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Oral MEK 1/2 Inhibitor Trametinib in Combination with AKT Inhibitor GSK2141795 in Patients with Acute Myeloid Leukemia with RAS Mutations: A Phase II Study

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Abstract

With proven single-agent activity and favorable toxicity profile of MEK-1/2 inhibition in advanced leukemia, investigation into combination strategies to overcome proposed resistance pathways is warranted. Resistance to MEK inhibition is secondary to upstream hyperactivation of RAS/RAF or activation of the PI3K/PTEN/AKT/mTOR pathway. This phase II multi-institution CTEP-

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CLINICAL PRACTICE POINTS

This is the first study to explore clinical relevance of the combination of ATP-competitive pan-AKT inhibitor GSK2141795, targeting the PI3K/AKT pathway, and MEK inhibitor trametinib in *RAS*-mutated relapsed/refractory acute myeloid leukemia. Based on determination of modest single-agent activity of MEK inhibition, exploration of combinatorial inhibition of the proposed resistance pathway PI3K/AKT was pursued. Preclinical data revealed that dual inhibition yielded additive cell killing in AML cell lines with *RAS* mutations. Despite preliminary biological efficacy of this combination, clinical benefit was not observed. Correlative evaluation of activation status and modulation of the MEK and AKT pathways revealed expected target modulation of pERK and pS6 with significant and near significant downmodulation of each, respectively. This study reveals that MEK and AKT inhibition with trametinib and GSK 2141795 at the investigated doses was insufficient to yield clinical benefit in advanced leukemia patients. This study highlights that this combination is not efficacious in this clinical setting despite preclinical evidence supporting its use. Further exploration of this combination in advanced leukemia should include additional cytotoxic and/or epigenetic therapy in an effort to augment clinically relevant responses through possible elimination of other compensatory pathways.

sponsored study was conducted to determine efficacy and safety of the combination of ATP-competitive pan-AKT inhibitor GSK2141795, targeting the PI3K/AKT pathway, and MEK inhibitor trametinib in RAS-mutated relapsed/refractory acute myeloid leukemia (AML). The primary objective was to determine the proportion of patients achieving a complete remission (CR). Secondary objectives included assessment of toxicity profile and biologic effects of this combination. Twenty-three patients with *RAS*-mutated AML received the combination. Two dose levels were explored (dose level 1: 2 mg trametinib, 25 mg GSK2141795 and dose level 2: 1.5 mg trametinib, 50 mg GSK2141795). Dose level 1 was identified as the recommended phase II dose. No CRs were identified in either cohort. Minor responses were recognized in 5 patients (22%). The most common drug-related toxicities included rash and diarrhea, with dose-limiting toxicities of mucositis and colitis. Longitudinal correlative assessment of the modulation of MEK and AKT pathways using reverse-phase protein array and phospho-flow analysis revealed significant and near-significant down-modulation of pERK and pS6, respectively. Combined MEK and AKT inhibition had no clinical activity in patients with *RAS*-mutated AML. Further investigation is required to explore the discrepancy between activity of this combination on leukemia cells and the lack of clinical efficacy.

MICROABSTRACT

To augment modest single-agent activity of MEK inhibition, combinatorial inhibition of the proposed resistance pathway PI3K/AKT was explored. Preclinical data evaluating leukemia cells in the presence of MEK/AKT inhibition demonstrated consistent inhibition of pERK, variable effects on pAKT, and significant blockade of pEBP1 and pS6, downstream targets of AKT. Despite preliminary biological efficacy of this combination, we demonstrate that trametinib combined with GSK2141795 was not associated with clinically relevant antileukemic activity in 23 patients. Correlative evaluation of activation status and modulation of the MEK and AKT pathways revealed expected target modulation of pERK and pS6 with significant and near significant downmodulation of each, respectively. Further exploration of the discrepancy between preclinical activity of this combination in leukemia cells and lack of clinical efficacy is needed.

