#3503. CG-806, a First-in-Class pan-FLT3/pan-BTK inhibitor, Exhibits Broader and Greater Potency Than Ibrutinib Against Primary and Cultured Malignant B Cells

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APTOSE BIOSCIENCES

Abstract

Mantle cell lymphoma (MCL), chronic lymphocytic leukemia (CLL), follicular lymphoma (FL), and diffuse large B-cell lymphoma (DLBCL) account for >70% of B-cell lymphoma. Targeting Bruton tyrosine kinase (BTK) with ibrutinib in B-cell malignancies led to a paradigm shift in therapy. However, primary resistance to ibrutinib has been observed in ~30% of MCL patients; more than 50% of patients with CLL and MCL treated with ibrutinib discontinue treatment due to intolerance or emergence of resistant disease (Woyach *et al.*, 2017, Shpilberg *et al.*, 2018). CG-806 is an oral small molecule non-covalent pan-FLT3/pan-BTK inhibitor designed to address the shortcomings of ibrutinib. It is in development for acute myeloid leukemia (AML) and B-cell lymphoma.

CG-806 inhibited cell proliferation and induced apoptosis with a potency that was 50-6,000 times greater than that of ibrutinib when tested against 14 established malignant B-cell lines *in vitro*. When tested against 124 samples freshly isolated from the bone marrow of CLL patients the median IC_{50} for CG-806 was 0.11 µM and the median for ibrutinib was 4.09 µM, respectively, p<0.001. Since stromal-mediated signaling plays an important role in malignant B-cell survival and chemoresistance, the apoptotic effect of CG-806 was further analyzed on cultured and primary malignant B-cells in the presence of stromal cells. CG-806 produced a similar dose dependent apoptotic effect on Mino cells, and MCL cell line, in the presence or absence of human stromal HS-5 cells indicating that its potency was not impaired by factors released by these stromal cells. Most importantly, CG-806 dose-dependently induced apoptosis in ibrutinib-refractory primary MCL samples in the presence of CD40L-expressing stromal cells (n=4). Whereas, 0.1 µM and 1 µM CG-806 caused about 25% and 45% apoptotic cell death, respectively, 1 µM ibrutinib induced less than 10% cell death under the same culture conditions. CG-806 inhibited malignant B-cell colony formation 18-1000 fold and migration towards SDF1α 2-fold more effectively than ibrutinib. Given the role of activated B-cell receptor (BCR) and NFκB pathways in lymphoma, CG-806 was tested for it's ability to impair signaling in these pathways. CG-806 produced cell line dependent and dose/time dependent decreases in the phosphorylation of BTK, PLCγ2, PI3K, AKT, mTOR, and ERK. These effects were correlated with induction of PARP cleavage and cell cycle arrest.

We conclude that CG-806 inhibits driver and rescue pathways to directly and potently kill a broad range of malignant B-cells, including both established cell lines and freshly isolated patient samples, thereby distinguishing CG-806 from ibrutinib and supporting clinical development of CG-806 in patients with CLL and other B-cell malignancies intolerant, resistant, or refractory to ibrutinib.

Woyach J. J Clin Oncol. 2017 May 1;35(13):1437-1443.
Shpilberg O. Br J Haematol. 2018 May;181(3):306-319.

Materials and Methods

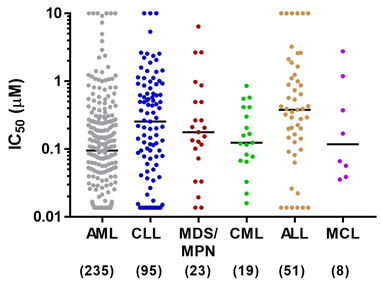
- Cytotoxicity assay - Cell viability of primary patient cells and cultured cell lines was measured by MTS assay and graphed as percent vehicle control.
- Cell migration towards SDF-1α was assayed in trans-well plates with the concentrations of CG-806 listed.
- Protection against CG-806 and ibrutinib by HS-5 stromal cells was assayed in either trans-well/indirect or co-culture/direct contact format.
- Colony forming assay – Number of colonies present after 10 days growth in Methocult™ media with Vehicle, CG-806, or ibrutinib at the concentration listed was calculated as percent vehicle control.

Disclosures: Zhang: Aptose Biosciences Inc.: Employment. Local: Aptose Biosciences Inc.: Employment. Benbatoul: Aptose Biosciences Inc.: Employment. Folger: Aptose Biosciences Inc.: Employment. Sheng: Aptose Biosciences Inc.: Employment. Rice: Aptose Biosciences Inc.: Employment.

CG-806 Kills Primary Malignant Cells from Patients: IDH1^{mutant} AML & FLT3-ITD AML are Hyper-Sensitive

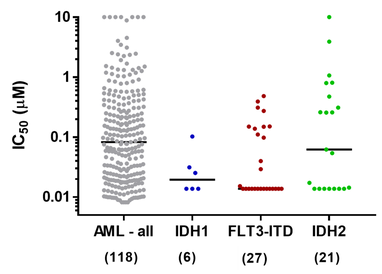
A.

