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Alternatively to targeting CD47, Zhao et al propose that targeting its macrophage ligand SIRPα may be more efficacious. We agree that targeting SIRPα is a viable therapeutic strategy and warrants further exploration. Compared with CD47, SIRPα expression is restricted,[8] thus antibody targeting of SIRPα may lead to less toxicity. Both we and Zhao et al have shown that blocking anti-SIRPα antibodies eliminate tumor cells in vitro.[3-5] In addition, a recombinant SIRPα Fc-fusion protein may also be a viable therapy.[9] However, 2 challenges to targeting SIRPα exist. First, therapeutic inhibition of SIRPα on all SIRPα-expressing phagocytes may lead to nonselective phagocytosis. Second, the human SIRPα gene contains many polymorphisms.[10] Thus, anti-SIRPα antibodies may differentially interact with differing SIRPα alleles resulting in variable therapeutic efficacy, which Zhao et al has preliminarily shown.[4] Agents targeting SIRPα may need to be individualized according to each patient's SIRPα allotype to achieve a response. This is in contrast to targeting CD47 whereby no human polymorphisms at the contact interface with SIRPα have yet been identified.

In conclusion, we previously demonstrated that anti-CD47 antibody can eliminate tumor cells through the FcR-independent mechanism of CD47-SIRPα blockade, while not excluding the possibility of FcR-dependent ADCC. Initial preclinical toxicity studies are limited. Further investigation will determine whether targeting CD47 and/or SIRPα represents a viable cancer therapeutic strategy.

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Conflict-of-interest disclosure: I.W., M.P.C., and R.M. have filed US Patent Application serial no. 12/321,215 enttiled "Methods for manipulating phagocytosis mediated by CD47" and US Patent Application serial no. PCT/US2010/048992 entitled "Synergistic anti-CD47 therapy for hematologic cancers."

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To the editor:

Improved classification of *MLL-AF9*–positive acute myeloid leukemia patients based on *BRE* and *EVI1* expression

The 5-year overall survival (OS) rate of patients with acute myeloid leukemia (AML) containing an *MLL-AF9* fusion gene is approximately 40%.[1,2] We recently showed in 2 independent cohorts that the prognosis among *MLL-AF9* positive patients can be refined based on *BRE* mRNA expression.[3] *MLL-AF9* positive patients with outlier high *BRE* expression exhibited a superior outcome (5-year OS of 80% and 64% for the 2 cohorts, respectively) while patients with normal *BRE* expression exhibited a very poor outcome (5-year OS of 0% and 7%, respectively). Thus, *BRE* expression may be used for refined risk stratification among *MLL-AF9* positive cases. However, the identification of patients with high *BRE* expression with routinely available techniques such as qPCR is difficult as the fold-difference between normal and high *BRE* expression is small.[3]

High *EVI1* expression occurs in approximately 10% of AML cases and is associated with an inferior outcome. High *EVI1* expression has recently been associated with *MLL* rearrangements including *MLL-AF9*.[4-6] To determine the correlation between *BRE* and *EVI1* expression, we reanalyzed the 2 *MLL-AF9* cohorts for which we reported high *BRE* expression.[3,7,8] This showed that high *BRE* and high *EVI1* expression are mutually exclusive (Figure 1A). Hence, the poor prognosis of the patients lacking high *BRE* expression could be explained by *EVI1* expression (eg, see Figure 2C in Noordermeer et al[3]). Of note, in both *MLL-AF9* cohorts we identified a few cases without high *BRE* or *EVI1* expression (Figure 1A). As the number of these patients is low, additional studies are required to reliably determine their prognosis.

To study whether the mutually exclusive expression of *BRE* and *EVI1* is accompanied by distinct expression profiles, we performed unsupervised genome-wide cluster analysis. The results showed that among *MLL-AF9* patients, high *BRE* expressing patients clustered apart from *EVI1*-positive patients (Figure 1B). Although the *EVI1*-positive patients were separated from the *BRE*-positive patients, they did not share highly similar expression profiles, while *BRE*-positive patients did. Indeed, in an unsupervised cluster analysis of the total AML cohort, *MLL-AF9* positive patients with high *EVI1* expression clustered only partially, showing modest similarities in expression profiles (supplemental Figure 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). This was in contrast to patients with high *BRE* expression that were almost completely confined to one