Keywords

AML; drug resistance; developmental therapeutics; neoplasia-myeloid leukemias and dysplasias; phase II clinical trials

INTRODUCTION

After decades of effort to improve upon historical outcomes, acute myeloid leukemia (AML), the most common acute leukemia in adults, remains a challenging disease to treat.^{1,2} With a 5-year survival of only around 30%, more effective treatment strategies are necessary.¹ Relapsed/refractory AML presents an even greater challenge, as the outcome for these patients is poor, and few effective treatment options are available in this setting.³ Progress has been made through the exploration and elucidation of the cytogenetic and genomic landscape to better characterize the heterogeneity and complexity of AML.^{2,4,5} With refined understanding of the molecular-genetic defects underlying the pathogenesis of AML, development of novel molecularly targeted agents is ongoing.^{6,7}

Through detailed characterization of the molecular landscape of AML, the RAS/RAF/MEK/ERK and PI3K/AKT/mTOR pathways have been implicated in the pathogenesis.⁸ Mutations of genes in the RAS/RAF/MEK/ERK pathway, which regulates cell survival, proliferation, differentiation, and motility, are well defined in AML.⁸ *RAS* mutations are identified in 10–25% of AML patients, and an additional 37% of patients have mutations in *FLT3*, an upstream receptor tyrosine kinase known to activate the RAS pathway.^{9,10} With a significant percentage of AML patients harboring mutations involved in the RAS/RAF/MEK/ERK pathway, this pathway is acknowledged as a prime therapeutic target.^{8,11} The oral small-molecule MEK inhibitor selumetinib was evaluated in a phase II trial of relapsed/refractory AML.¹² Despite a modest response rate, with responses in 17% of patients in the *FLT3* wild-type cohort, the toxicity profile was favorable and further evaluation of combination strategies with MEK inhibition was proposed.¹² The MEK inhibitor trametinib produced a better single-agent response rate in a phase I/II trial in relapsed/refractory AML, with an overall response rate of 28%, including a 12% complete remission in *RAS*-mutated patients.^{13,14}

Compensatory activation of parallel signaling through the PI3K/AKT/mTOR pathway has emerged as a mechanism of resistance to MEK inhibition.^{15,16} As it is known that the PI3K signaling pathway plays a critical role in oncogene-mediated tumor growth and proliferation and has regulatory functions in cell survival, apoptosis, protein synthesis, and glucose metabolism,^{17–19} constitutive activation of the PI3K pathway has been implicated in the pathogenesis and progression of a wide variety of neoplasms, including AML.^{19–23} Apoptosis is restored in AML cells *in vitro* when the PI3K/AKT signaling pathway is inhibited.²⁴ Further, AKT is an integral downstream target of PI3K that transduces the proliferative and anti-apoptotic signals of this pathway, and therefore is a proposed specific downstream target for inhibition of the PI3K pathway.²⁵ MEK inhibition in *RAS*-mutated cancers has been shown to result in upregulation of AKT phosphorylation.^{26,27} Inhibition of the downstream target AKT is a proposed mechanism to overcome resistance to MEK inhibition. Preliminary data revealed that combination of the prototypical MEK inhibitor selumetinib and AKT inhibitor MK-2206 resulted in additive cell killing in AML cell lines with *RAS* mutations, including MV4;11 cells with *KRAS* mutation and HL-60 cells with *NRAS* mutation.²⁸ *In vivo* studies have also shown that dual inhibition of PI3K/AKT and MEK pathways is effective in tumors with reliance on RAS signaling.^{29–31}

In this trial, GSK2141795, an ATP-competitive, pan-AKT kinase inhibitor that inhibits the AKT pathway and proliferation of tumor cells *in vitro* and *in vivo*,²⁵ is used to explore combination therapy with MEK inhibition in an effort to overcome resistance to single-agent MEK inhibition. With preclinical data supporting the additive effect of MEK and AKT inhibition in *RAS*-mutated AML cell lines, it was hypothesized that AML patients with *RAS* mutations would obtain clinical benefit from the combination of MEK and AKT inhibition.

The overall goal of this clinical trial was to determine efficacy and safety of trametinib in combination with GSK2141795 in patients with *RAS*-mutated relapsed/refractory AML and to characterize modulation of MAPK and AKT signaling pathways (NCT01907815).

METHODS

Patients with *RAS*-mutated AML who experienced relapse or were refractory to standard chemotherapy were eligible for this study.

