



BCL2A1: A Novel Target in Refractory/Relapsed AML with *FLT3*-ITD/D835 Double Mutations

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Abstract

BCL2A1 exerts a pro-survival function in *FLT3*-ITD/D835-double mutant acute myeloid leukemia (AML) cells and may be a putative novel therapeutic target in *FLT3*-mutant AML. The combination of gilteritinib, which reduced BCL2A1 with venetoclax, was highly synergistic in *FLT3*-ITD/D835-double mutant cells..

Background & Objective

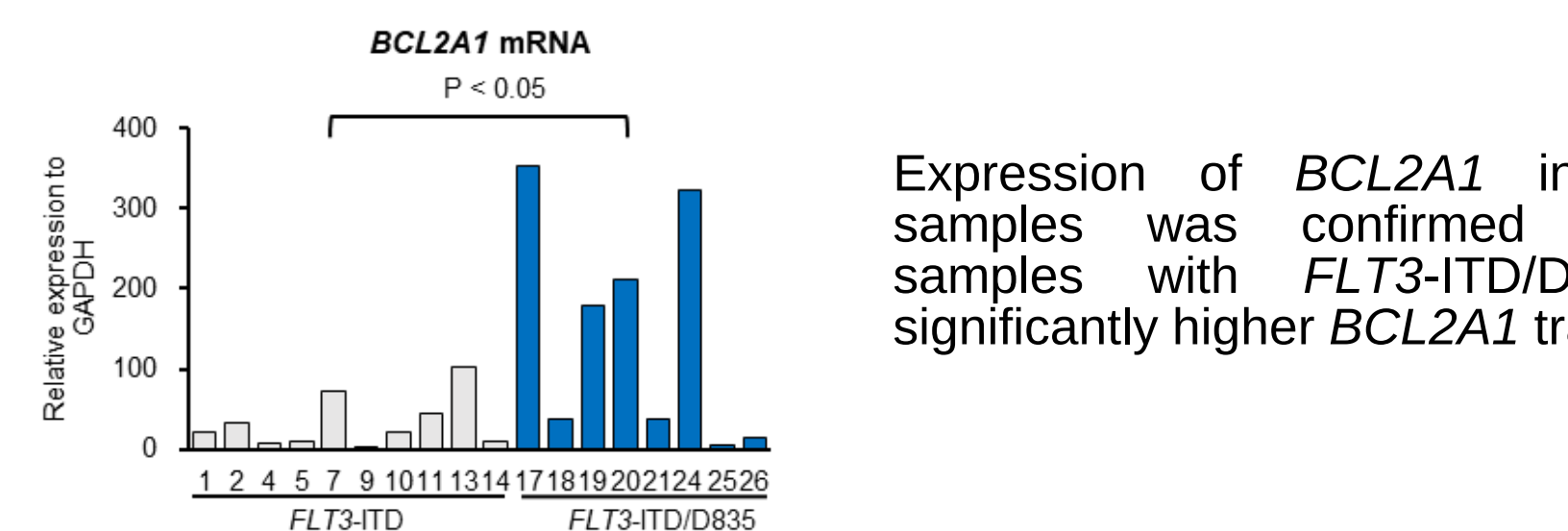
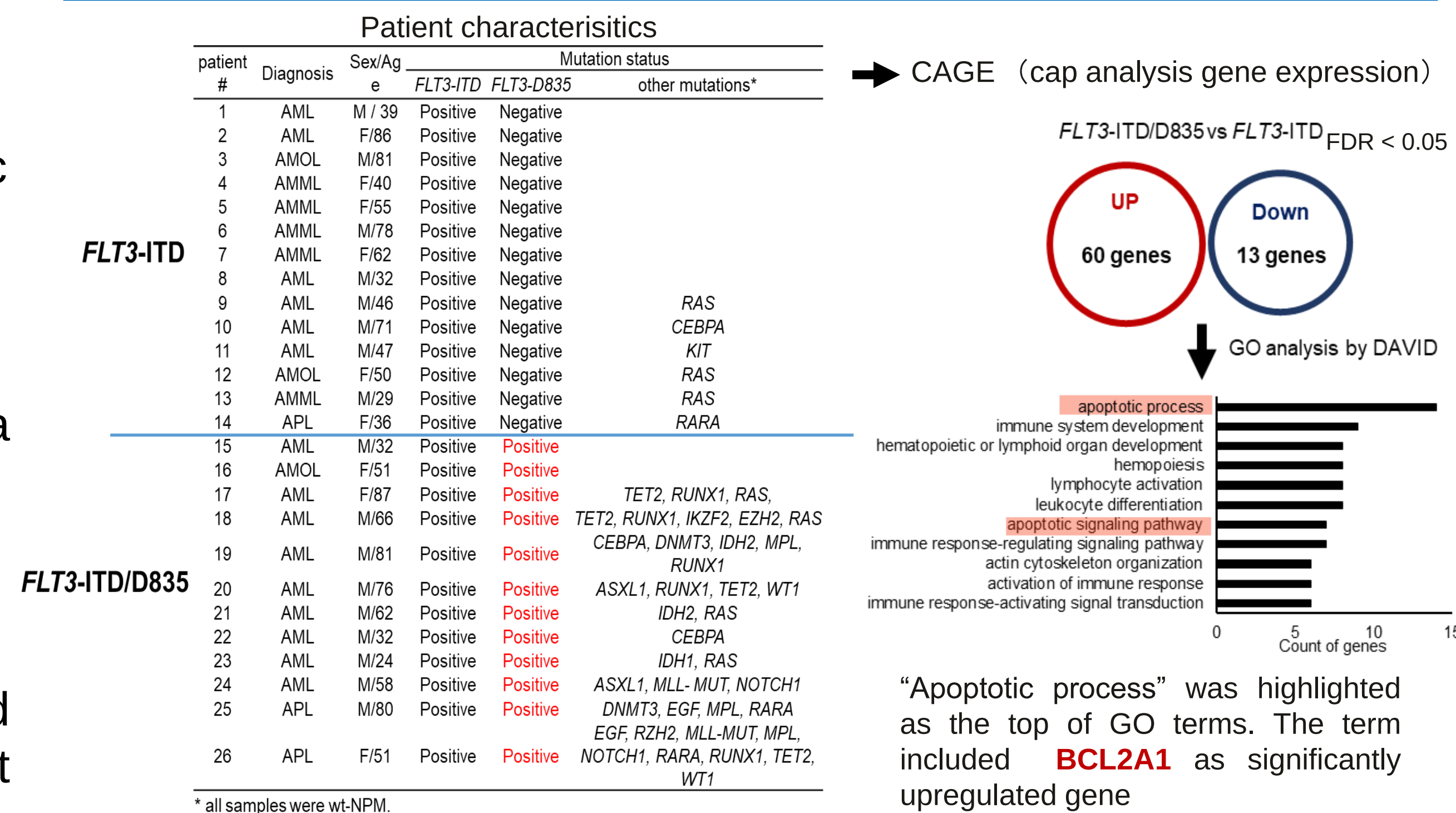
While *FLT3* tyrosine kinase inhibitors (TKI) emerged as a new therapeutic option in AML with internal tandem duplications in the juxtamembrane domain of *FLT3* (*FLT3*-ITD), the prognosis of *FLT3*-mutated AML is still poor because heterogeneous clonal populations with additional acquired *FLT3* and *Ras* mutations emerge and cause drug resistance. *FLT3*-ITD plus a point mutation at residue D835 in the tyrosine kinase domain (*FLT3*-ITD/D835) often cause resistance or decrease sensitivity to TKIs. In this study, we investigated new drug targets in AML with *FLT3*-ITD and TKD double mutations.

