combinations with other agents looking for deep response as primary outcome. The results of the INNOVATE study, which randomized WM patients to ibrutinib and rituximab versus rituximab alone, are eagerly awaited (NCT02165397). Given the relatively benign toxicity profile of ibrutinib, combinations with monoclonal antibodies, proteasome inhibitors, alkylators, and other agents are likely to be well tolerated have greater efficacy at inducing deep responses in WM patients.

DISCLOSURE OF INTERESTS

JJC has received honoraria and/or research funds from Abbvie, BeiGene, Gilead, Janssen, Millennium, and Pharmacyclics. SPT has received honoraria and/or research funds from Pharmacyclics. All other authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

JJC, TD and SPT took care of the patient and gathered the data. AK, MD, NT, LX, and ZRH performed the MYD88 L265P mutational testing. JJC drafted the manuscript. All authors read and approved the manuscript.

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Genetic biomarkers of sensitivity and resistance to venetoclax monotherapy in patients with relapsed acute myeloid leukemia

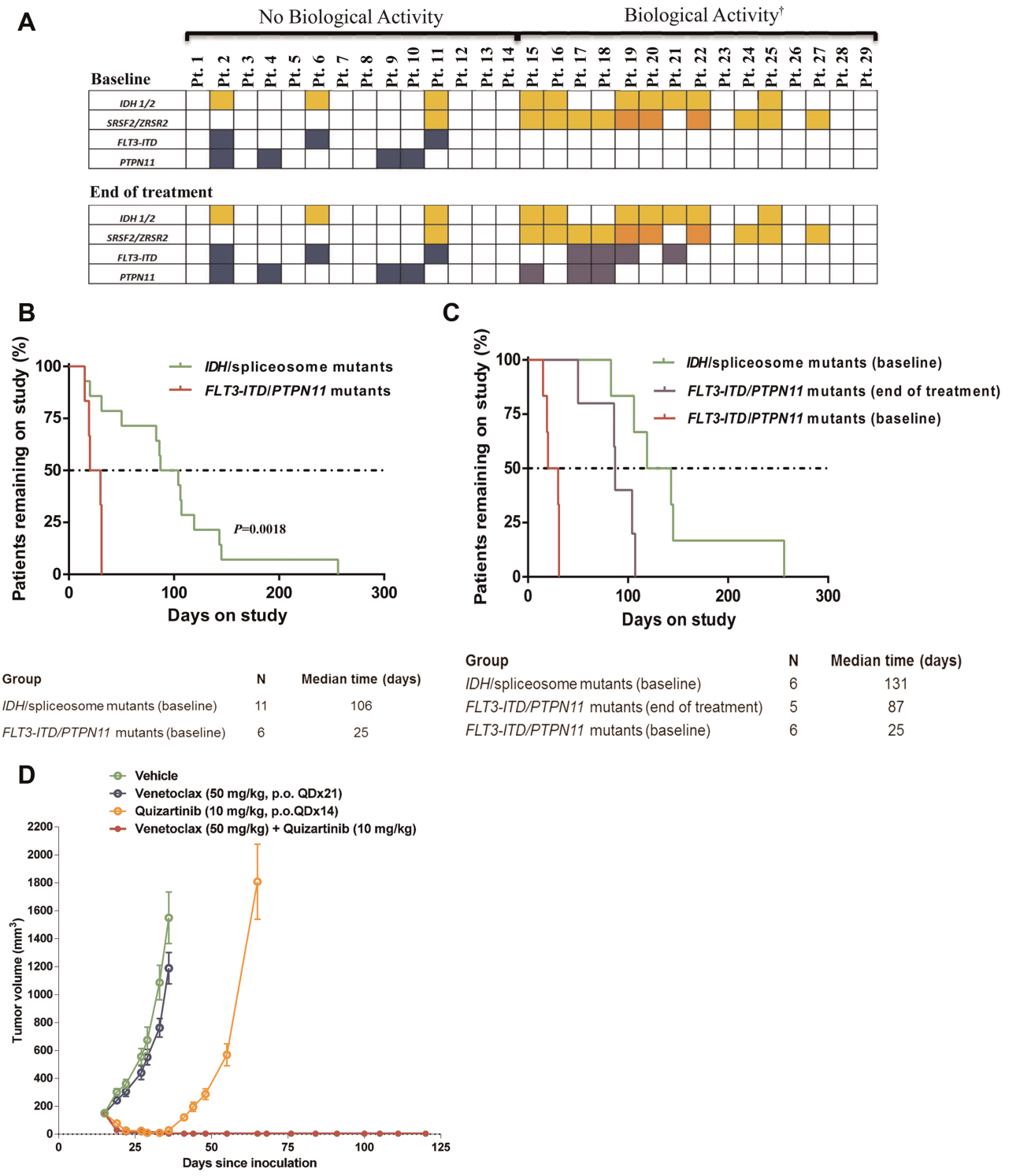
To the Editor:

Acute myeloid leukemia (AML) is a heterogeneous malignancy characterized by chromosomal aberrations and somatic mutations that identify biologically distinct subsets and guide risk stratification for therapy.[1] Treatment-associated changes in clonal architecture are common in AML, with emergence or clearance of specific sub-clones driving sensitivity and resistance to therapy. Therefore, the molecular characterization of emerging clones may facilitate the selection of optimal targeted therapies and rational combinations.

Venetoclax, a selective BCL-2 inhibitor, induced a complete response or complete response with incomplete blood recovery (CR/CRi) in 6/32 (19%) patients with AML who either had relapsed/refractory disease or were medically unfit for intensive chemotherapy.[2] In this report, we present a comparison of genetic biomarkers observed in pre- and post-treatment specimens from 29 of the 32 patients enrolled on this phase II study. Measurable reduction in bone marrow (BM) blast counts was observed in 15/29 (52%) of the patients, including CR/CRi in 6, a ≥50% reduction in BM blasts in 5, and a more modest blast reduction of <50% in 4 (Supporting Information Figure 1). The remaining patients (14/29, 48%) had no blast reduction.

We investigated the presence of somatic mutations commonly associated with AML in baseline and end-of-treatment samples. DNA isolated from blood and bone marrow specimens was analyzed by next-generation sequencing using the TruSight Myeloid panel (Illumina), the FoundationOne Heme panel (Foundation Medicine), or whole exome sequencing (MD Anderson Cancer Center, Khalifa Institute). Comparison of mutations at baseline and end of treatment is shown in Figure 1A.

At baseline, 10/29 (34%) patients had mutations in isocitrate dehydrogenase 1/2 (*IDH1/2*) genes. Of these, 7 (70%) had a reduction in BM blasts, including 3 CR/CRi. At baseline, 11/29 (38%) patients had spliceosome mutations in *SRSF2* or *ZRSR2*. Ten (88%) of these patients had a decrease in BM blasts, including 3 CR/CRi. Seven patients had both *IDH1/2* and spliceosome mutations with BM blast reductions observed in 6 (86%). In total, 11/14 (79%) patients with



Group	N	Median time (days)
IDH/spliceosome mutants (baseline)	11	106
FLT3-ITD/PTPN11 mutants (baseline)	6	25

Group	N	Median time (days)
IDH/spliceosome mutants (baseline)	6	131
FLT3-ITD/PTPN11 mutants (end of treatment)	5	87
FLT3-ITD/PTPN11 mutants (baseline)	6	25

FIGURE 1 Mutations affecting response to venetoclax in AML. **A**, Mutations observed pre-therapy and end of treatment with single agent venetoclax therapy. Yellow indicates *IDH* or *SRSF2* mutations; orange indicates *ZRSR2* mutations; blue indicates *FLT3-ITD* or *PTPN11* mutations; purple indicates newly detected *FLT3-ITD* or *PTPN11* mutations; blank cells indicate specific mutation was not detected, †Biological activity defined as any reduction in BM blast count while on venetoclax therapy; **B**, Time on study for patients with mutations associated with intrinsic sensitivity (pre-therapy *IDH*/spliceosome mutants) and intrinsic resistance (pre-therapy *FLT3-ITD/PTPN11* mutants) to venetoclax; **C**, Time on study for patients with mutations associated with intrinsic sensitivity, intrinsic resistance, and acquisition of mutations associated with acquired resistance (*FLT3-ITD/PTPN11* mutants) to venetoclax; **D**, Tumor growth inhibition by venetoclax plus quizartinib in mice xenografted with *FLT3-ITD*$^{+}$ MV-4–11 cells

mutations in *IDH1/2* or *SRSF2/ZRSR2* had evidence of BM blast reduction, including 4 CR/CRi, implicating these as possible markers of sensitivity to venetoclax (Figure 1A).