Study Objectives

The primary objective of this study was to determine the proportion of AML patients with *RAS* mutations achieving complete remission (CR) or CR with incomplete recovery of platelets (CRp) as best response within 4 cycles of therapy with trametinib in combination with GSK2141795. Secondary objectives were to determine the disease-free survival and duration of response in patients achieving CR/CRp, assess the toxicity profile of trametinib in combination with GSK2141795, and characterize the biologic effects of trametinib in combination with GSK2141795 in leukemia cells.

Study Design

This was a single-arm, open label, multi-institution, phase II CTEP-sponsored study of the MEK inhibitor trametinib in combination with the AKT inhibitor GSK2141795 in AML patients with *RAS* mutations. The trial consisted of two parts, including a run-in phase to evaluate the safety of three combination dose levels and a second phase evaluating the efficacy of the combination dose identified in the run-in phase.

Dose levels are shown in Table 1. Dose levels 1 and 2 were shown to be safe in patients with solid tumors.³² To assess the safety of the combination in the AML study population, dose levels 1 and 2 were evaluated simultaneously. Patients were randomized into either level 1 or 2 with equal probabilities. Dose level 3 was to be tested only after dose levels 1 and 2 were concluded to be safe. The initial goal was to enroll 6 patients in dose level 1 and 6 patients in dose level 2 to obtain data on safety and on biomarker modulation. If 2 out of 6 patients experienced DLTs, the dose level would be considered too toxic. A modified 3+3 design was used to determine a recommended phase II dose (RP2D), which was based on multiple factors, including tolerability, efficacy and biomarker modulation of the targeted pathways.

With the goal of evaluating efficacy in the second phase, a total of 33 patients was planned, including the 6 patients treated at the R2PD in the run-in phase. Response assessment was planned for the first four cycles of therapy. Patients without progressive disease or with CR/CRp or deemed to be deriving clinical benefit could continue study treatment beyond 4 cycles. The target CR/CRp rate was 35%. The study was considered a failure and the combination rejected if the CR/CRp was 15% or lower.

Bone marrow evaluations were obtained at baseline (within 30 days prior to initiation of the study drugs), on cycle 1 day 28 (+/- 7 days) and then every 4 weeks (+/- 7 days). Once CR was achieved, bone marrow evaluation was obtained every 2–3 months, or as clinically indicated to assess for response.

Treatment Regimen

Patients received trametinib and GSK2141795 continuously orally daily in 28-day cycles. At dose level 1, trametinib was dosed at 2 mg daily and GSK2141795 at 25 mg daily.

Trametinib was decreased to 1.5 mg daily and GSK2141795 increased to 50 mg daily for dose level 2. Dose level 3 was planned with trametinib at 2 mg daily and GSK2141795 50 mg daily, but no patients were enrolled at this level.

Eligibility

Patients 18 years or older with a histologically confirmed diagnosis of AML, relapsed or refractory to standard chemotherapy, were enrolled. Patients were required to have a *RAS* mutation (*NRAS* codon 12, 13, 61 or *KRAS* codon 12, 13, 61) identified by a CLIA-certified laboratory. Other inclusion criteria included: ECOG performance status of 0–2, life expectancy >4 weeks, serum creatinine 1.5 mg/dL or calculated creatinine clearance 60 mL/min, bilirubin 1.5 mg/dL, SGOT or SGPT 2.5X upper limit of normal (ULN), fasting glucose 150 mg/dL, and left ventricular ejection fraction (LVEF) institutional lower limit of normal and at least 50%. All patients provided written informed consent.

Biomarker and Correlative Assessment

Peripheral blood and bone marrow samples were collected to assess the biologic correlates of the treatments administered.

Molecular Analysis—For patients treated at MD Anderson Cancer Center (MDACC), *RAS* mutation testing was performed with the Actionable Cancer Gene Scan CLIA panel in the MDACC Molecular Diagnostics Laboratory. Genes tested clinically included *KRAS*, *NRAS*, *FLT3*, *NPM1*, *CEBPA*, *DNMT3A*, *IDH1*, *IDH2*, and *KIT*. Testing was performed based on an internal laboratory algorithm using either a CMS53 panel, which interrogates mutation hot-spots in 53 cancer-related genes or a CMS28 panel, which interrogates entire coding sequences of 28 cancer-related genes. These panels are described in further detail in the supplemental appendix.

