

# CG'806, a First-in-Class FLT3/BTK Inhibitor, Exhibits Potent Activity Against AML Patient Samples with Mutant or Wild Type FLT3, as well as Other Hematologic Malignancy Subtypes

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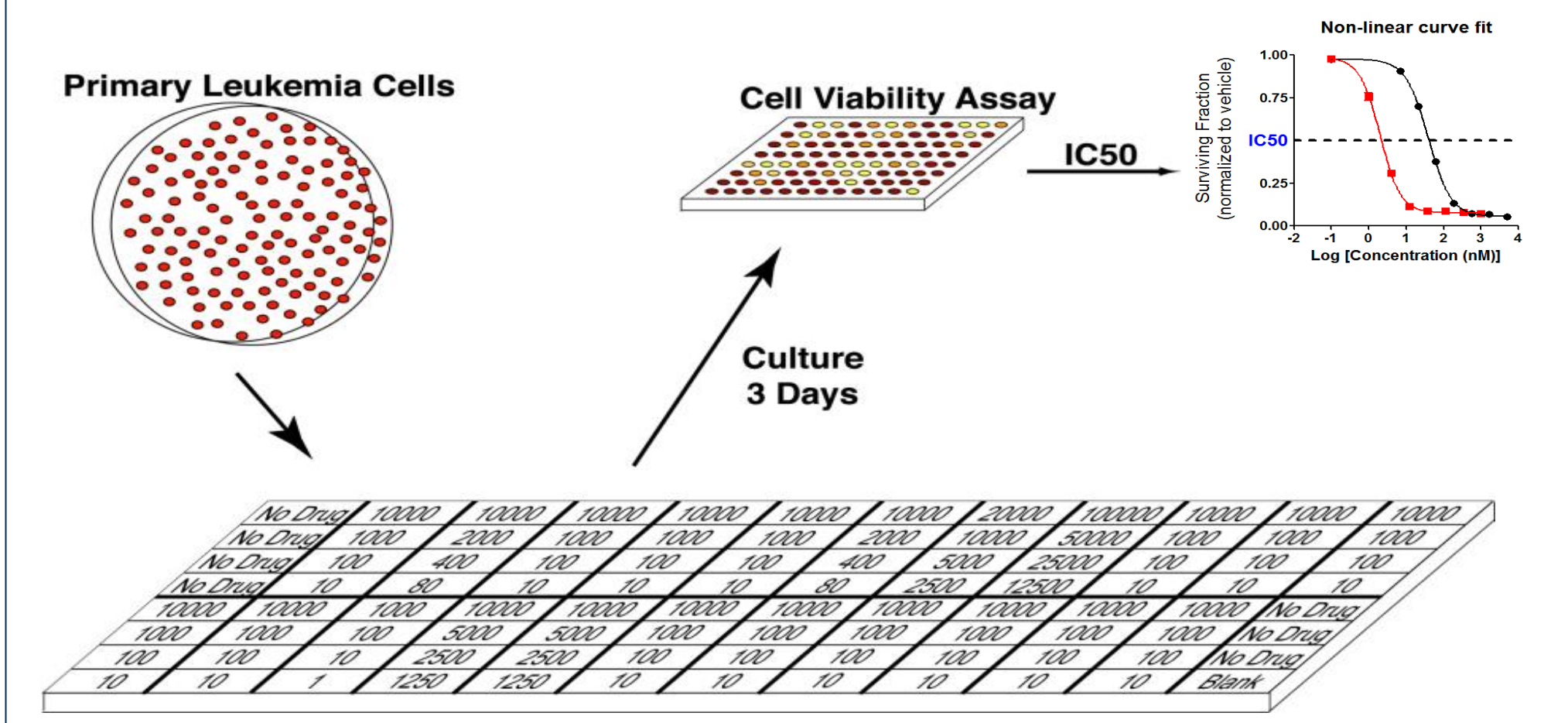