Among 14 patients who did not have a decrease in BM blasts on venetoclax treatment, 3 (21%) had FMS-like tyrosine kinase-3-internal tandem duplication (*FLT3-ITD*) and 4 (29%) had protein tyrosine

phosphatase, non-receptor type 11 (*PTPN11*) mutation at baseline, with 1 having both. Three patients had baseline mutations in both the *IDH*/spliceosome and *FLT3-ITD/PTPN11* groups and these three were the only patients who harbored *IDH*/spliceosome mutations and did not have BM blast reductions on venetoclax. The median time on study was 106 days (range, 50–256) for the *IDH*/spliceosome$^+$ (n = 11) and 25 days (range, 15–31) for *FLT3-ITD/PTPN11*$^+$ patients (n = 6) (P = .0018, Wilcoxon) (Figure 1B). These data suggest that *FLT3-ITD* or *PTPN11* mutations in AML may produce intrinsic/primary resistance to venetoclax.

We also performed mutational analysis on matched end-of-treatment samples from 20 patients at the time of AML progression/therapy termination. The *IDH1/2*, *SRSF2/ZRSR2*, *FLT3-ITD*, and *PTPN11* mutations identified prior to treatment were still present at the end of therapy in all patients. Notably, in 5 *IDH*/spliceosome$^+$ patients that were negative for *FLT3-ITD/PTPN11* mutations at baseline, *FLT3-ITD* (n = 2), *PTPN11* (n = 1), or both (n = 2) mutations were now detected in the end-of-treatment samples. The median time on study for these five patients was 87 days (range, 50–107) as compared to 131 days (range, 83–256) for the six patients who had *IDH*/spliceosome mutations at baseline and did not acquire *FLT3* or *PTPN11* mutations (Figure 1C). Furthermore, two patients in whom venetoclax initially induced BM blast reductions had both *FLT3-ITD* and *PTPN11* mutations newly detectable at the end of treatment in different subclones, based on allele frequency.

Based on our sequencing findings, we assessed the combination of venetoclax with the small-molecule FLT3 inhibitor quizartinib in the *FLT3-ITD*+ mutant xenograft model MV-4–11 (Supporting Information Methods). *In vitro*, the MV-4–11 cells were sensitive to BCL-2 inhibition by venetoclax.[3] However, similar to our clinical observations, venetoclax did not inhibit the growth of these tumors when implanted *in vivo*. Daily dosing of quizartinib induced tumor regressions in this model, although the tumors regrew following cessation of therapy. Strikingly, co-treatment with venetoclax and quizartinib induced similar tumor regressions as quizartinib alone but with significantly increased durability, preventing tumor re-emergence for up to 3 months post-cessation of treatment (Figure 1D). These data suggest that combining venetoclax with FLT3 inhibitors could be highly effective for the treatment of FLT3-mutated AML and may also prevent the emergence of FLT3-mutated, venetoclax-resistant sub-clones in patients who do not have an already detectable FLT3 mutation.

In summary, our data suggest that *SRSF2/ZRSR2* and *IDH1/2* mutations may predict sensitivity to venetoclax therapy in AML. Chan et al. previously demonstrated that *IDH1/2* mutations can sensitize leukemic cells to venetoclax.[4] However, of the 10 *IDH1/2*-mutated AML samples assessed in this trial, 7 had co-occurring spliceosome mutations, making it difficult to determine whether only one or both of these mutations together predict for venetoclax sensitivity. Recent findings suggest that *IDH2* and *SRSF2* mutations cooperate to induce a lethal transplantable myeloproliferative neoplasm.[5] Additionally, *SRSF2* mutation is known to induce alternative splicing of genes involved in the apoptotic pathway, a possible link to venetoclax sensitivity.[6] We note that *FLT3-ITD* or *PTPN11* mutations may confer primary and secondary resistance to venetoclax. Consistent with this, previous studies have shown that *FLT3-ITD* or *PTPN11* mutations can enhance the expression of anti-apoptotic BCL-2 relatives like BCL-X_L and MCL-1.[7,8] When combined with venetoclax, the FLT3 inhibitor quizartinib induced more durable responses in *FLT3-ITD+* tumor-bearing mice than either agent alone (Figure 1D). Thus, simultaneous targeting of BCL-2 and FLT3 may be one approach to overcome primary resistance and prevent emergence of secondary resistance to venetoclax therapy in AML patients.