For patients enrolled at the University of Chicago and University of Maryland, *RAS* mutation testing was performed using a single-base extension mutation assay called SNaPshot™ performed in a CLIA certified molecular diagnostics laboratory at the University of Chicago.³³ Samples obtained at the University of Maryland were sent to the University of Chicago to complete this testing.

Biomarker Evaluation—Reverse-Phase Protein Array (RPPA) analysis was used to measure effects of treatment on activation of MAPK and AKT/mTOR in AML blasts/CD34+ cells. Details of the RPPA procedure are provided in the supplemental appendix.

For this correlative analysis, peripheral blood samples (30 cc) were collected on day 1 (pre-dose; prior to day 1 drug administration), 24 hours after 1st dose (i.e. on day 2; within 60 minutes prior to day 2 drug administration), and on day 15 (irrespective of time of drug administration) during Cycle 1 of therapy. Peripheral blood samples (30 cc) were also collected at the time of disease assessment on cycle 1 day 28 (+/– 7 days), at time of follow-up bone marrow evaluations, and at time of disease progression. Bone marrow samples (15 cc) were collected before therapy (at screening) and at the time of disease assessment on day 28 (+/– 7 days).

RPPA was used to determine: 1) protein expression of the known upstream regulators of the PI3K/AKT pathway, such as the phosphatases PP2A and PTEN (total and phospho), and P53, 2) activation status and modulation of the AKT/mTOR pathway [AKT (Ser473)/(Thr308), p70S6K (Thr389), S6RK, 4EBP1 (Thr70)/(Thr37/46)], total and phospho-mTOR, PRAS40, and FOXO1, 3) downstream apoptotic modulators known to be inhibited by PI3K/mTOR and MEK inhibitors (Bim, Mcl-1, Bcl-2, Bad, XIAP, survivin), 4) effects of trametinib and GSK2141795 on activation of mutant *RAS* and other signaling pathways [total and phospho-ERK (p44/42), FLT3, STAT-3 and STAT-5].^{34–39}

Complementary flow cytometry analysis was performed to measure effects of treatment on activation of MAPK and AKT in AML blast cells and in AML CD34+ cells. Phospho-ERK (pERK) was used to assess inhibition of the MAPK pathway, and pAKT, pS6 and p4EBP1 were used to evaluate AKT blockade. Maximum percentage change from baseline in total and phospho-proteins using mean fluorescence intensities (MFI) by flow cytometry was assessed for available patient samples at multiple time points. Flow cytometric assessment was based on methods previously described.⁴⁰

RESULTS AND DISCUSSION

Study Population

A total of 24 patients (MDACC n=17; University of Maryland n=4; University of Chicago n=3) with *RAS*-mutated AML were enrolled between October 2013 and January 2016. One patient (MDACC) signed the informed consent form but elected not to participate in the study. Thus, a total of 23 patients received the study treatments. Baseline characteristics (N=23) are shown in Table 2. Median age was 69 years (range, 21–80). Fourteen patients (61%) had an antecedent hematologic disease (AHD) (myelodysplastic syndrome, n=9; myeloproliferative neoplasm, n=1; chronic myelomonocytic leukemia, n=4), with 12 of the 14 having received prior therapy for their AHD diagnosis. Median bone marrow blast percentage was 36% (range, 9–83%), with only 3 patients having a bone marrow blast percentage less than 20. The median number of prior therapies, including treatment for AHD and AML, was 3 (range, 1–14): cytarabine (n=21), hypomethylating agents (n=15), fludarabine (n=3), clofarabine (n=4) and cladribine (n=7), either alone or in combination. Five patients had undergone a prior allogeneic stem cell transplant (allo-SCT). Cytogenetic analysis showed a diploid karyotype in 7 patients (30%), adverse cytogenetic aberrations (including chromosome 5 and/or 7 abnormalities, inversion 3, and complex) in 9 patients (39%), and other aberrations in 5 patients (22%). All patients had a *RAS* mutation, as required for enrollment in the trial. *NRAS* mutation was identified in 15 patients, *KRAS* mutation in 5 patients, and both *NRAS* and *KRAS* in 3 patients. Eight of 17 MDACC patients had co-existing mutations, with details demonstrated in Table 2.