Materials & Methods

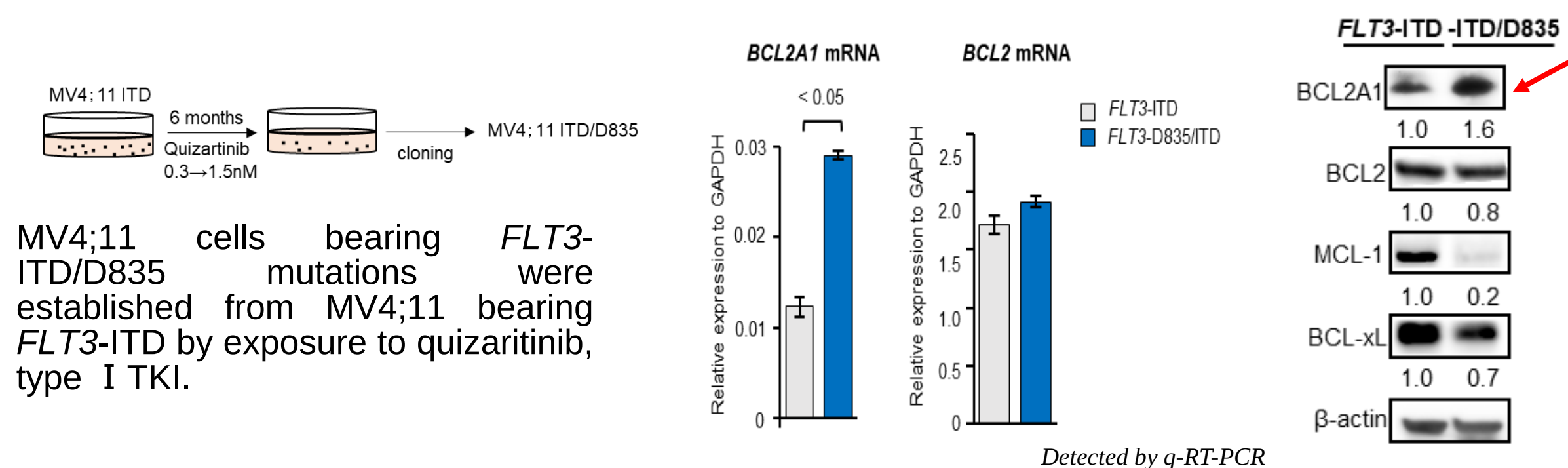
We carried out cap analysis of gene expression (CAGE) in primary AML cells from 26 patients to characterize transcriptome differences between *FLT3*-ITD-positive and *FLT3*-ITD/D835 double-positive primary AML cells. Selected genes were confirmed and validated with immunoblotting and RT-qPCR using *FLT3*-ITD-positive and *FLT3*-ITD/D835-positive MV4-11 cells. Furthermore, we generated *FLT3*-ITD-postive cells with overexpression of the selected genes using lentivirus vectors.