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CONFLICT OF INTEREST

Vivian Ruvolo, Zixing Wang, and Ken Chen: nothing to disclose. Evelyn McKeegan was an employee of AbbVie, Inc. at the time of the study and may own stock. Marina Konopleva: consulted for and received research grants from AbbVie Inc. and Genentech. Naval Daver: received research grants from AbbVie Inc. and Genentech. Brenda Chyla, Kelly Doyle, Xin Huang, Andrew Souers, Joel Leverson, Jalaja Potluri, Erwin Boghaert, Anahita Bhathena, Relja Popovic: employees of AbbVie Inc. and may own stock.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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Impact of intranasal fentanyl in nurse initiated protocols for sickle cell vaso-occlusive pain episodes in a pediatric emergency department

To the Editor:

Although pain is a universal feature of sickle cell disease (SCD), there is limited evidence to guide management for vasoocclusive pain episodes (VOE). In 2014, the National Heart, Lung, and Blood Institutes (NHLBI) published new guidelines recommending rapid evaluation and treatment of VOE in the acute care setting, with timely pain assessments and repeat analgesia as needed to control pain. Despite these guidelines, delays in administration of parenteral analgesia are common in pediatric emergency departments (ED).[1]

Fentanyl is a potent, synthetic narcotic analgesic with a rapid onset and short duration of action. It is a strong agonist at the μ-opioid receptors, approximately 100 times more potent than morphine. Intranasal fentanyl (INF) has an onset of action of about 5–10 minutes, peaking within 30 minutes. INF 1.4 mcg/kg equates to intravenous (IV) fentanyl 1 mcg/kg (approx. 70% bioavailability),[2–6] and can provide rapid and powerful analgesia in the ED without the need for IV access. Current evidence suggests that INF is a safe and effective method of pain management for children in a variety of clinical settings, and is commonly used in the ED to control acute pain.[2] A quality improvement (QI) initiative by Kavanagh and colleagues was the first to use INF in the management of VOE in children with SCD and demonstrated improvements in time-to-first-parenteral-opioid dose, together with improved time-to-second-opioid and time-to-ED disposition. Of interest, ED discharge rates also increased from 32% to 48%, without an increase in 24-hour readmission or adverse outcomes like respiratory depression.[3] A recently published randomized placebo-controlled controlled trial involving 49 children with SCD presenting to an ED with moderate-severe pain randomized to INF (2 μg/kg, maximum 100 μg) had a greater decrease in median pain score at 20 minutes compared to normal saline placebo. We therefore evaluated the addition of INF to a nurse-initiated protocol at the Egleston Pediatric ED at Children's Healthcare of Atlanta (CHOA) for the management of SCD/VOE on time-to-first-parenteral-opioid dose administration, ED length of stay (LOS), and admission rates compared to: (1) historical control data prior to implementation of the INF protocol and (2) those who were not treated with INF during the study period. (See Supplement Methods Section for details on setting, participants, the CHOA nurse-initiated SCD-pain protocol [Supporting Information Figure 1], education initiatives utilized to ensure team buy-in, data collection, statistical analysis, and limitations.) All children with an established diagnosis of SCD (all genotypes) between the ages of 2–18 years presenting to the ED with VOE treated with intravenous (IV) opioids were eligible. Electronic medical record data were collected for a 6-month period before and after implementation of INF use to the nurse-initiated protocol. Patient/family and nursing satisfaction with INF was obtained through a Likert Scale Survey.

A total of 248 SCD visits for moderate-to-severe VOE occurred during the 6-month pilot period. Of those, 228 patients received parenteral opioids (92%), of whom 180 (79%) received INF. Of the 48 patients who did not receive INF (INF− group), 36 were not offered INF without explanation for the clinical protocol deviation, while 12 refused INF. Mean age of the 228 patients treated with parenteral opioids was 12 ± 5 years, 56% were female, and 65% had HbSS (See Supporting Information Table 1 for patient clinical characteristics). Patients in the INF− group had similar gender and hemoglobin genotype, but were older than patients in the INF+ group (13.4 ± 4.0 vs. 11.7 ± 4.5 years, $P = 0.01$).

Triage pain scores were similar in all groups and improved significantly at the time of ED disposition, without a significant difference in the INF+ vs. INF− groups (Supporting Information-Figure 2). Mean time-to-first-parenteral-opioid decreased significantly in the INF+ group compared to historical controls (29 ± 15 vs. 35 ± 18 minutes, $P < 0.01$, $n = 228$) and the INF− group (77 ± 44 minutes, $P < 0.001$; $n = 48$). The ED LOS between the INF+ group and historical controls was similar, but lower in the INF− group. Admission rates were similar in the INF+ group and historical controls but significantly higher in the INF− group (48% and 45% vs. 71% respectively, $P = 0.004$; Table 1).

No adverse events including over-sedation or respiratory depression occurred during the study. The most common side effects included complaints of nasal burning and irritation after administration.