CG-806, a pan-FLT3 / pan-BTK inhibitor, demonstrates superior potency against cells from IDH-1 mutant and other non-favorable risk groups of AML patients.

Hongying Zhang[1], Andrea Local[1], Jeffrey W. Tyner[2], Stephen E. Kurtz[3], Beth Wilmot[4], Shannon Mcweeney[4], Brian J. Druker[5], Stephen B. Howell[6], William G. Rice[1].
[1]Aptose Biosciences, Inc, San Diego, CA; [2]Oregon Health & Science University, Knight Cancer Institute, Portland, OR; [3]Knight Cancer Institute, Division of Hematology and Medical Oncology, Oregon Health & Science university, Portland, OR; [4]Knight Cancer Institute, Division of Bioinformatics and Computational Biology, Oregon Health and Science University, Portland, OR; [5]Knight Cancer Institute, Oregon Health & Science University, Portland, OR; [6]UC San Diego Moores Cancer Center, San Diego, CA

APTOSE BIOSCIENCES

AACR 2019, abstract # 1323

Abstract

CG-806 is a pan-FLT3 / pan-BTK inhibitor that is more potent (IC_{50} = 0.08 µM, n=265, p < 0.001) than other FLT3 inhibitors including midostaurin, sorafenib, sunitinib, dovitinib, quizartinib, crenolanib and gilteritinib against acute myeloid leukemia (AML) primary patient samples containing wild-type or mutated FLT3. CG-806 has significant activity against AML cell lines with or without FLT3 internal tandem duplication (ITD) / tyrosine kinase domain (TKD) mutations and in mouse AML xenograft models. Oral CG-806 has a desirable safety profile in the pre-IND studies of rodent and dog 28-day GLP toxicology, rodent respiratory and central nervous system safety, and the bacterial reverse mutation assay. The current study explored the relationship between genetic abnormalities in bone marrow and peripheral mononuclear cells isolated from AML patients and sensitivity to CG-806 using an ex vivo cytotoxicity assay. To correlate CG-806 sensitivity with clinical status, gene abnormalities and expression levels, whole exome sequencing (n=118) and RNA sequencing (n=111) were performed. CG-806 was equally potent against cells from patients in the adverse, intermediate and favorable risk groups (2017 ELN risk stratification), and cells from patients with relapsed or transformed AML (WHO classification) were as sensitive as those from patients with de novo AML. CG-806 had equivalent potency in cases of TP53 WT and TP53 mutations, whereas cases with TP53 mutations were resistant (FDR-corrected p<0.1) to most other FLT3 inhibitors including midostaurin, sorafenib, sunitinib, dovitinib, quizartinib and crenolanib. CG-806 had similar potency in cases with ASXL1 or SRSF2 mutations compared to WT, whereas sunitinib and crenolanib appeared resistant to ASXL1 and SRSF2 mutations, respectively. As expected, patient samples with FLT3-ITD mutation were more sensitive to CG-806 as compared to FLT3 WT (FDR-corrected p<0.01); in addition, cases with high ITD allelic ratio, including concurrent mutations with NPM1 and DNMT3A, had greater sensitivity than cases with low allelic ratio. Most unexpectedly, all 6 specimens containing IDH1 R132 mutations demonstrated significantly greater sensitivity to CG-806 relative to WT (FDR-corrected p<0.01), yet there was no increased sensitivity of IDH-2 mutant cells to CG-806. In conclusion, CG-806 demonstrated potency in primary AML patient samples across all AML subgroups including relapsed /refractory /transformed AML and those with genetic abnormalities related to poor prognosis. While patient samples with FLT3-ITD mutations were expected to have greater sensitivity to CG-806, the most surprising correlation was the sensitivity of patient samples with IDH1 R132 mutations. These features of CG-806 warrant investigation in the clinical setting.

Materials and Methods

***Ex Vivo Drug Sensitivity* Assay:** Inhibitor activity was assessed by an *ex vivo* assay to determine sensitivities of drugs on freshly isolated primary patient samples. Cell viability was assessed after 72-hour culture using a tetrazolium-based MTS assay and IC50 and are-under-curve (AUC) values calculated as a measure of drug sensitivity. Under the culture conditions used here, the cells retain viability (>90%), but do not proliferate.