## INTRODUCTION

- ❖ Fms-like receptor tyrosine kinase 3 (FLT3) is expressed in ~90% of acute myeloid leukemia (AML), almost all B-lineage acute lymphoblastic leukemia (B-ALL), and some other hematologic malignancy subtypes.
- ❖ ~1/3 of AML patients harbor constitutive activating internal tandem duplication in FLT3 (FLT3-ITD), which is associated with very poor prognosis. Additional acquired point mutations have been identified, commonly at activation loop residue D835 and "gatekeeper" residue F691, which are related to drug resistance and relapse.
- ❖ CG'806, a first-in-class small molecule multi-kinase inhibitor against FLT3 and Bruton's tyrosine kinase (BTK), is under development as a next-generation agent for the treatment of FLT3-driven AML. We previously demonstrated that CG'806 retains full activity against Ba/F3 cells housing FLT3-ITD and/or point mutations, including D835G, D835Y, D835H and F691L.
- ❖ We now evaluate the potency of CG'806 on various hematologic malignancy cell lines and patient primary bone marrow specimens.

## Ex Vivo Drug Sensitivity Assay

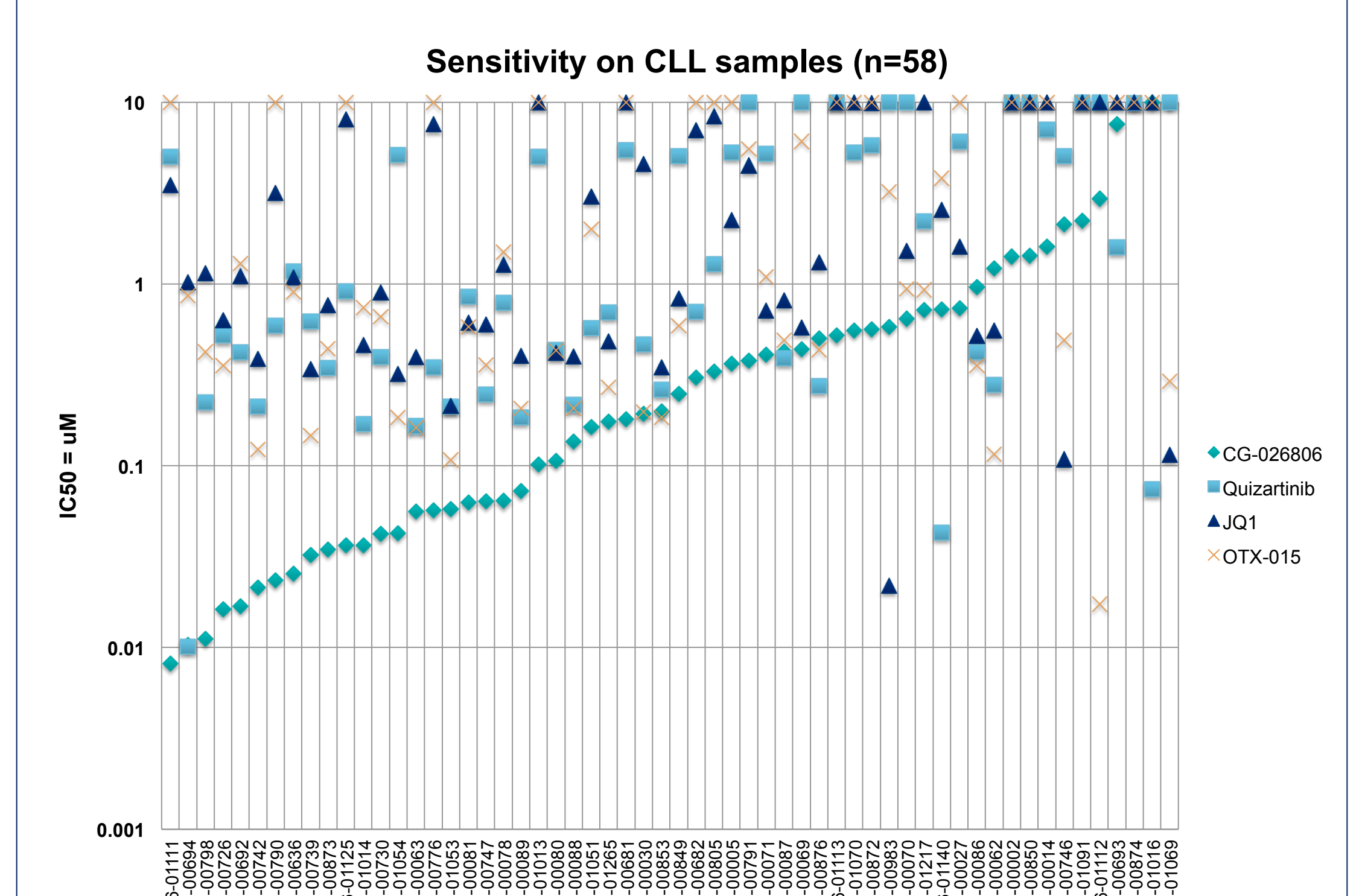
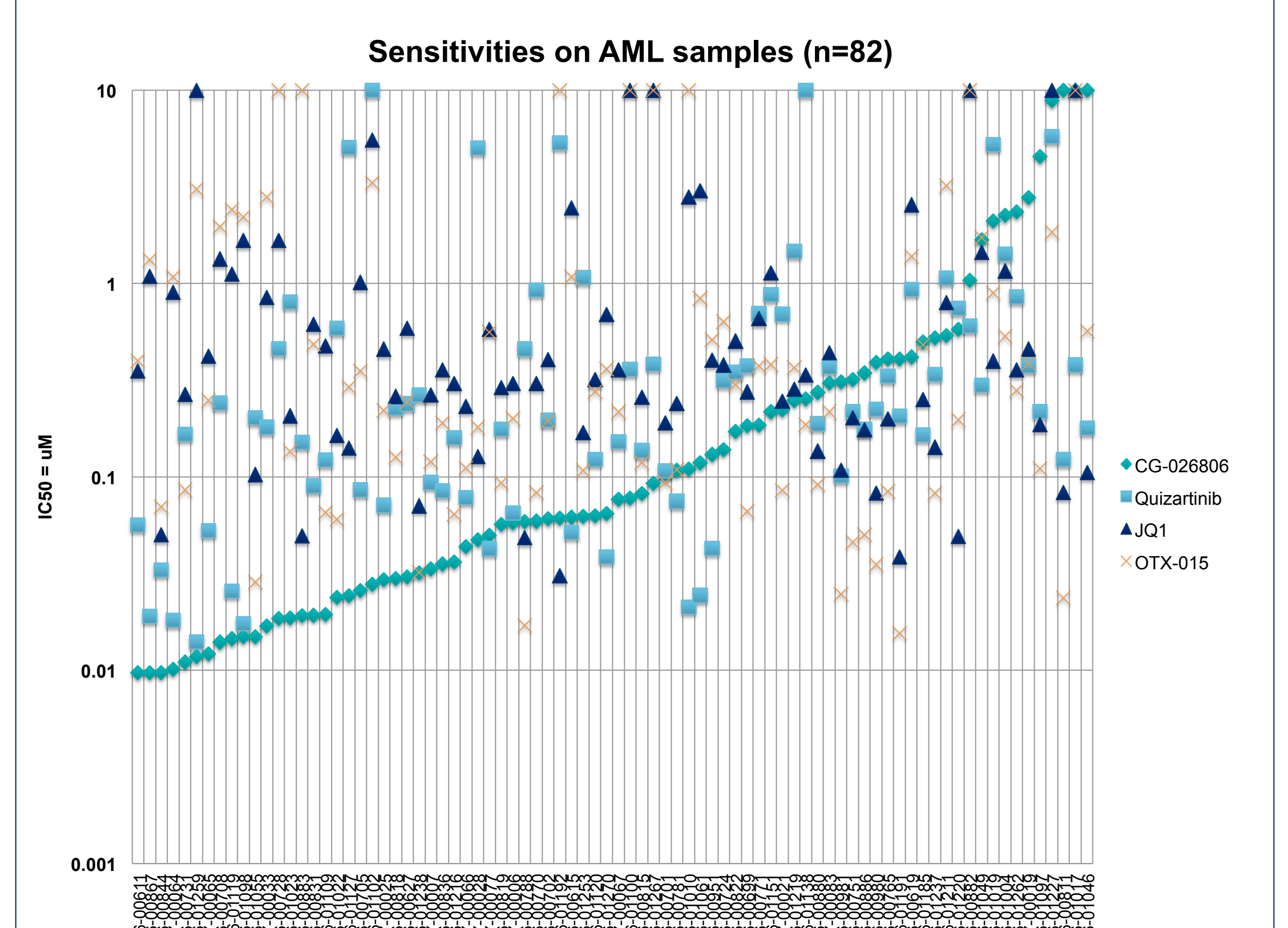
- ❖ Primary patient mononuclear cells were derived from 172 patients diagnosed with AML (n=82), acute lymphoblastic leukemia (ALL, n=17), myelodysplastic syndrome/myeloproliferative neoplasms (MDS/MPN, n=15), or chronic lymphocytic leukemia (CLL, n=58).
- ❖ We used an *ex vivo* drug sensitivity assay to determine the activity of CG'806, FLT3 inhibitor quizartinib, and bromodomain (BET) inhibitors JQ1 and OTX-015 across concentrations of each agent. Combinations were tested at a fixed, equimolar ratio over the same dose range. Cell viability was assessed using a colorimetric, tetrazolium-based MTS assay after a 3-day culture, and IC50 values were calculated.