Patient Enrollment and Determination of Recommended Phase 2 Dose

In the run-in phase of the study, 13 patients were enrolled: 6 patients were treated at dose level 1, and 7 patients at dose level 2. One of the 6 patients at dose level 1 had a DLT (grade 3 mucositis). One of the 6 patients at dose level 2 had DLT (grade 3 mucositis and colitis). Drug was discontinued due to grade 3 nausea, vomiting, and diarrhea on day 18 of planned

28 days for another patient at dose level 2. In discussion with the CTEP, it was concluded that dose escalation to dose level 3 could not occur since 2/6 DLT were determined at dose level 2. Dose level 1 was declared the recommended phase II dose (RP2D). Eleven additional patients were then enrolled at dose level 1 during the phase II portion of the study. Two of these eleven patients enrolled at dose level 1 had a DLT, one with grade 3 diarrhea and one with grade 3 esophageal necrosis.

Drug Delivery

The median duration on study was 66 days (range, 15–214). Four patients were not evaluable for DLT as they received study drugs for only 15, 16, 18, and 20 days before progression of disease and non-drug related toxicities that required discontinuation of study drugs. Three of these patients were at dose level 1, and one at dose level 2. Discontinuation of therapy was due to progression of disease (n=14), DLT as described above (n=5), adverse events determined unrelated to drug (n=1, lipase/amylase elevation), and death (infection, n=2 and respiratory failure, n=1).

Treatment Outcome

No patient obtained CR or CRp. The study was closed early due to lack of clinical activity. Since no significant response was observed in any enrolled patient, duration of response and disease-free survival could not be assessed. Five patients had minor hematologic responses, and 2 additional patients had unconfirmed minor responses. Of these 7 patients, 86% (N=6) had a mutation in *NRAS*. Only 1 patient with an unconfirmed minor response expressed concomitant mutations in *TET2*, *RUNX1*, and *ASXL1* along with *NRAS* mutation. The remaining 6 patients carried only a single *RAS* mutation.

At dose level 1, minor hematologic improvements in platelet count (defined as improvement to greater than $100 \times 10^9/L$, HI-P) were seen in 2 patients, with 1 of the 2 patients also having >50% reduction in absolute PB blasts lasting >8 weeks (HI-PB). Two patients in dose cohort 1 had unconfirmed HI in BM blast count (defined as >50% decrease in BM blast count lasting >8 weeks); responses were unconfirmed because only a 4-week BM was available for review. At dose level 2, three patients had minor hematologic improvements (N=2 with >50% decrease in BM blasts lasting >8 weeks and N=1 with HI-PB). Regarding the 3 patients with a low baseline blast percentage in the marrow at initiation of this study, HI-PB was noted in 1 patient.

Of the 11 patients who remained on study for >70 days, 64% (N=7) reported improvement in symptoms and/or had minor responses while on study despite lack of objective CR. Symptom improvement was identified in 1 patient with HI-P, 2 patients with HI-PB, and 1 patient with no response.

Median overall survival was 3 months (range, 1–16). For patients with confirmed minor HI (N=5), median duration on therapy was 3.8 months (range, 3.6–5.5), and median OS was 4.8 months (range, 3.6–6.6). Though the primary objective was not met, secondary objectives of toxicity and biologic effects of the combination were explored.

Safety Findings

Toxicities at least possibly related to drug are shown in Tables 3 and 4. The most common drug-related toxicities included diarrhea (52%), maculopapular rash (39%), mucositis (22%), and nausea/vomiting (26%). Most toxicities were mild (grade 1–2). Grade 3–4 toxicities occurred in 39% of patients. Grade 3 toxicities included rash (13%), mucositis (9%), diarrhea (17%), nausea/vomiting (9%), hypertension (4%), infection (4%), esophageal necrosis (4%), and colitis (4%). One patient experienced grade 4 toxicity with febrile neutropenia at dose level 1. Another patient was admitted on C1D21 for a possibly related gastric hemorrhage (grade 4), causing a possibly related grade 4 anemia; this patient received study drug at dose level 1 for only 20 days before declining further study.

