The Pan-FLT3/BTK Multi-kinase Inhibitor CG'806 Induces AML Killing in FLT3-mutant and Wild Type Cells, and Exerts Synergistic Pro-apoptotic Effects with Concomitant Targeting of Anti-apoptotic Bcl-2 and/or Mcl-1.

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Abstract: 3348

Background

Internal tandem duplication (ITD) or point mutations of the Fms-like tyrosine kinase 3 (FLT3) are present in approximately 30% of patients with acute myeloid leukemia (AML) (Thiede et al., 2002). The ITD mutation is associated with very poor disease prognosis (Kottaridis et al., 2003) and additional acquired mutations of FLT3, including the D835 or the "gatekeeper" F691 mutation, have been identified in patients who developed resistance to FLT3 inhibitors (Man et al., 2012; Smith et al., 2012). In addition, the upregulation of FLT3 ligand (FL), resulting from conventional chemotherapy, or bone marrow (BM) microenvironmental-mediated protection of AML cells are also associated with resistance to FLT3-targeted therapy (Sato et al., 2011).

CG'806 is a first-in-class inhibitor that demonstrates sub-nanomolar activity against FLT3, Bruton's tyrosine kinase (BTK), aurora kinases, and certain other kinase family members in cell-free systems and in human and murine leukemia cells (Zhang et al., 2017). Here, we evaluated the anti-leukemia effect of CG'806 in leukemia cell lines harboring FLT3 ITD and TKD mutations, and further in a murine leukemia model engrafted with murine Baf3-FLT3-ITD cells. In addition, we investigated combinatorial regimens with Bcl-2 family inhibitors for enhancing the anti-leukemia effects of CG'806 against FLT3-mutated AML.

Materials and Methods

Cell Lines: Baf3 cells harboring FLT3 wild type (WT), ITD mutations and/or TKD domain mutations were used for evaluating anti-leukemia effects of CG'806, an oral, small molecular multi-kinase inhibitor of BTK/FLT3/AurK.

IC_{50}s and EC_{50}s: The 50% inhibitory concentration (IC_{50}) for cell growth inhibition (using Trypan blue dye exclusion method) and the 50% effective concentration (EC_{50}) for apoptosis induction (using flow cytometry for measuring annexin V positivity) were calculated using CalcuSyn (BioSoft, Cambridge, UK).

Immunoblot Assays: AML cells were treated with CG'806 at indicated concentrations and collected for immunoblot analysis.

Animal study: Murine leukemia model was established by injecting Baf3-FLT3-ITD cells via tail vein into NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NOG) mice. CG'806 (10mg/kg or 100mg/kg) treatment started from day 4 of leukemia cells engraftment via oral gavage following Qd x 5 days and 1 day off schedule. The anti-leukemia effect was assessed by measuring tumor burden. The vehicle served as control. The survival was estimated by the Kaplan-Meier method.

Combinatorial treatment: Leukemia cells were exposed in CG'806, Bcl-2 antagonist ABT-199 and/or Mcl1 inhibitor A1210477 for 48 h. Apoptosis induction were evaluated by measuring annexin V positivity using flow cytometry.

Results

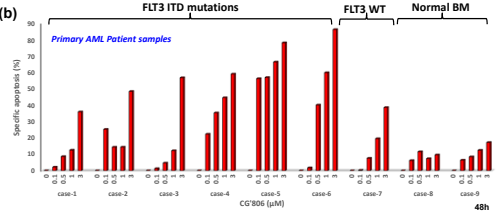
CG'806 Exerts Effective Anti-leukemia Effects in Both FLT3 Mutant and Wild Type Leukemia Cell Lines, and Primary AML Samples As Well

(a) Effectiveness of CG'806 Against Acute Leukemia Cells Harboring FLT3 Mutations or Wild Type.