## Primary AML and CLL Patient Samples Are Sensitive to Single-Agent CG'806

- ❖ Primary AML and CLL samples showed broad sensitivity to CG'806, with median IC50 values of 0.07 μM and 0.22 μM for AML and CLL, respectively.

% of samples sensitive to CG'806 (IC <sub>50</sub> < 0.1 μM)	
AML	CLL
59% (48/82)	40% (23/58)

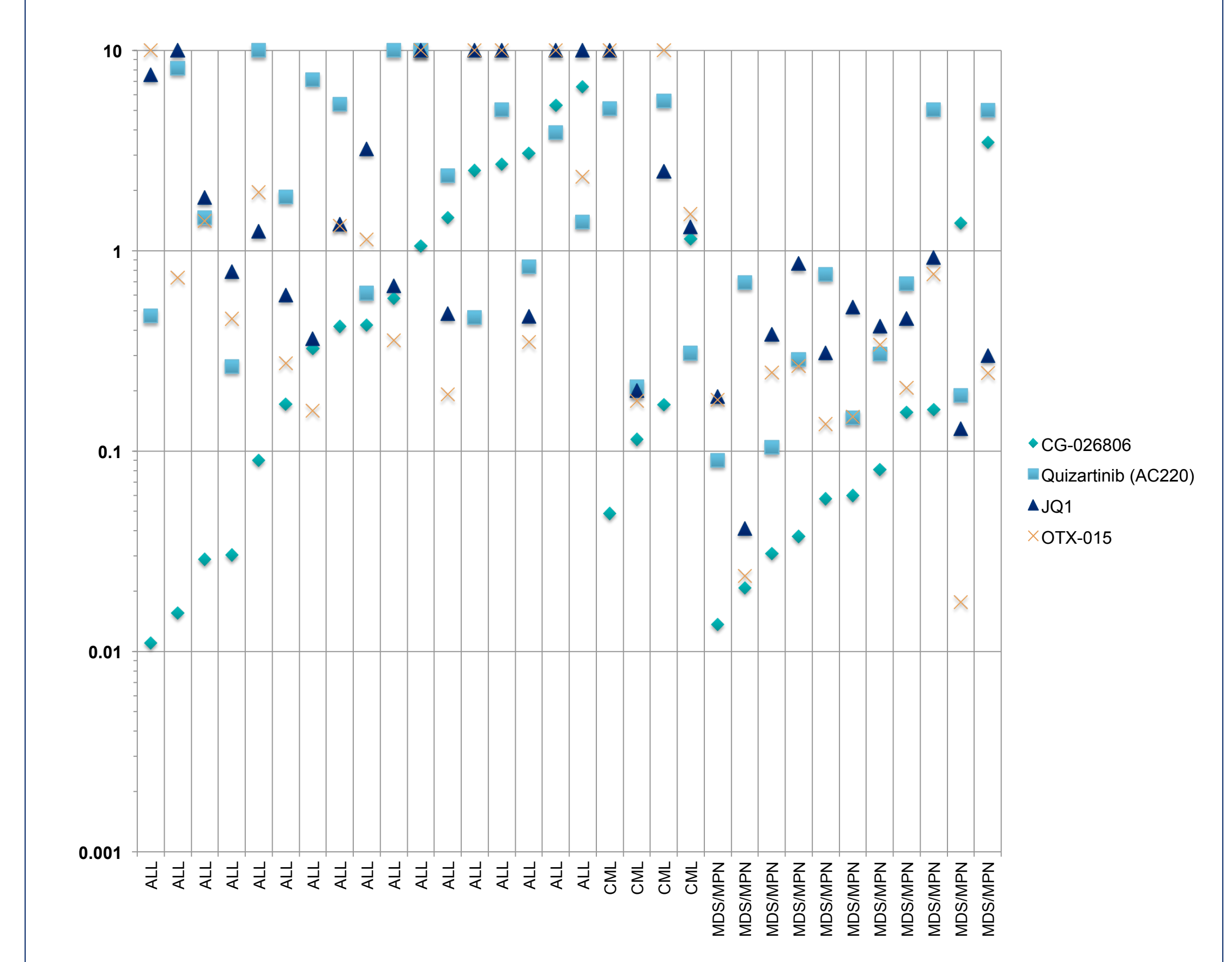


- ❖ FLT3 mutational status was known for 38 of the AML patient samples. FLT3-ITD+ AML samples tended to have enhanced sensitivity to CG'806 compared to the FLT3-WT samples

Sensitivities of AML samples with known FLT3 status to CG'806	
FLT3-ITD+ (n=8)	WT (n=30)
Median IC <sub>50</sub> = 0.02 μM	Median IC <sub>50</sub> = 0.12 μM

