CG'806, a First-in-Class FLT3/BTK Inhibitor, Exerts Superior Potency Against AML Cells Harboring FLT3-ITD, TKD, and Gatekeeper Mutant or Wild-Type FLT3

Weiguo Zhang[1], Hongying Zhang[2], Andrea Local[2], William G. Rice[2], Charlie Ly[1], Guopan Yu[1], Stephen B. Howell[3] and Michael Andreeff[1, 4]

[1]Section of Molecular Hematology and Therapy, Department of Leukemia, The University of Texas M. D. Anderson Cancer Center, Texas; [2]Aptose Biosciences, San Diego, California; [3]University of California, San Diego, California, Moores UCSD Cancer Center, La Jolla; The University of California at San Francisco, San Francisco, California, and [4]Department of Leukemia, The University of Texas M. D. Anderson Cancer Center, Texas, USA

Background

The receptor tyrosine kinase *FLT3* gene can undergo a series of mutations, including the activating internal tandem duplication (ITD) in the juxtamembrane region and point mutations in the tyrosine kinase domain such as at the activation loop residue D835 *(Thiede et al., 2002)*. FLT3 is widely accepted as a prime target for acute myeloid leukemia (AML) therapy, as the FLT3-ITD mutation is present in approximately 24% of AML patients and it is associated with very poor prognosis *(Kottaridis et al., 2003)*. However, additional acquired mutations of *FLT3*, including D835 or "gatekeeper" F691 mutations that have been identified in patients who showed resistance/relapse with the FLT3 inhibitors sorafenib or quizartinib *(Man et al., 2012; Smith et al., 2012)*, can render most FLT3 inhibitors ineffective. We also reported that aberrant upregulation of other parallel pro-survival signaling pathways may render AML resistant to FLT3-targeted therapy *(Zhang et al., 2014)*. *In addition*, targeting Aurora with Alisertib has also demonstrated encouraging clinical efficacy in recent Phase I trial against AML *(Fathi et al., 2017)*. CG'806 as a small molecule multi-kinase inhibitor against FLT3, Aurora and BTK kinases that is under development to treat FLT3-driven AML. A single test concentration of 25 nM in a 583 kinase panel, an IC_{50} analysis against 176 kinases, and a *Kd* analysis against 483 kinases illustrated the ability of CG'806 to target the entirety of FLT3-mutant enzymes and to inhibit additional kinases (e.g., BTK, AURK, STE group, and TRK/AXL/DDR group). Therefore, we hypothesize that CG'806 may provide potential for targeting FLT3-mutated AML, especially beneficial for targeting relapsed/refractory AML with FLT3 mutations.

Materials and Methods

Cell Lines: Anti-leukemia effects of CG'806 were evaluated in human or murine leukemia cell lines with FLT3 wild type (wt), FLT3 -ITD mutations, FLT3 TKD domain mutations or ITD plus TKD mutations.

IC_{50}s and EC_{50}s: Cell viability was assessed using the Trypan blue dye exclusion method or MTS assay, and apoptosis was determined via FACS by annexin V positivity. The 50% inhibitory concentration (IC_{50}) for cell growth inhibition and the 50% effective concentration (EC_{50}) for apoptosis induction were calculated using CalcuSyn (BioSoft, Cambridge, UK).

Immunoblot Assays: Cells were treated with CG'806 and collected for cell lysates. The total, and phosphorylated, levels of the indicated proteins were determined by western blot.

Animal study: Balb/c mice were injected (SQ) with human FLT3-ITD-mutated leukemia cells MV4-11, and treated orally (q.d.) with the indicated doses of CG'806 for 14 d. The anti-leukemia effect was assessed by measuring tumor burden. Oral toxicity was evaluated by monitoring body weight, etc. CG'806 concentrations in plasma were measured at the indicated time points after dosing at the first day.

Results

1) CG'806 Exerts Profound Cell Growth Inhibitory and Apoptotic Effects in FLT3-mutant, Including ITD, D835, Gatekeeper F691, and FLT3-WT AML Cells Mediated by Suppression of FLT3, BTK, and AURKs