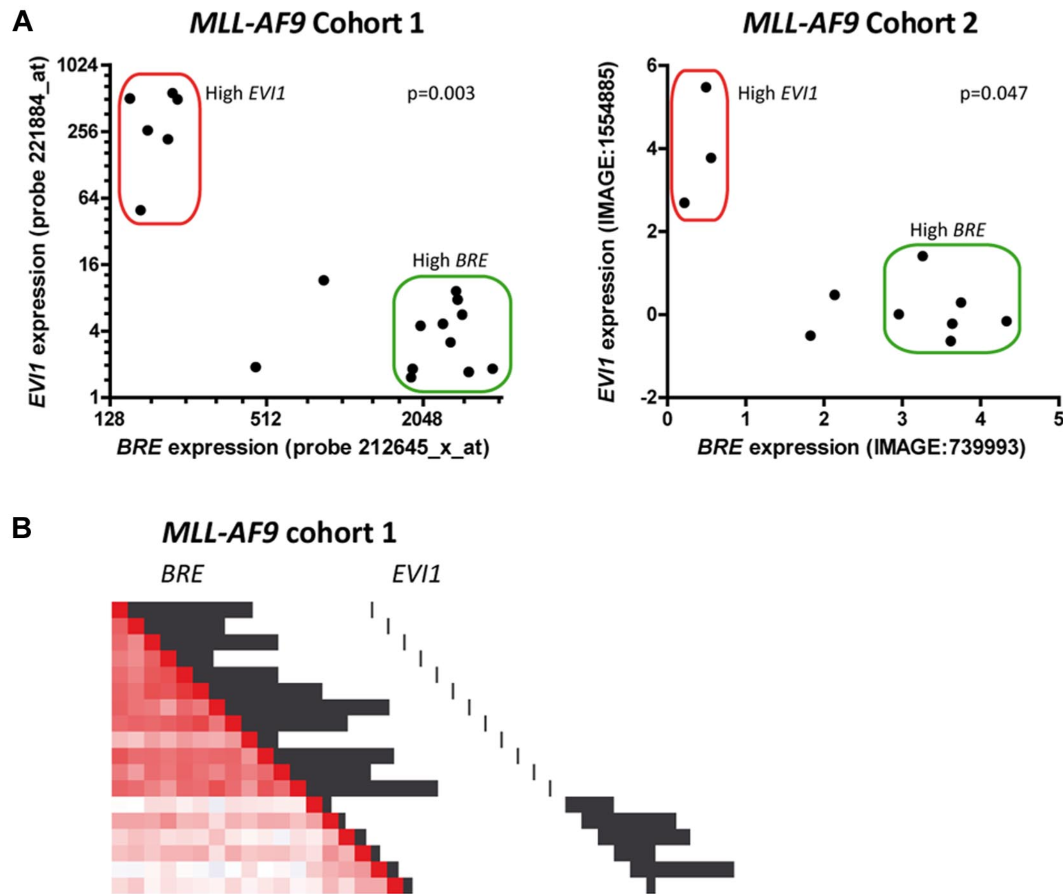

Figure 1. High *BRE* and high *EVI1* expression are mutually exclusive in *MLL-AF9* leukemia. (A) *BRE* expression was plotted against *EVI1* expression for *MLL-AF9* positive cases in 2 separate *MLL-AF9* cohorts.[7,8] In the first cohort (left plot), 33% (6/18) of the samples showed high *EVI1* expression, and 55.6% (10/18) showed high *BRE* expression. In the second cohort (right plot), 27.3% of the samples showed high *EVI1* expression (3/11), and 54.5% showed high *BRE* expression (6/11). Both cohorts contained 2 patients with neither high *BRE* nor high *EVI1* expression. High *BRE* expression was defined as described before.[3] High *EVI1* expression was defined as the expression of the upper 10% of the total cohort. *P* values for negative correlations were calculated using Spearman correlation tests. (B) *EVI1*-positive patients cluster apart from high *BRE* expressing patients among *MLL-AF9* positive patients in unsupervised clustering analysis. However, *EVI1*-positive patients show less similar expression profiles among each other compared with high *BRE* expressing patients (indicated by faint red color compared with bright red color, respectively). Unsupervised clustering was performed on the first cohort as described elsewhere[9] and clustering is represented as pairwise correlations between samples with a gradient from red to blue indicating degree of correlation (bright red: high correlation, blue: poor correlation). Black bars represent relative *BRE* (212645_x_at) and *EVI1* (221884_at) expression, as indicated.

distinct AML cluster, as described before.[3] Thus, *MLL-AF9* positive patients with high *BRE* expression seem to represent a specific AML subclass with highly similar expression profiles, while the *MLL-AF9* positive patients with high *EVI1* expression do not.

In our previous study, 40% of the *MLL-AF9* positive cases were missed by routine cytogenetics.[3] In addition, *MLL-AF9* positive cases with high *EVI1* expression lack chromosomal rearrangements encompassing the *EVI1* locus on chromosome 3.[4,5] Therefore, risk stratification of these patients could be improved by molecular screening for *MLL-AF9*–positivity and *EVI1* overexpression, in addition to routine cytogenetics.

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Contribution: S.N. and B.R. designed the study; S.N., D.M. and M.S.

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performed the analyses; S.N. and D.M. wrote the manuscript, which was critically revised by M.S., L.B., J.J. and B.R.; and data interpretation was performed by all coauthors.