Infection and/or fever were recognized in 65% of patients. Infection, fever, sepsis, and febrile neutropenia were often classified as unrelated or unlikely related adverse events. Fourteen infection events were observed, as well as 4 occurrences of sepsis, 4 episodes of febrile neutropenia, and 11 fevers. Only 1 incidence of infection was reported as possibly related to the drug combination, and 1 episode of febrile neutropenia was reported as probably related.

Though dose level 3 was planned with trametinib at 2 mg and GSK2141795 at 50 mg, dose level 2 was determined too toxic and no further dose escalation was pursued.

Mutational Analysis

Concomitant mutations were identified along with *RAS* in 8 of 17 MDACC patients and are listed in Table 2. There was no significant difference between median OS of patients with multiple mutations versus patients with *RAS* mutations alone. When *KRAS*-mutant patients were compared to *NRAS*-mutant patients, no significant survival difference was demonstrated. Three patients had both *KRAS* and *NRAS* mutations. For the second longest surviving patient (13 months), concomitant mutations in *NPM1*, *IDH2*, *DNMT3A* were identified. This patient also went on to 3 additional lines of therapy after discontinuation of study drug. The longest survivor (16 months) also carried an *NPM1* mutation in addition to an *NRAS* mutation.

RPPA Analysis

The effect of the drug combination on leukemia cells was explored using RPPA analysis, performed using methodology referenced in the supplemental material.⁴¹ RPPA was used to determine the activation status and modulation of the MEK and AKT pathways by the drug combination. Data were available from bone marrow (BM) for 20 patients: 13 from MDACC, 4 from University of Maryland, and 3 from University of Chicago. Peripheral blood (PB) samples were available for 19 patients: 12 from MDACC, 4 from University of Maryland, and 3 from University of Chicago. Longitudinal samples were available for each patient. Samples were profiled with RPPAs on 303 antibodies. RPPA data are provided as supplementary files. Overall, using RPPA, consistent and significant downregulation of downstream targets of AKT and MEK, as observed in preclinical studies, was not seen in patients, and there was no significant differential expression of proteins among different time points.

Phospho-flow Analysis

Baseline and serial (day 2, 15, and 28) bone marrow and peripheral blood samples were available for 8 patients to evaluate MAPK inhibition using pERK and AKT blockade using p4EBP1, pAKT, and pS6. Significant inhibition was observed in pERK in peripheral blood blasts ($p=0.0005$) and CD34+ peripheral blood blasts ($p=0.006$) between day 1 and day 15/28, shown in Figure 1. There was a trend toward significant inhibition in pS6 in peripheral blood blasts ($p=0.059$) and CD34+ peripheral blood blasts ($p=0.07$) between day 1 and day 15/28, demonstrated in Figure 2. Mixed results were identified for p4EBP1, though inhibition was noted consistently in 4 bone marrow and peripheral blast samples. Slight increase of pAKT was observed in 4 bone marrow samples, which is similar to flow analysis of other tumor types under AKT blockade.

Minor responders ($N=5$) were compared to non-responders ($N=3$) with available data, and no significant differences were seen when evaluating pERK, p4EBP1, pAKT, and pS6 by flow. Phospho-flow from dose cohort 1 ($N=3$) and dose cohort 2 ($N=5$) were compared, and again, no significant differences were seen between the two groups. Plots of flow data are provided in the supplementary file.

DISCUSSION

This trial was the first study to evaluate the MEK 1/2 inhibitor trametinib in combination with the AKT inhibitor GSK2141795 in patients with *RAS*-mutated AML. The intent of this analysis was to evaluate the tolerability, preliminary efficacy, and biologic activity of trametinib in combination with GSK2141795 in *RAS*-mutated AML to guide the feasibility and design of future therapeutic investigations using combinatorial MEK and AKT inhibition. The RP2D was identified as dose level 1, with trametinib dosed at 2 mg daily and GSK2141795 dosed at 25 mg daily. Despite preclinical evidence that this combination effectively kills leukemia cells, the combination at dose levels 1 and 2 did not prove effective in patients. The study was closed due to lack of clinical activity after 23 patients were enrolled.