FLT3 Mutational Status: For AML samples, mutational status of FLT3 for internal tandem duplications (FLT3-ITD) was assessed by PCR using forward primer 5'- AGCA ATT TAG GTA TGA AAG CCAGCTA – 3' and reverse primer 5' - CTT TCA GCA TTT TGA CGG CAA CC - 3'. PCR products were detected by capillary electrophoresis and quantified. Mutational status for FLT3-Tyrosine Kinase Domain (TKD) point mutations was determined by whole exome sequencing.

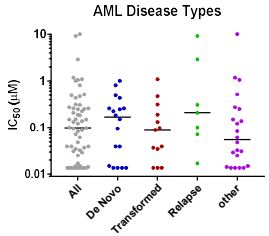
Whole exome sequencing and RNA sequencing and data processing: refer to Tyner, J. W. et al. *Nature* 562, 526–531 (2018)

Disclosures: H. Zhang: None. A. Local: None. J.W. Tyner: Research Funding; Aptose Biosciences, Inc. S.E. Kurtz: None. B. Wilmot: None. S. Mcweeney: None. B.J. Druker: Consultancy, Equity Ownership and Membership on an entity's Board of Directors or advisory committees; Aptose Biosciences, Inc. S. Howell: Membership on an entity's Board of Directors or advisory committees; Aptose Biosciences, Inc. W. Rice: Equity Ownership and Patents & Royalties; Aptose Biosciences, Inc.

CG-806 has superior potency against primary AML cells from patients in various risk groups

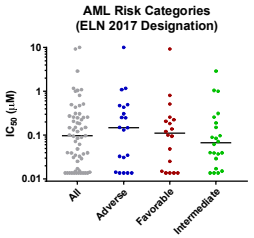
A.

Primary cells from patients with different **AML disease types (WHO classification)** are equivalently and highly sensitive to CG-806 (median IC_{50} = 0.098 µM).



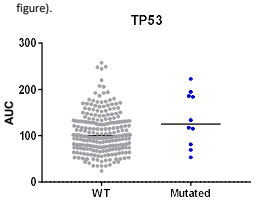
B.

Primary cells from AML patients at different **ELN (2017) risk categories** are equivalently and highly sensitive to CG-806.



C.

CG-806 has equivalent potency in primary AML cells with **TP53** WT and TP53 mutations, whereas cases with TP53 mutations were resistant to most other FLT3 inhibitors (highlighted in yellow in the right figure).



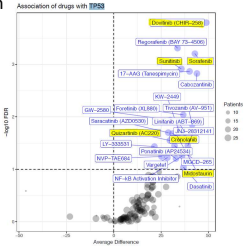
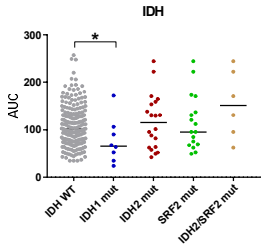


Figure adapted from Tyner, J. W. et al. *Nature* 562, 526–531 (2018)

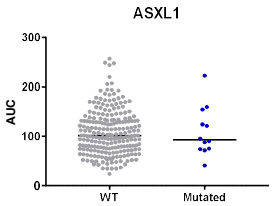
D.

AML cells from patient samples with **mutated IDH1** are more sensitive to CG-806 as compared to the cases of IDH WT or IDH2 mutations (p < 0.05).



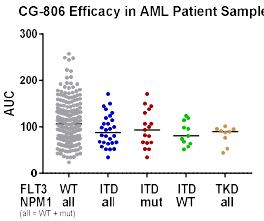
E.

CG-806 has equivalent potency in primary AML cells with **ASXL1** WT and ASXL1 mutations.



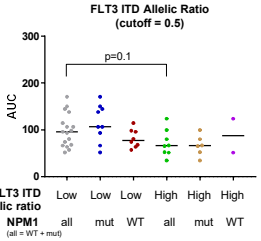
AML cells from patients with FLT3 ITD and TKD mutations are hypersensitive to CG-806

A. Primary samples from AML patients with FLT3 mutations (ITD or TKD), with or without concurrent mutations of NPM1, are more sensitive to CG-806 as compared to FLT3 WT (p < 0.05).