Primary Cells From Patients with **Diverse Hematologic Malignancies** Highly Sensitive to CG-806



B.

Primary Cells from **AML** Patients with **IDH-1** Mutation or **FLT3-ITD** Mutation Hypersensitive to CG-806 Cytotoxicity



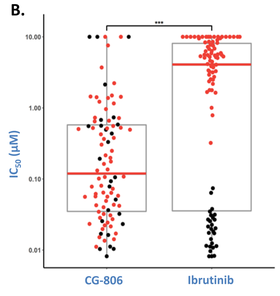
CG-806 kills a broad panel of malignant primary patient samples and cell lines. A) CG-806 potency against primary patient samples from AML and B-cell malignancies. B) CG-806 IC_{50} was measured ex vivo against AML patient samples expressing FLT3-ITD and IDH mutations.

CG-806 Superior to Ibrutinib In Vitro Against Malignant B-Cell Lines and Primary Samples from CLL Patients

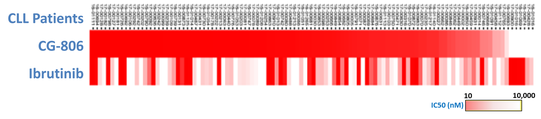
A.

Disease Type	Cell Line	IC_{50} (µM)		Fold difference
		CG-806	Ibrutinib	
MCL	Mino	0.006	18.2	3033
	Granta-519	0.020	29.9	1495
	Jeko-1	0.197	23.6	120
B-ALL	RS411	0.002	12.4	6200
	MHH-Call4	0.026	22.8	877
Burkitt's	Ramos	0.014	22.0	1571
	Daudi	0.236	23.9	101
ABC-DLBCL	OCI-Ly3	0.830	29.0	35
	U2932	0.632	8.9	14
	SU-DHL2	0.744	27.0	36
GCB-DLBCL	BJAB	0.882	1.8	2
	SU-DHL6	0.083	1.2	14
	RL	0.667	17.0	25
FL	DOHH2	0.003	0.441	147

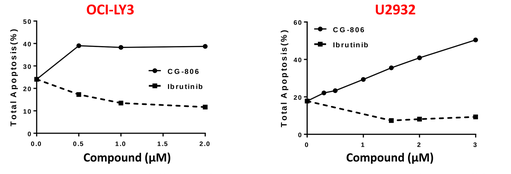
B.



C. CG-806 Superior to Ibrutinib at Killing Primary CLL Cells



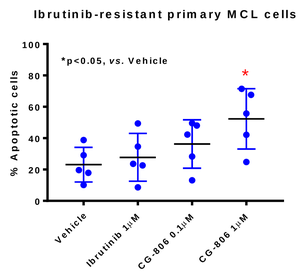
D. CG-806 Superior to Ibrutinib at Killing DLBCL Cell Lines



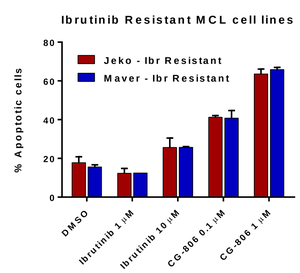
CG-806 superior to ibrutinib in killing malignant primary CLL patient samples, B-cell lines, and ibrutinib-insensitive DLBCL B-cell lines. A) CG-806 IC_{50} was measured in vitro against a panel of cancer cell lines by MTS assay B) CG-806 and ibrutinib sensitivity measured in 95 CLL patient samples. Median IC_{50} 0.114 µM and 4.09 µM respectively (***Wilcoxin signal rank test p<0.0001). Ibrutinib resistant samples are colored red. C) CG-806 and ibrutinib activity ex vivo against primary patient CLL samples. Heatmap D) Apoptosis was assessed with Annexin V / PI staining after 24 hr treatment of ibrutinib-insensitive B-cell line OCI-Ly3 and ibrutinib-sensitive cell line U2932 with either Vehicle or indicated concentrations of CG-806 or ibrutinib.

CG-806 Kills Ibrutinib-Resistant MCL Patient Cells

A.



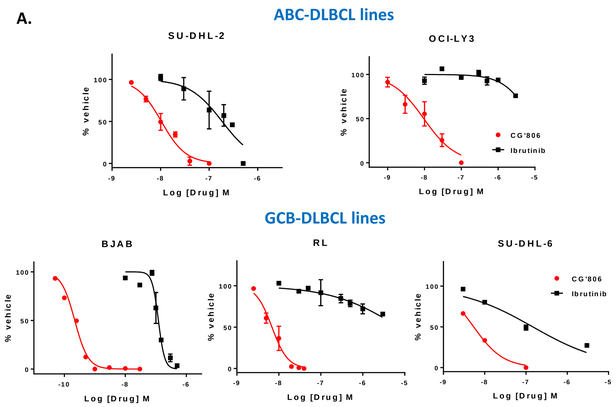
B.