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To the editor:

Bcl-x_L–inhibitory BH3 mimetic ABT-737 depletes platelet calcium stores

Recently, several studies have been published in *Blood* that have examined the effect of Bcl-x_L–inhibitory BH3 mimetics on platelet function and lifespan.[1-3] These reports agree that 2 related BH3 mimetics, ABT-737 and ABT-263, induce platelet apoptosis. This is characterized by caspase-3 activation and caspase-dependent phosphatidylserine externalization, and may underlie the thrombocytopenia observed during administration of these drugs.[2,4] However, in addition, the study by Vogler et al made 2 specific observations regarding the effect of BH3 mimetics on platelet calcium signaling: first, that they induce a transient calcium signal; and second, that prolonged treatment with BH3 mimetics depletes intracellular calcium stores.[1] In contrast, Schoenwaelder and Jackson, in correspondence to the editor, dispute these observations.[5]

We have also investigated the effect of ABT-737 on platelet calcium signaling. In agreement with Vogler et al, we find that ABT-737 (10μM) can induce a calcium signal (Figure 1A). However, the signal that we detect is small relative to that induced by thrombin. The maximum increase in cytosolic calcium concentration above basal was estimated as ~ 50nM, and was variable between platelets from different donors. For clarity, we have

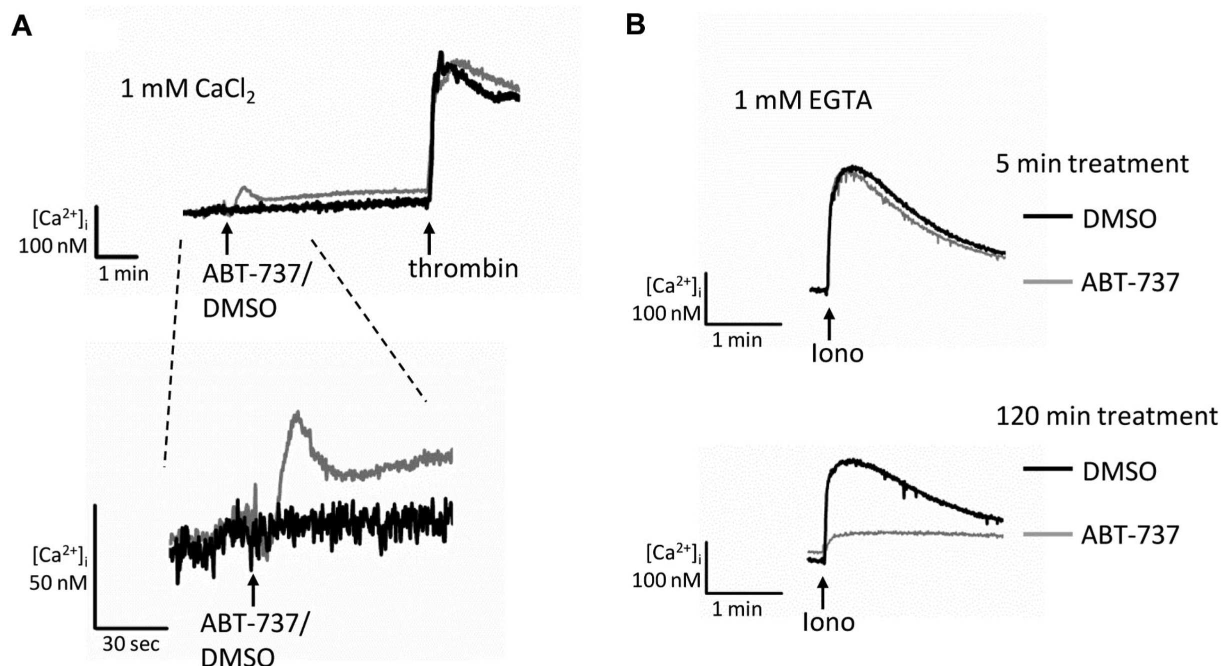

Figure 1. Effect of ABT-737 on platelet calcium signaling. (A) Washed platelets loaded with Fura-2 (1×10^8/mL) were treated with ABT-737 (10μM; gray trace) or DMSO (black trace) in the presence of extracellular $CaCl_2$ (1mM), followed by stimulation with thrombin (0.1 U/mL). The initial section of the traces are expanded and separated below to show the small ABT-737–induced signal. Fluorescence was calibrated in terms of cytosolic calcium concentration to estimate the magnitude of the response.[6] (B) Platelets in modified Tyrode buffer were treated with ABT-737 (10μM) or DMSO or for the indicated time then treated with EGTA (1.2mM) followed by ionomycin (Iono; 1μM) to artificially deplete calcium stores. Traces are representative of at least 3 independent experiments. All experiments were performed at 37°C under stirring conditions.