Although this trial did not meet its primary endpoint, exploration of secondary objectives of toxicity and biologic effects was pursued. Fifteen out of 23 evaluable patients (65%) experienced at least possibly related adverse events, with 71% of events grade 2. At the RP2D, diarrhea and rash were seen most often. Rash and diarrhea have been well defined as overlapping toxicities of MEK and AKT inhibitors, and our results support an expected and manageable toxicity profile.^{42–46} Feasibility and tolerability have been shown in other advanced tumors using AKT and MEK inhibitors individually and in combination.^{12,13,32,42,43,45,47–49} This study is the first to evaluate MEK and AKT inhibitor combination therapy in relapsed/refractory AML. Dose escalation could not progress to dose level 3 as an unacceptable level of toxicity was determined at dose level 2.

Though minor responses of hematologic improvement were seen in 5 patients, no CR/CRps were obtained. Despite lack of objective clinical effect, patients continued study drugs for a median of 2 cycles. Many patients (64%) who remained on study >70 days (beyond 2 cycles) did report subjective symptomatic improvement or had minor responses to therapy.

Failure to achieve clinical effect with this combination has several possible causes. Heterogeneity of relapsed/refractory AML likely underlies a limited response to this targeted therapeutic strategy.⁵⁰ Targeted agents often rely on pathway dependence to induce clinical activity, and it is likely that multiple other concomitant protein pathways are important to the pathogenesis of *RAS*-mutated AML. Incomplete target inhibition could also have contributed to the lack of clinical effect in this study population.⁵¹ Further, tumor adaptation to compensatory survival pathways also contributes to limited efficacy of targeted regimens.⁵²

Our correlative studies did not reveal significant trends to define the biologic mechanisms contributing to the lack of clinical efficacy in this trial. RPPA analysis was used to explore the biologic effects of this combination in this study population. Despite extensive analysis evaluating direct protein targets, downstream protein targets, and compensatory proteins of MAPK and AKT, no significant up- or downregulation was identified. Each patient had unique protein expression findings, though there were trends consistent with expected downregulation in selected targets of MAPK and AKT in 10% of evaluated patients. There are many possible reasons why the expected biologic effects were not recognized in this study population, including the concept that the maximum tolerated clinical dose might not reflect the dosing necessary to produce the desired biologic effect of this combination. Further, protein dysregulation is expected in a heavily pre-treated population with relapsed/refractory AML, and many factors could contribute to unanticipated changes in investigated pathways.⁵³ Finally, technical issues of protein stability upon storage and differences in sample preparation at different centers could have contributed to lack of reproducible data.

Though RPPA did not reveal conclusive results regarding target modulation, expected modulation of pERK and pS6 was observed in flow cytometric analysis of samples from patients treated at MDACC, with significant and near-significant down-modulation by day 15 or 28, respectively. As an important recognized substrate of MEK, pERK has been shown to be elevated in >75% of AML patients, and even more so in relapsed/refractory AML.^{11,54,55} Prior studies have shown that down-modulation of pERK induces apoptosis of primary AML blasts.⁵⁶ Investigation of single-agent MEK inhibitor, selumetinib, revealed that 2 of 4 patients evaluated for pERK levels over time had notable decrease, with only one patient also having a clinical response to the agent.¹² pERK stands as a useful biomarker for MEK inhibition, and although expected pERK down-modulation was seen in our study population, clinical benefit was not observed. pS6 is a known downstream target of AKT, and flow cytometry revealed decrease in pS6 following AKT inhibition. p4EBP1 is another downstream target of AKT, and overall there was a noted decrease in p4EBP1 over time in 4 patient samples. Two patients had profound decrease in pS6 and p4EBP1 in their peripheral blasts by day 15/28; these results support expected downstream response to AKT inhibition. Generally, less inhibition of pS6 and p4EBP1 was seen in BM compared to PB cells, consistent with our prior reports of microenvironment-triggered stimulation of AKT signaling.^{57,58} Significant differences in p-AKT over time were not observed. Overall, flow analysis revealed effects of MEK and AKT inhibition are consistent with expected downstream effects of dual blockade. However, this did not translate into clinical efficacy, suggesting that dual blockade with the dose schema described is insufficient for effective treatment of relapsed/refractory AML.