Results

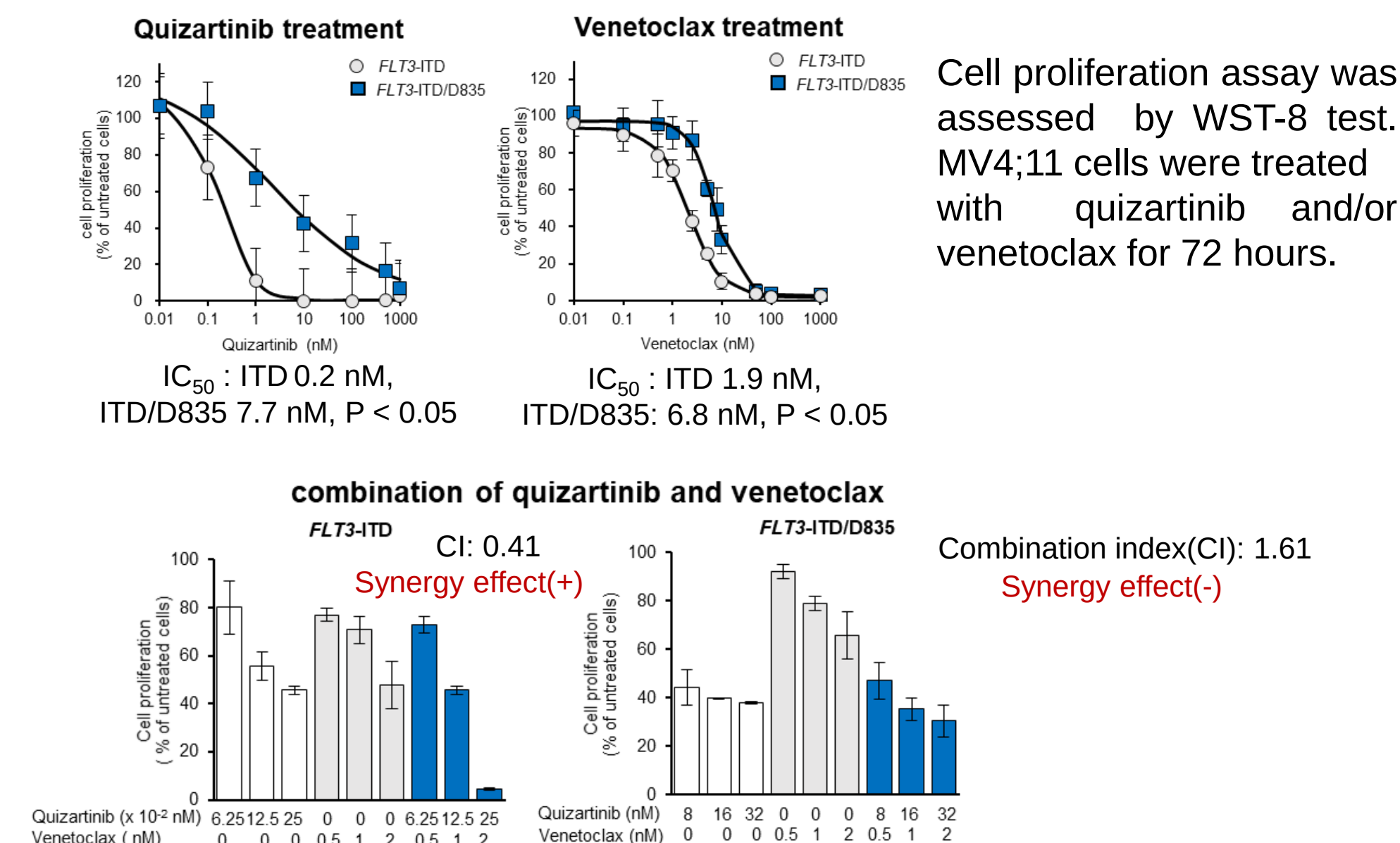
AML cells harboring *FLT3*-ITD/D835 expressed increased BCL2A1 gene transcripts



