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

Ekaterina Kim[1], Hongying Zhang[2], Mariela Sivina[1], Alicia Vaca[1], Philip A. Thompson[1], Nitin Jain[1], Alessandra Ferrajoli[1], Zeev Estrov[1], Michael J. Keating[1], William G. Wierda[1], William G. Rice[2], Michael Andreeff[1], and Jan A. Burger[1]

[1] The University of Texas M.D. Anderson Cancer Center, Department of Leukemia, Houston, TX; [2] Aptose Biosciences, Inc., San Diego, CA.

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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-covalent pan-BTK/pan-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

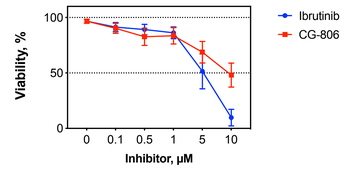
Peripheral blood mononuclear cells from patients with CLL were cultured for 14 days until outgrowth of nurse-like cells (NLC). Before treatment with inhibitors, non-adherent cells were harvested and re-plated on autologous NLC at a concentration of $1x10^7$ cells/mL. MEC-1 CLL cell line was incubated with escalating doses of CG-806 or ibrutinib for 72 hours. The $IC_{50}$ of CG-806 and ibrutinib were measured using TACS® 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 DiOC6/propidium iodide. For immunoblotting, CLL cells were pre-treated with CG-806 or ibrutinib for 2 hours and then stimulated with anti-IgM 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 CCL3 and CCL4 concentration in the supernatants by ELISA.

## Disclosures

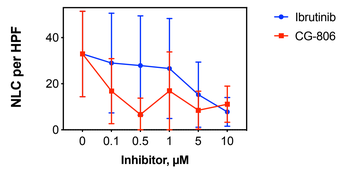
Zhang – Aptose Biosciences, Inc (Employment)
Thompson – AbbVie, Pfizer, Pharmacyclics (Research Funding); Amgen (Consultancy and Research Funding); Genentech, Gilead (Consultancy and Honoraria)
Jain – AbbVie, Janssen Pharmaceuticals, Inc (Consultancy); Pharmacyclics (Consultancy and Research Funding)
Wierda – Genzyme (Consultancy); AbbVie, Acerta, Cyclacel, Genentech, Gilead, GSK/Novartis, Janssen, Juno, Karyopharm, Kite, Loxo, Miragen, Oncternal, Pharmacyclics, Sunesis, Xencor (Research Funding)
Rice – Aptose Biosciences, Inc (Employment, Equity Ownership and Membership of an Entity's Board of Directors or Advisory Committees)
Andreeff – Daiichi-Sankyo, Amgen, AstraZeneca (Consultancy and Research Funding); Jazz Pharmaceuticals, Celgene, 6 Dimensions Capital (Consultancy); Aptose Biosciences, Inc (Equity Ownership and Membership of an Entity's Board of Directors or Advisory Committees); Reata, Eutropics, SentiBio, Oncoceutics, Oncolyze (Equity Ownership); Breast Cancer Research Foundation, CPRIT, NIH/NCI (Research Funding); Centre for Drug Research & Development, Cancer UK, NCI-CTEP, German Research Council, Leukemia Lymphoma Foundation (LLS), NCI-RDCRN (Rare Disease Clin Network), CLL Foundation, BioLineRx (Membership of an Entity's Board of Directors or Advisory Committees)
Burger – Aptose Biosciences, Inc, Gilead, Pharmacyclics (Research Funding); Janssen Pharmaceuticals (Consultancy)
Kim, Sivina, Vaca, Ferrajoli, Estrov, Keating – no relevant financial relationships to disclose

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

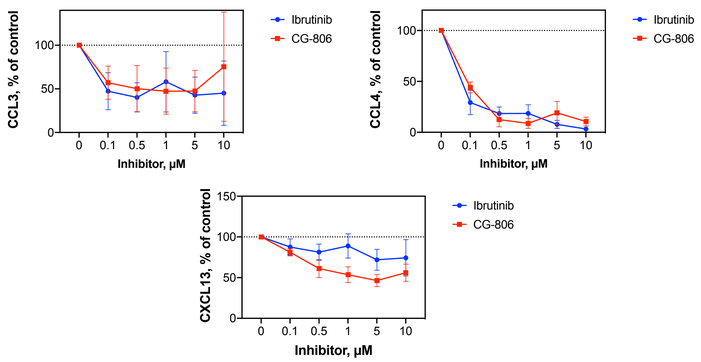
### A. CG-806 dose-dependently inhibited viability of primary CLL cells in co-culture with NLC