CG-806 is effective against Ibrutinib resistant primary patient cells and cell lines. A) Ibrutinib resistant primary patient samples were cultured with stromal cells and treated with CG-806 or ibrutinib (at the concentrations listed) for 24 hr. Apoptosis was assayed by Annexin V staining N=5. B) Ibrutinib resistant cell lines were treated with either ibrutinib or CG-806 for 24 hr and apoptosis measured by Annexin V staining.

CG-806 Inhibits Clonogenicity, Migration, and Stromal Protection more Efficiently than Ibrutinib

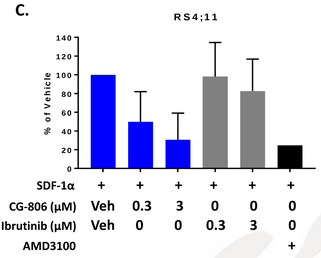
A.



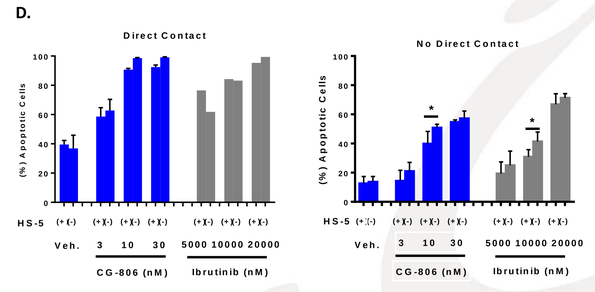
B.

Cell Line		IC_{50} (µM) CFU Assay	
		CG-806	Ibrutinib
ABC-DLBCL	SU-DHL-2	0.01	0.18
	OCI-LY3	0.01	8.11
GCB-DLBCL	SU-DHL-6	0.005	0.15
	RL	0.007	7.2
	BJAB	0.0002	0.12

C.



D.



CG-806 is more potent than Ibrutinib at inhibiting malignant B-cell colony formation, migration, and inducing apoptosis in the presence of stromal cells. A) CG-806 and ibrutinib inhibition of DLBCL colony formation in Methocult™ media after 10 days growth. Average of 2-4 replicates expressed as % vehicle treated. B) Comparison of CG-806 and Ibrutinib IC_{50} values in CFU assays. C) Inhibition of RS4;11 cell migration towards SDF-1α. N=4 for CG-806 and ibrutinib, graphed as % vehicle. D) Mino cells were incubated with HS-5 stromal cells in either direct contact (N=2) or trans-well assays (N=3) then CG-806 and ibrutinib dependent apoptosis was measured by Annexin V / PI staining after 72 or 48 hr respectively.

CG-806 Exhibits Favorable Safety Profile in GLP Toxicity and Toxicokinetic Studies

28-Day GLP Oral Gavage (Twice Daily) Repeat Dose Toxicity and Toxicokinetic Study with CG-806 in Mice and Dogs with a 2-Week Recovery

Mice:

Doses Tested:	60, 200, 600 mg/kg/day
Adverse findings:	
Clinical Signs	None
Food Consumption:	None
Clinical Pathology:	None
Anatomic Pathology:	None
NOAEL:	600mg/kg/day (highest dose tested)

Dogs:

Doses Tested:	60, 120, 240 mg/kg/day
Adverse findings:	
Clinical Signs	None
Food Consumption:	None
Clinical Pathology:	None
Anatomic Pathology:	None
Electrocardiogram (ECG)	No Changes
NOAEL	240mg/kg/day (highest dose tested)

Secondary Safety Evaluations:

Ames Genotoxicity Assay	Clean
Mouse Respiratory Safety Study	Clean
Mouse CNS Safety Study	Clean
Dog Cardiovascular Safety Study	Clean

Summary

- **Highly sensitive AML patient bone marrow cells:**
  - **IDH-1 mutant**
  - **FLT3-ITD mutant**
- **CG-806 is more potent than ibrutinib ex vivo:**
  - **Killing primary CLL patient cells**
  - **Inhibiting malignant B-cell clonogenicity**
  - **Inhibiting malignant B-cell migration**
  - **In stromal cell co-cultures**
  - **Ibrutinib-resistant primary MCL cells**
  - **Ibrutinib-resistant B-cell lines**
- **CG-806 shows favorable safety profile in IND-enabling GLP toxicity and toxicokinetic studies**
- **Potency and safety profile of CG-806 support its clinical investigation in patients with:**
  - **FLT3 inhibitor-resistant AML**
  - **IDH-1 mutant AML**
  - **CLL and other B-cell malignancies intolerant, resistant or refractory to ibrutinib or other BTK inhibitors**