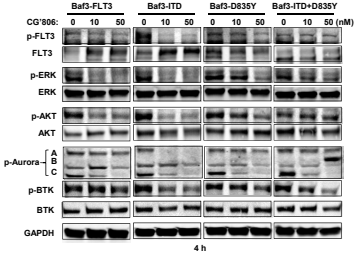
Cell Line	FLT3 Mutations	IC_{50} (nm)	95% Lower/Upper	EC_{50} (nm)	95% Lower/Upper
Ba/F3-FLT3	---(WT)	9.49	6.04/14.9	23.22	15.66/34.43
Ba/F3-ITD	ITD	0.30	0.07/1.29	5.60	2.31/13.58
Ba/F3-D835G	D835G	0.12	0.02/0.89	4.30	2.20/8.41
Ba/F3-D835Y	D835Y	8.26	4.18/16.30	15.46	8.22/29.09
Ba/F3-ITD+691	ITD+F691L	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	ITD+D835Y	9.72	5.46/17.30	22.01	9.51/50.95
Ba/F3-ITD+D835H	ITD+D835H	6.74	3.71/12.26	25.82	14.25/46.78

* 72h Treatment

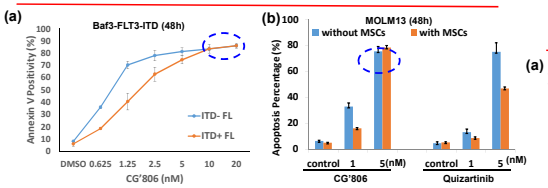
(b)



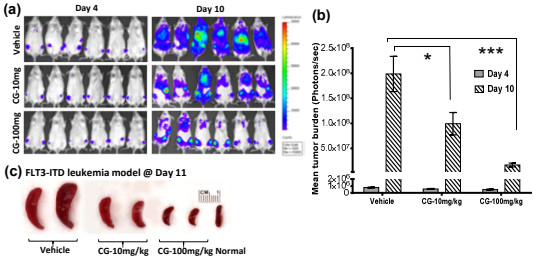
CG'806 Profoundly Suppresses Targeted Proteins Phospho-FLT3, -BTK, -Aurora, and Downstream Effectors Phospho-ERK and -AKT

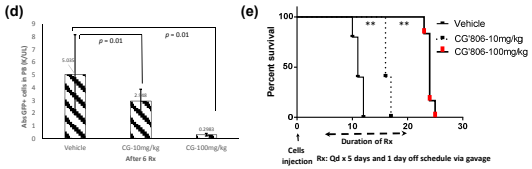


CG'806 Abrogates FLT3 Ligand- and MSCs-mediated Protection, and Triggers Apoptosis Induction in FLT3-mutated AML Cells

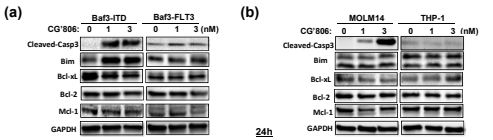


CG'806 Significantly Reduces Leukemia Burden and Extends Survival in a Dose-dependent Manner in Baf3-ITD Cell-engrafted Leukemia Model

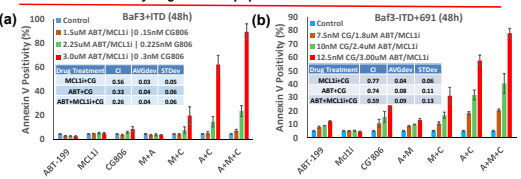




CG'806 Triggers ITD-mutated AML Cells Apoptosis by Increasing Cleaved-Casp3 and Bim, But No Effects on Anti-apoptotic Mcl-1/Bcl-2



CG'806 Combined with the Bcl-2 antagonist ABT199 and/or Mcl1 inhibitor A1210477 Exerts Synergistic Pro-apoptotic Effect in ITD- or F691-mutated Cells



Conclusions

- **CG'806 is effective in both FLT3 WT and mutant AML cells, including those harboring dual ITD plus D835/F691 mutations, which is apparently mediated through suppression of FLT3/BTK/AurK and their downstream signaling pathways.**
- **CG'806 Exerts pro-apoptotic effects in primary AML cells, but not in normal bone marrow cells.**
- **CG'806 significantly reduces disease burden, circulating blasts, and extends survival in a dose-dependent manner in Baf3-ITD-engrafted murine AML model.**
- **FL or BM stromal cells do not protect AML cells from CG'806-induced cell killing.**
- **Concomitant blockade of FLT3, Bcl-2 and/or Mcl-1 induces synergistic pro-apoptotic effects in FLT3-mutant leukemic cells including those resistant to quizartinib.**

* H. Zhang and W. Rice are employees of Aptose Biosciences; M. Andreeff serves on Aptose Biosciences SAB.