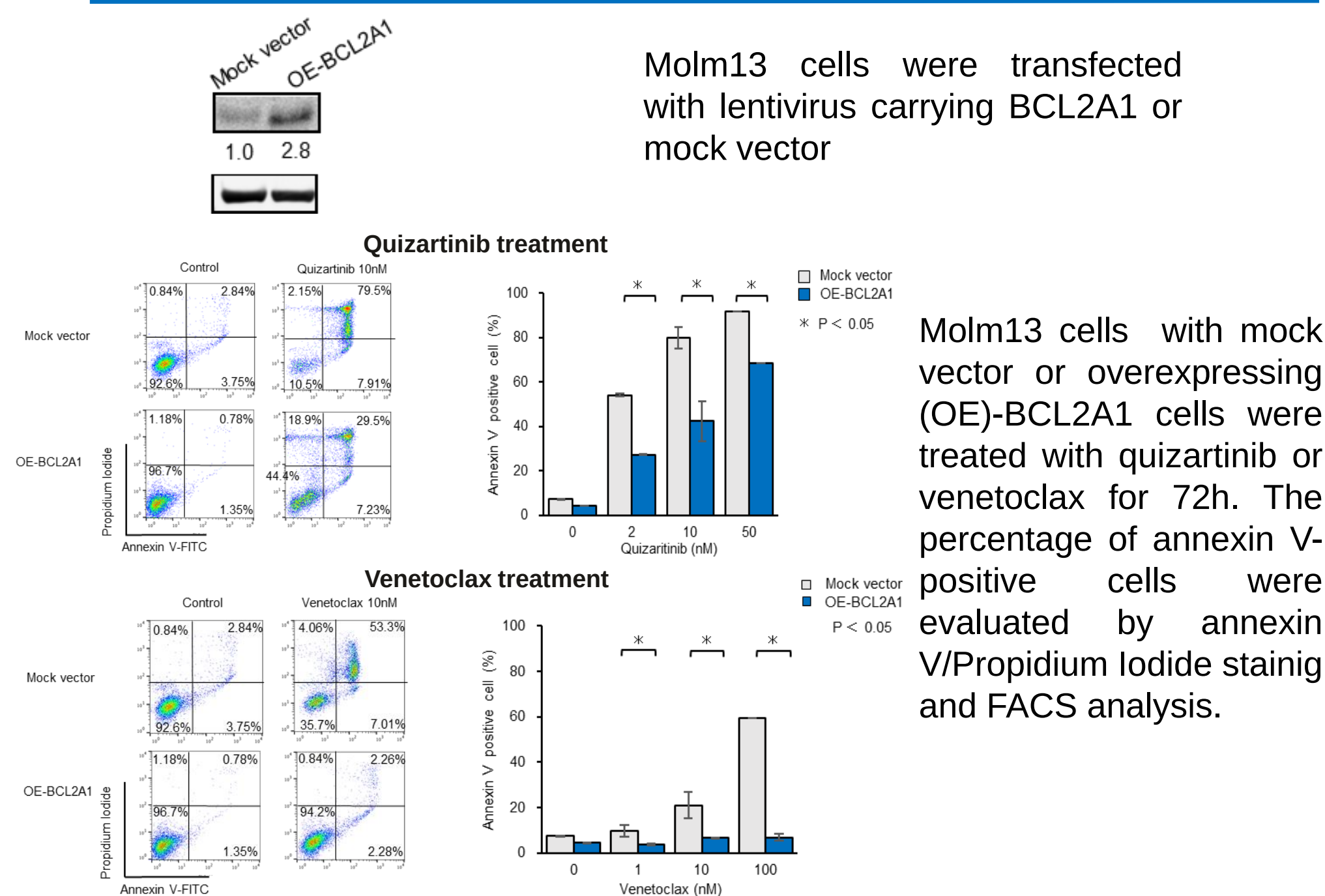
Higher gene transcript and protein levels of BCL2A1 in *FLT3*-ITD/D835 compared to *FLT3*-ITD MV4;11 cells