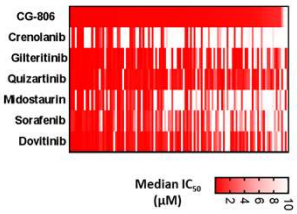


B. Sensitivity of primary cells from AML patients generally related to FLT3 ITD high allelic ratio (IC_{50} = 0.03 µM) vs. low allelic ratio (IC_{50} = 0.11 µM).

2017 ELN Risk category	NPM1	FLT3 ITD allelic ratio (cutoff = 0.5)
Favorable	Mutated	High
	Mutated	Low
Intermediate	Wild type	High
	Wild type	Low
Adverse	Mutated	High
	Wild type	High



C. CG-806 has greater potency to kill AML cells from patients than other FLT3 inhibitors.



Drug	Median IC50 (µM)
CG-806	0.102
Quizartinib	0.305
Gilteritinib	0.307
Dovitinib	0.620
Midostaurin	0.887
Sorafenib	2.479
Crenolanib	10.000

D. CG-806 has greater potency to kill Ba/F3 cells transfected with various FLT3 mutations than other FLT3 inhibitors.

FLT3 Inhibitor	IC_{50} in Transfected Ba/F3 cells (nM, n=3)				
	ITD	D835Y	ITD-F691L	WT	ITD-D835Y
CG-806	0.5	8.8	10.0	11.3	19.3
Quizartinib	2.2	2089.0	115.3	1956.0	246.4
Gilteritinib	26.5	472.5	98.4	500.3	6.8
Crenolanib	35.0	888.9	257.6	2617.0	31.7

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

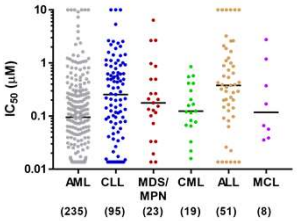
Adverse Findings	Doses Tested	
	60, 200, 600 mg/kg/day	60, 120, 240 mg/kg/day
	Mice	Dogs
Clinical Signs	None	None
Food Consumption	None	None
Clinical Pathology	None	None
Anatomic Pathology	-	None
Electrocardiogram (ECG)	-	No Changes

Secondary Safety Evaluation

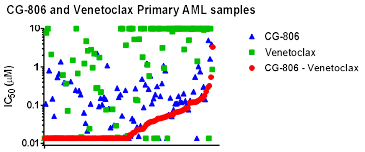
Ames Genotoxicity Assay	Clean
Mouse Respiratory Safety Study	Clean
Mouse CNS Safety Study	Clean
Dog Cardiovascular Safety Study	Clean

CG-806 potently kills diverse hematologic malignant cells and synergizes with Venetoclax

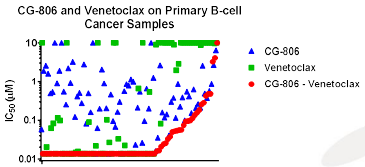
A. Primary cells from patients with diverse hematologic malignancies are highly sensitive to CG-806



B. CG-806 enhances killing of primary cells from AML patients when combined with Venetoclax.



C. CG-806 enhances killing of primary cells from B-cell cancer patients when combined with Venetoclax.



Conclusions

- **CG-806 demonstrates significant potency against primary AML cells from patients including:**
  - **Relapsed and transformed AML (WHO classification)**
  - **Adverse, intermediate and favorable risk groups**
  - **TP53 mutations**
  - **FLT3-ITD mutations: ITD (high and low allelic ratio, with and without concurrent NPM1 mutations)**
  - **FLT3-TKD mutations**
  - **IDH-1 mutations**
  - **ASXL1 mutations**
- **CG-806 kills AML cells more potently than other FLT3 inhibitors**
- **CG-806 potently kills primary cells from CLL B-cell cancer patients**
- **CG-806 enhances killing of primary AML cells and B-cell cancer cells when combined with Venetoclax**
- **CG-806 shows a 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:**
  - **Relapsed/refractory AML**
  - **FLT3 inhibitor-resistant AML**
  - **IDH-1 mutant AML**
  - **TP53 mutant AML and other non-favorable risk groups of AML**
  - **CLL and other B-cell malignancies**