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

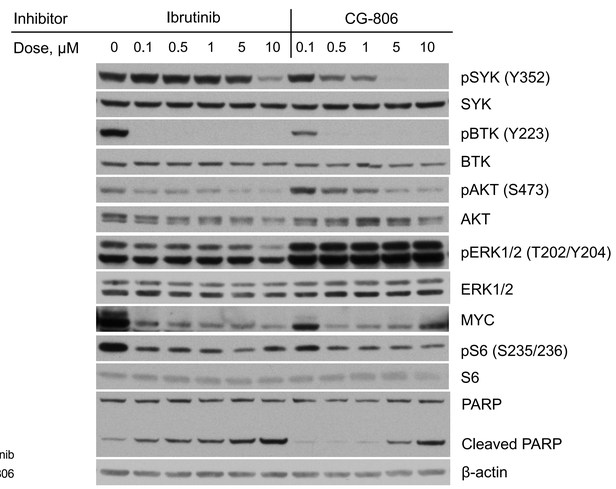



### C. CG-806 dose-dependently reduced CCL3, CCL4, CXCL13 in CLL/NLC co-culture



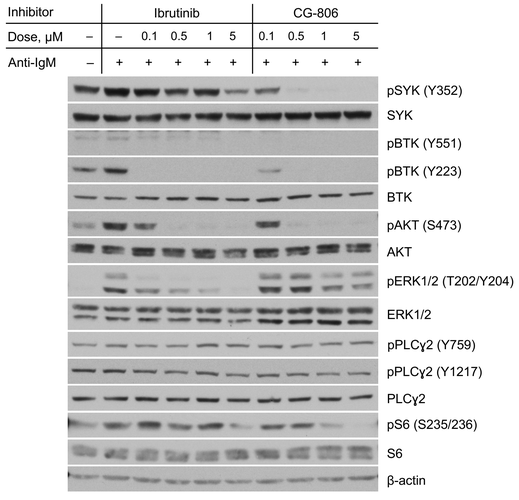

**GC-806 is active against CLL cells co-cultured with NLC.** (A) A fraction of viable nonadherent cells in CLL/NLC co-culture as measured by flow cytometry after 120 hours of exposure to CG-806 or ibrutinib. The mean with 95% CI, N=12. (B) Adherent NLC from the same co-cultures were counted under the microscope. The mean with 95% CI, N=12. (C) The levels of CCL3 and CCL4 were dramatically reduced in CLL/NLC co-culture after 24 hours of exposure to CG-806 or ibrutinib. The mean ± SEM, N=4. The level of NLC-derived CXCL13 was reduced as well under the same conditions. The mean ± SEM, N=4.

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



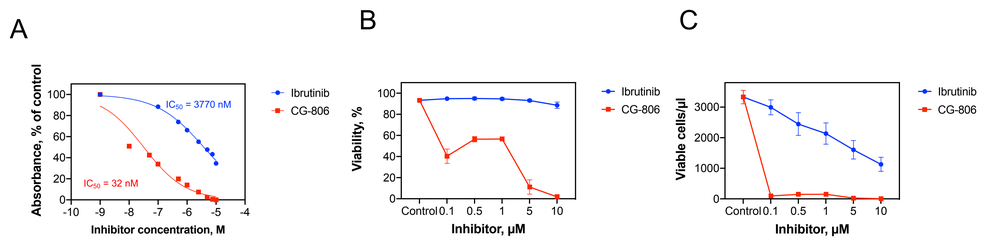

**GC-806 inhibits BCR signaling in CLL cells co-cultured with NLC.** CLL PBMC co-cultured with NLC were treated with GC-806 or ibrutinib for 24 hours. Presented here is a representative Western blot analysis.

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




**GC-806 inhibits BCR signaling in CLL cells.** CLL PBMC were pretreated with GC-806 or ibrutinib for 2 hours and then stimulated with anti-IgM antibody for 15 minutes. A representative Western blot analysis.

## CG-806 is more potent than Ibrutinib to induce apoptosis of MEC-1 CLL cells




**GC-806 induces apoptosis in MEC-1 CLL cells.** (A) $IC_{50}$ of GC-806 and ibrutinib in MEC-1 CLL cells were measured by XTT proliferation/viability assay after 72 hours of exposure to the increasing concentrations of inhibitors. (B) Fraction of viable MEC-1 cells and (C) absolute number of viable cells were determined by flow cytometry after 72 hours of incubation with CG-806 or ibrutinib. The mean ± SD.

## 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.**