CG'806, a First-in-Class Pan-FLT3/Pan-BTK Inhibitor, Demonstrates Superiority to Other FLT3 and BTK Inhibitors Against Primary Patient Samples

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Abstract # 791

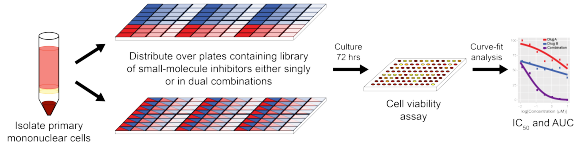
Introduction

While acute myeloid leukemia (AML) is a complex and heterogeneous malignancy, the most common mutation is the internal tandem duplication (ITD) of FLT3 occurring in ~30% of AML patients. Several FLT3 inhibitors have transient clinical benefit, lasting only 3-4 months, often due to emergence of drug resistant clones with additional mutations in FLT3. Thus, there is need for a pan-FLT3 inhibitor to control all mutant forms of FLT3, and to suppress diverse oncogenic clones. Likewise, overexpression of Bruton's tyrosine kinase (BTK) is a driver of chronic lymphocytic leukemia (CLL). Ibrutinib, a covalent BTK inhibitor approved for CLL and certain other B-cell malignancies, is limited by resistance resulting from mutation at cysteine residue 481 to serine (BTK-C481S), prompting a need for new agents to inhibit all forms of BTK.

CG'806 is a small molecule that inhibits specific clusters of related kinases, including the FLT3 cluster (FLT3 wild type (WT), FLT3-ITD and tyrosine kinase domain (TKD) point mutations, including D835G, D835Y, D835H, F691L and CSF1R), the BTK cluster (BTK WT, BTK-C481S, BLK and ITK), the TRK cluster (TRK-A/B/C) and others. We profiled CG'806 on primary samples from patients with AML, CLL and other hematologic malignancies to determine its activity and potency relative to other FLT3 inhibitors. CG'806 and ibrutinib were compared directly for sensitivities on primary CLL samples and various B-cell and other hematologic malignancies.

CG'806 exerted greater cell killing potency on a broader subset of AML samples relative to other FLT3 inhibitors, including midostaurin, gilteritinib, quizartinib, sorafenib, crenolanib, and dovitinib. CG'806 had greater activity on CLL primary samples than ibrutinib, which may be due to the dual activity of CG'806 on CSF1R (IC_{50}=0.6nM), a recently described target in CLL that provides a pro-tumor signal from nurse-like monocyte/macrophage lineage cells.

Ex Vivo Functional Assay



- 384-well format using 10,000 mononuclear cells/well
- Cell viability (MTS) performed on day 3
- Drugs tested in 7-point concentration series

***Methods*:**

***Ex Vivo Drug Sensitivity* Assay**
Inhibitor activity was assessed by an *ex vivo* assay to determine sensitivities of CG'806 and other FLT3 and BTK inhibitors on freshly isolated primary patient samples. Cell viability was assessed after 72-hour culture using a tetrazolium-based MTS assay and IC50 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 CCA GCTA – 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.

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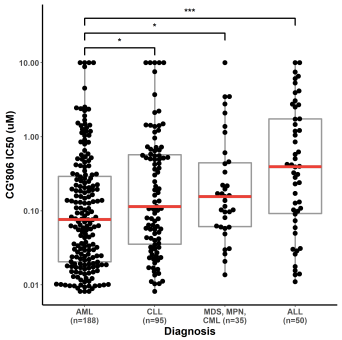
Sensitivity of CG'806 on Primary Patient Samples

Diagnosis Subgroups & # Patient Samples Tested			
AML	CLL	MDS/MPN	ALL
188	95	35	50

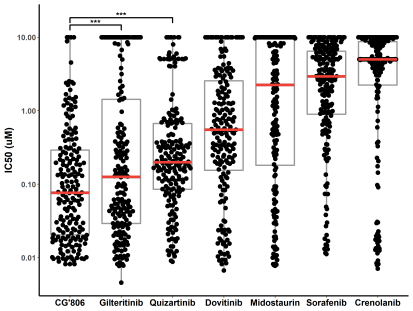
Across the four general subtypes of hematologic malignancies, primary patient samples were broadly sensitive to CG'806.

Median IC_{50}s for CG'806 were 0.076 µM and 0.114 µM on primary AML and CLL cells, respectively. CG'806 had median IC_{50}s of 0.156 µM and 0.395 µM on primary MDS/MPN and ALL cells, respectively.

Comparison between diagnosis subgroups by Mann-Whitney test indicates significant differences (* p<0.05; *** p<0.001).