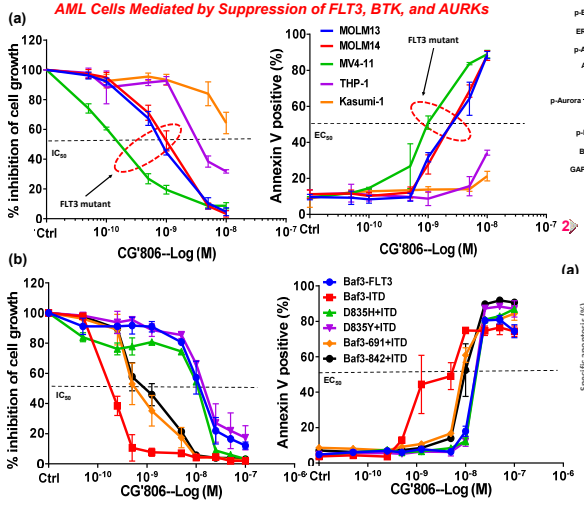

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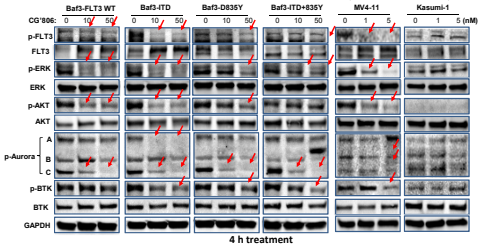
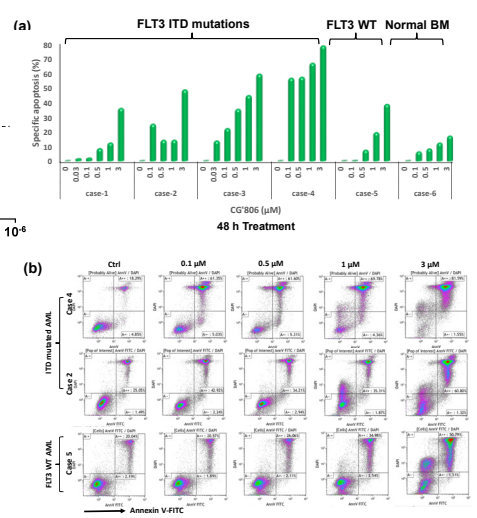
Table 1. Effectiveness of CG'806 Against Acute Leukemia Cells Harboring WT or FLT3 Mutations .

	Cell Line	Characteristics	IC_{50} (nM)	95% Lower/Upper	EC_{50} (nM)	95% Lower/Upper
Murine Cells	Ba/F3-FLT3	FLT3-WT	9.49	6.04/14.9	23.22	15.66/34.43
	Ba/F3-ITD	FLT3-ITD	0.30	0.07/1.29	5.60	2.31/13.58
	Ba/F3-D835G	FLT3-D835G	0.12	0.02/0.89	4.30	2.20/8.41
	Ba/F3-D835Y	FLT3-D835Y	8.26	4.18/16.30	15.46	8.22/29.09
	Ba/F3-ITD+691	ITD+F691L	0.43	0.31/0.61	14.65	8.84/24.28
	Ba/F3-ITD+842	ITD+Y842C	0.73	0.42/1.27	13.39	9.24/19.42
	Ba/F3-ITD+D835Y	FLT3-ITD+D835Y	9.72	5.46/17.30	22.01	9.51/50.95
	Ba/F3-ITD+D836H	FLT3-ITD+D835H	6.74	3.71/12.26	25.82	14.25/46.78
Human Cells	MOLM13	FLT3-ITD, t(9:11)	0.82	0.79/0.87	4.34	1.93/9.86
	MOLM14	FLT3-ITD, t(9:11)	0.92	0.76/1.11	3.90	2.61/5.84
	MV4-11	FLT3-ITD, t(4:11)	0.17	0.12/0.25	1.69	1.30/2.20
	THP-1	FLT3-WT, t(9:11)	3.88	2.16/6.98	NA*	
	Kasumi-1	FLT3-WT	21.99	16.38/29.53	NA*	

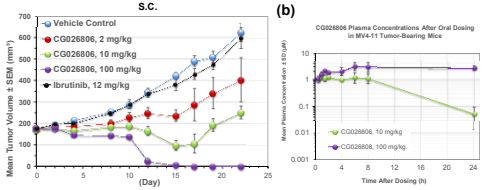
* NA = not reachable



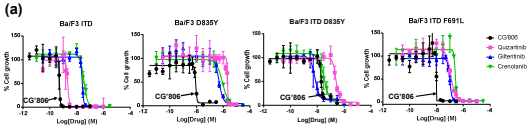
2) CG'806 Triggers Marked Apoptosis Induction in Primary AML Samples Harboring FLT3-ITD Mutations or WT FLT3



3) CG'806 Effectively Inhibits Leukemia Growth in a MV4-11 AML Xenograft Mouse Model with No Observed Oral Toxicity



4) CG'806 Shows Much Lower Half-maximal Inhibitory Concentration (IC_{50}) in AML Cells Compared to Other FLT3 Inhibitors



(b)

Table 2. Comparison of Antiproliferative Activity of CG'806 and Other FLT3 Inhibitors in FLT3 Mutated Cells.

FLT3 inhibitors	IC_{50} in Transfected Ba/F3 Cells (nM, n = 3)*			
	FLT3 ITD	FLT3 D835Y	FLT3 ITD+D835Y	FLT3 ITD+F691L
CG'806	0.5	8.8	19.3	10.0
Quizartinib	2.2	2089.0	246.4	115.3
Gilteritinib	26.5	472.5	6.8	98.4
Crenolanib	35.0	888.9	31.7	257.6

* Cell proliferation was measured with MTS Assay after 72h drugs treatment, and IC50s were calculated using GraphPad Prism 7.

Conclusions

- CG'806 exerts potent cell killing and induction of apoptosis in FLT3-mutated AML cells (i.e., ITD, TKD and gatekeeper mutants), including those unresponsive to other FLT3 inhibitors.
- CG'806 shows pronounced suppression of phosphorylation in target proteins such as FLT3, aurora kinase, and BTK in drug-sensitive cell lines.
- CG'806 triggers marked apoptosis in FLT3-ITD-mutated primary AML samples, but only minimal apoptosis in normal bone marrow cells.
- CG'806, administered orally, promotes tumor elimination in a S.C. MV4-11 xenograft murine (Balb/c) AML model without observable toxicity.
- CG'806 has lower nanomolar IC_{50}s in FLT3-inhibitor-resistant cell lines compared to quizartinib, gilteritinib, and crenolanib.
- CG'806 warrants further investigation for the treatment of patients with FLT3 mutated and WT AML.