK/ERK, AKT/S6 signaling in primary CLL cells stimulated with anti-lgM

**CG-806 is more potent than ibrutinib to induce apoptosis of MEC-1 CLL cells**

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**Conclusions**

- CG-806 broadly inhibits BCR signaling in CLL cells, resulting in CLL cell apoptosis and reduced proliferation.
- CG-806 targets elements of the CLL microenvironment, i.e. NLC, and thereby potentially targets pro-survival signals from the microenvironment.
- These findings support further development of CG-806 in B cell malignancies, including CLL patients who are intolerant, refractory or resistant to ibrutinib or other covalent or non-covalent BTK inhibitors.
- CG-806 is currently in a Phase 1a/b trial (NCT0389682) for patients with CLL/NHL B-cell cancers including those intolerant, resistant, or refractory to ibrutinib, other covalent or non-covalent BTK inhibitors, or other therapies.