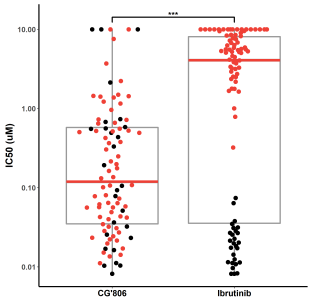
CG'806 has Greater Potency than other FLT3 inhibitors on Primary AML samples



CG'806 sensitivity was compared to FLT3 inhibitors on 188 AML patient samples. Median IC_{50} values are indicated in the table. The potency of CG'806 is significantly greater than all other FLT3 inhibitors tested (Wilcoxon Signed Rank test; *** p<0.001).

Drug	IC50 (uM)
CG'806	0.0765
Gilteritinib	0.125
Quizartinib	0.199
Dovitinib	0.551
Midostaurin	2.25
Sorafenib	2.93
Crenolanib	5.01

CG'806 has Greater Potency than Ibrutinib on Primary CLL specimens



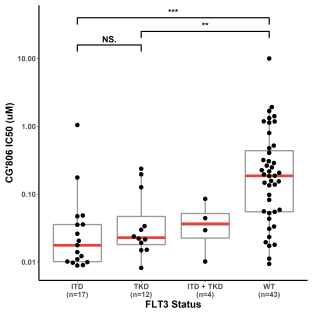
CG'806 sensitivity was compared to ibrutinib sensitivity on 95 CLL samples.

Median IC_{50} values for CG'806 and ibrutinib are 0.114 µM and 4.09 µM, respectively.

The difference is significant (Wilcoxon Signed Rank test; *** p<0.001).

CLL samples were broadly sensitive to CG'806, while the samples tended to segregate into groups sensitive or resistant to ibrutinib. Ibrutinib-resistant samples (IC_{50} >0.5 µM) are indicated in red.

CG'806 Sensitivity is Enhanced in AML samples with FLT3 mutations

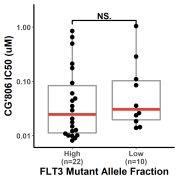


CG'806 sensitivity was compared across AML samples in the cohort for which FLT3 status was known. The median IC_{50} for samples with wild type FLT3 is 0.187 µM whereas FLT3-ITD or FLT3-TKD mutated samples had median IC_{50} values of 0.018 µM and 0.023 µM, respectively. Four patient samples had both mutations types (denoted ITD+TKD; median IC_{50} = 0.037 µM) yet remained sensitive to CG'806. Comparison between mutation type subgroups by Mann-Whitney tests indicate significant differences between ITD positive and wild type samples (p<0.001) and between TKD positive and wild type samples (p<0.01).

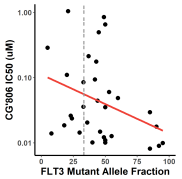
(** p<0.01; *** p<0.001; NS = not significant)

Correlation of CG'806 Sensitivity with FLT3-ITD allele frequencies

AML samples with FLT3-ITD mutations were classified as high (>33% allele frequency) or low (<33% allele frequency) following the ELN 2017 guidelines. The median IC_{50} for CG'806 on samples with high frequency FLT3-ITD is 0. 025 µM whereas the median IC_{50} on samples with low frequency FLT3-ITD is 0. 031 µM. Although the high frequency FLT3-ITD has a lower median IC_{50}, comparison by Wilcoxon Signed Rank test indicates there is no significant difference between high and low frequency groups. (NS = not significant)



IC_{50} values for CG'806 sensitivity were plotted against % Mutant Allele Fraction for FLT3-ITD positive samples. A modest correlation between increased CG'806 sensitivity (lower IC_{50} values) and higher allele frequencies is indicated by the trend line (red). Dashed line (grey) indicates 33% mutant allele frequency. (Spearman's r^2 = 0.039)



Conclusions

- CG'806 exerts potent cytotoxic activity on primary samples from patients with AML, CLL and other hematologic malignancies.
- The broad range of activity of CG'806 against hematologic malignancy cells results from its ability to inhibit multiple oncogenic pathways, supporting further development of CG'806 for AML, CLL and other hematologic malignancies.
- AML patient samples harboring FLT3-ITD and FLT3-TKD mutations demonstrated significantly enhanced sensitivity to CG'806 relative to wild type FLT3 patient samples. Increased sensitivity to CG'806 correlates with higher allele frequency for FLT3-ITD although the difference is not statistically significant within the sample size tested.
- These data reveal CG'806's enhanced activity relative to other kinase inhibitors currently approved for each indication and support further clinical development of CG'806 for both AML and CLL.