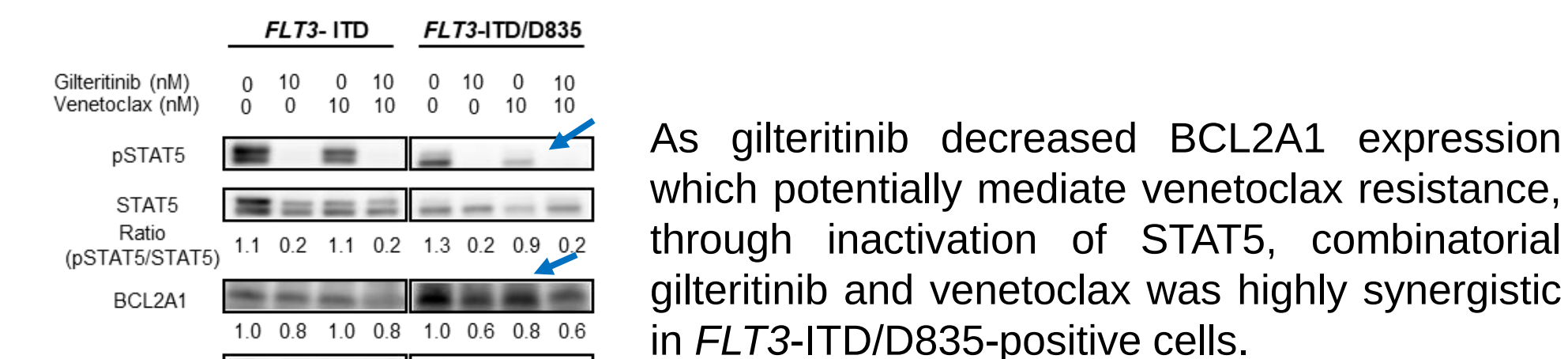
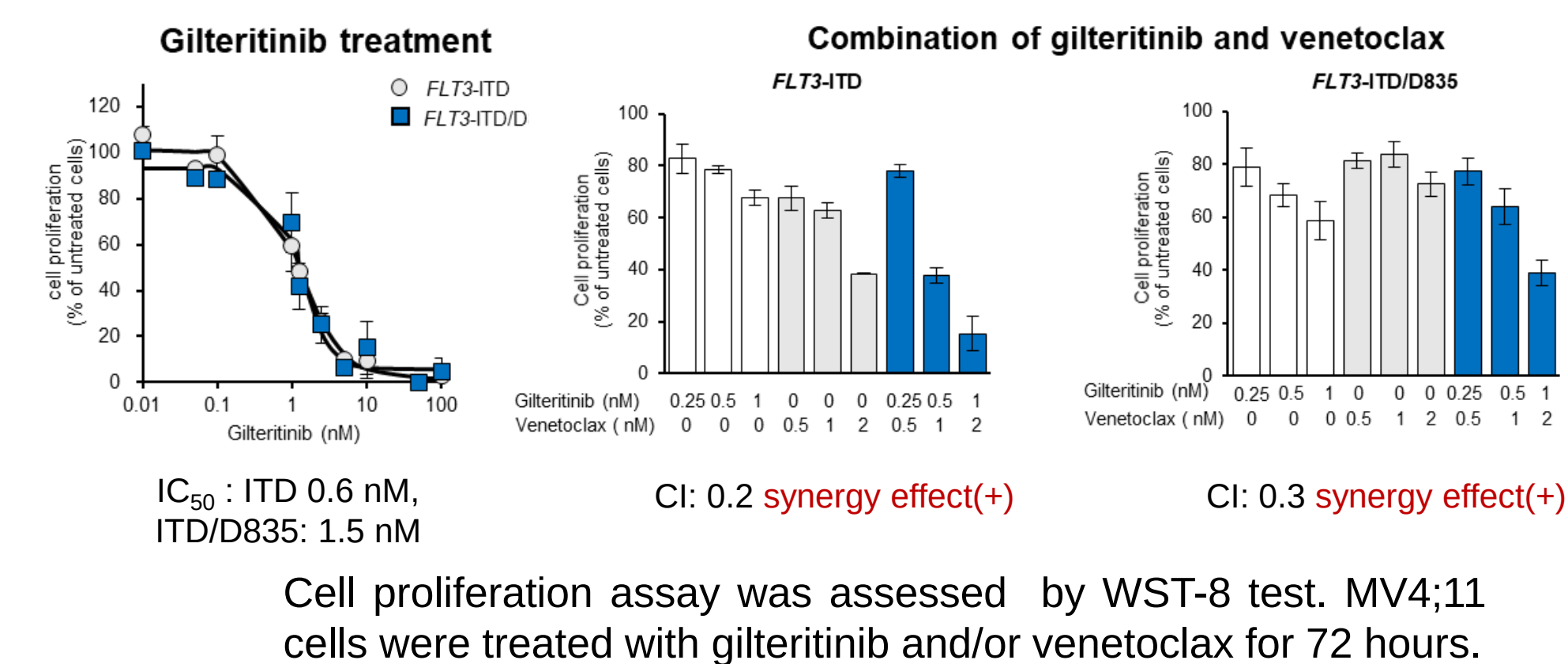
Cell growth inhibition by quizartinib and/or venetoclax, BCL2 inhibitor was less effective in *FLT3*-ITD/D835-positive MV4;11 cells