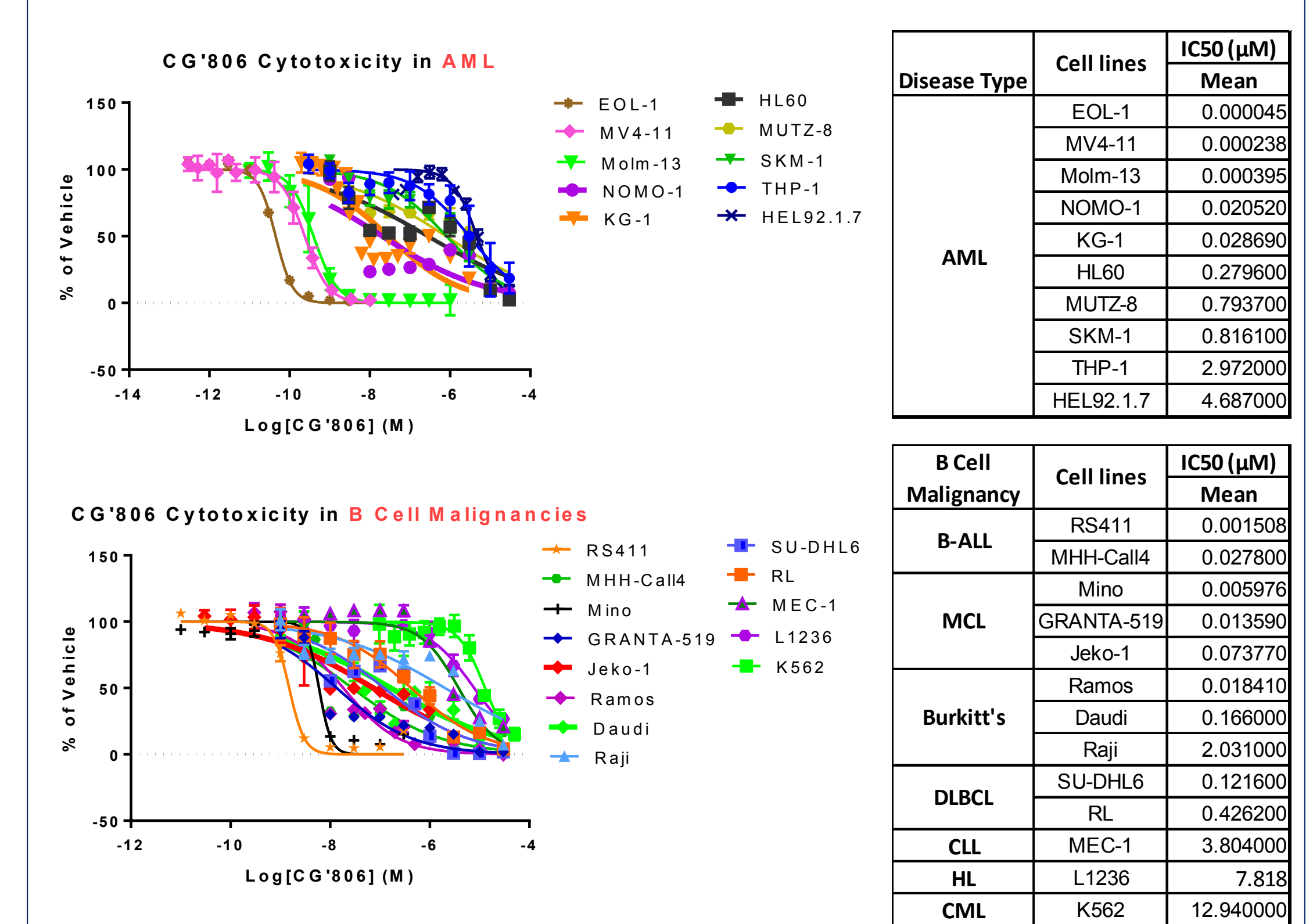
## ALL, CML, and MDS/MPN Samples Are Also Sensitive to CG'806

% of samples sensitive to CG'806 (IC <sub>50</sub> < 0.1 μM)		
ALL	CML	MDS/MPN
29% (5/17)	25% (1/4)	53% (8/15)



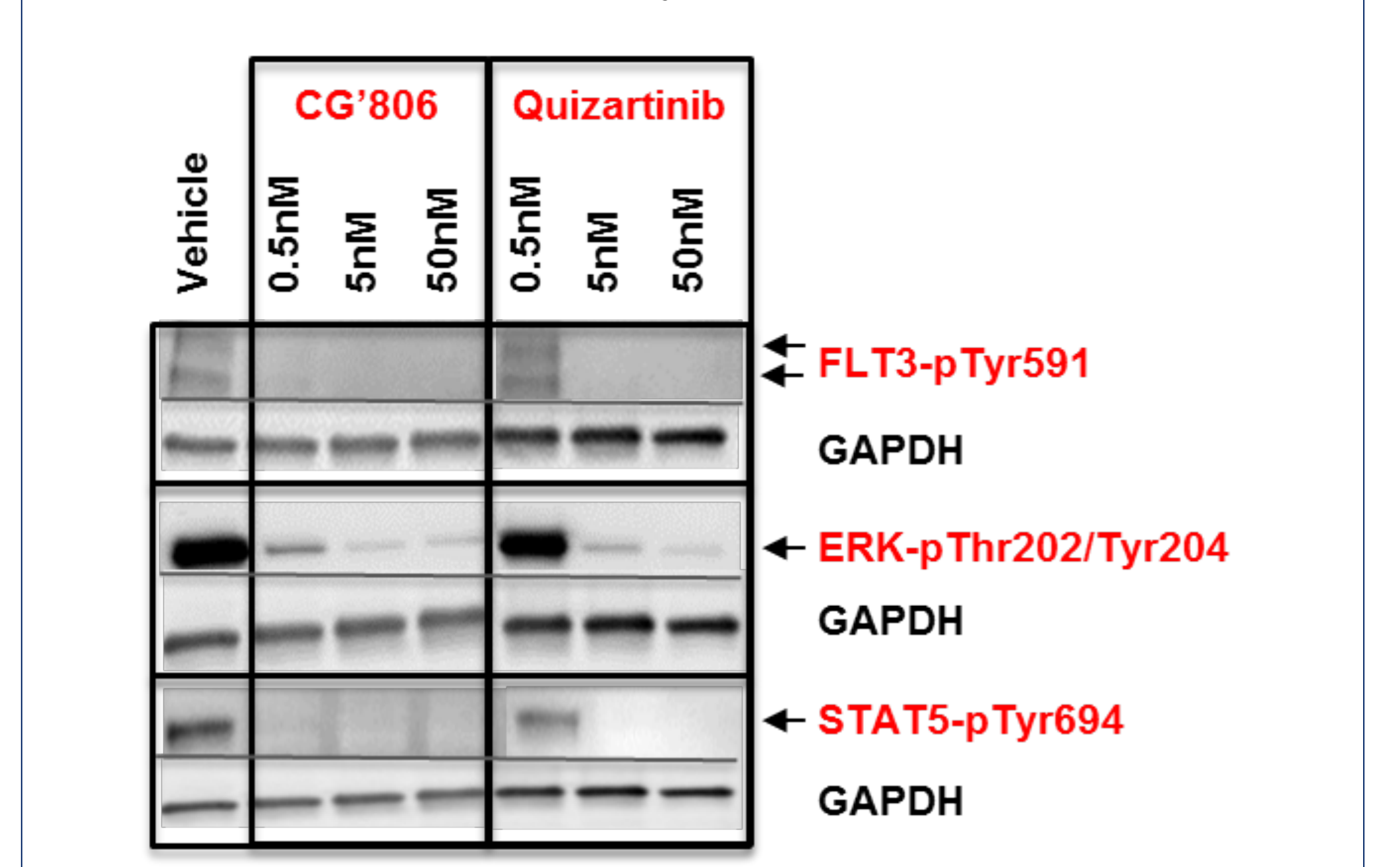
## CG'806 Inhibits Growth of AML and B-cell Malignancy Cell Lines

- ❖ AML (n=10) and B cell (n=13) malignant cell lines were treated with CG'806 or vehicle (DMSO) for 72 hours at 37°C, 5% CO<sub>2</sub>. Cell viability was quantified by MTS assay and to calculate IC50 values (n=3~10).



## CG'806 Inhibits FLT3 Signaling More Effectively than Does Quizartinib

- ❖ MV4-11 cells were treated with 500 pM CG'806, quizartinib, or vehicle (DMSO) for 1 hour and then subjected to Western blotting on FLT3 and its downstream signals (representative blots of three independent experiments).
- ❖ CG'806 completely inhibited phosphorylation of FLT3 and STAT5, whereas quizartinib only partially inhibited their phosphorylation.



## CONCLUSIONS

- ❖ CG'806 exhibits broad and potent activity against AML patient samples, as well as other hematologic malignancy subtypes.
- ❖ Median IC50 values for CG'806 indicate greater potency relative to Quizartinib and BET inhibitors across patient samples.
- ❖ Trend of greater sensitivity to CG'806 in FLT3 mutant AML cases compared with FLT3 wild type.
- ❖ These preclinical analyses of CG'806 support further development of this agent for hematologic malignancies.

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