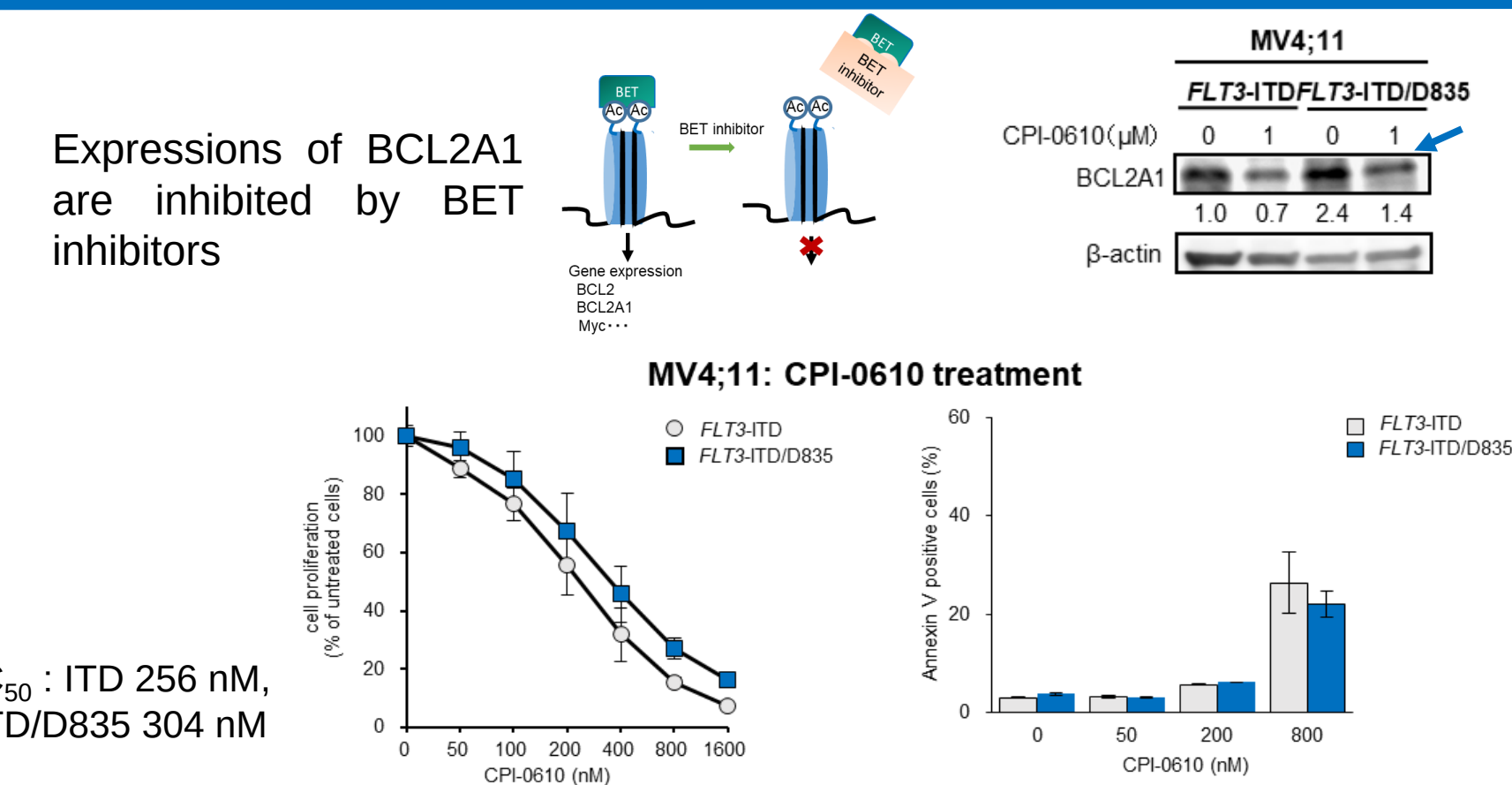
Overexpression of BCL2A1 in AML cells induced quizartinib and venetoclax resistance



Synergistic cell growth inhibition by gilteritinib, type I TKI plus venetoclax



CPI-0610, BET inhibitor suppressed BCL2A1 and exhibited anti-tumor activity



Conclusions

This study elucidates overexpression of BCL2A1 is associated with quizartinib-resistant *FLT3*-ITD/D835 mutant AML cells and identifies BCL2A1 as a putative novel therapeutic target. We also indicated gilteritinib combined with venetoclax, and CPI-0610 as promising drugs for these cells.