Though preclinical data support ongoing exploration of this combination in *RAS*-mutated AML, this first trial of MEK and AKT inhibition in relapsed/refractory *RAS*-mutant AML patients failed to demonstrate clinical benefit at tolerated doses. Exploration of this combination in solid tumor malignancies has also revealed little clinical benefit. Dosing of trametinib at 1.5 mg and GSK 2141795 at 50 mg was explored in BRAF wild-type melanoma, and though the combination was deemed safe, the best response was stable disease and all cohorts were closed to further accrual due to lack of efficacy.⁵⁹ This dosing combination also yielded limited clinical efficacy in triple-negative breast cancer, with only one unconfirmed partial response among 16 evaluable patients.⁶⁰ However, in multiple myeloma, improvement was seen in overall response rate when GSK2141795 was added sequentially to trametinib for patients who did not have at least a partial response to trametinib alone.⁶¹ Despite lack of efficacy of this combination in relapsed/refractory AML, combining this dual agent inhibition strategy to target the RAS/RAF/MEK/ERK and PI3K/AKT/mTOR pathways along with cytotoxic, targeted, or epigenetic therapy to eliminate any possible compensatory pathways could prove to be an effective treatment strategy. AKT and MEK pathways are now recognized as resistance pathways for venetoclax, an extremely important antiapoptotic agent approved for use in AML.^{62,63} Subsequently, future study of MEK/AKT inhibitors to potentially overcome resistance to selective BCL2 inhibition is encouraged. Acknowledging the toxicity of this regimen is pertinent and efforts to abrogate the severe toxicities are necessary to pursue continued use of this combination in future trials.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DISCLOSURES OF INTEREST

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Figure 1. Inhibition in pERK is identified in peripheral and bone marrow blasts following MEK inhibition.

Day 1 MFI value assigned as 100%, and later timepoints expressed as percentage MFI compared to day 1.

Figure 2. Inhibition in pS6 is identified in bone marrow and peripheral blood blasts following AKT inhibition.

Day 1 MFI value assigned as 100%, and later timepoints expressed as percentage MFI compared to day 1.

Table 1.

Protocol dose levels

Dose Level	Trametinib (mg)	GSK2141795 (mg)
1	2	25
2	1.5	50
3	2	50

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Table 2.

Baseline characteristics, N=23.

Characteristic	Median (%)/[Range]
Age (years)	69 [21–80]
60 years	17 (74)
Diagnosis	
AML – de novo	9 (39)
AML – post MDS/CMML/MPN	14 (61)
Performance Status	
0	3 (13)
1	18 (78)
2	2 (9)
Bone Marrow Blast Percentage	36 [9–83]
WBC $\times 10^9/L$	3.8 [0.2–138]
Platelets $\times 10^9/L$	15 [2–203]
Hemoglobin (g/dL)	9.0 [7.9–10.4]
Cytogenetics	
Diploid	7 (30)
Complex	4 (17)
–5/–7	2 (9)
Other adverse (–17p, –11q, inv3, –6q)	3 (13)
Miscellaneous	5 (22)
Missing	2 (9)
Prior Therapies *	3 [1–14]
Prior Stem Cell Transplant	5 (21)
RAS Mutations	
KRAS	5 (22)
NRAS	15 (65)
Both NRAS/KRAS	3 (13)
Other Mutations, N=17	
FLT3	1 (6)
IDH1/IDH2	2 (12)
RUNX1	1 (6)
DNMT3A	3 (17)
ASXL1	1 (6)
NPM1	1 (6)
CEBPA	1 (6)
PTPN11	2 (12)

* Prior therapies include stem cell transplantation.

Table 3.

Adverse events for dose level 1, N=16.

	Grade 1	Grade 2	Grade 3	Grade 4
	N (%)	N (%)	N (%)	N (%)
Maculopapular Rash	3 (19)		2 (13)	
Fatigue	1 (6)	1 (6)		
Mucositis	1 (6)	1 (6)	1 (6)	
Esophageal Necrosis			1 (6)	
Diarrhea	5 (31)	1 (6)	3 (19)	
Nausea	1 (6)			
Vomiting		2 (13)		
Hypertension		2 (13)	1 (6)	
Gastroesophageal Reflux		1 (6)		
Anorexia		1 (6)		
Cholecystitis		1 (6)		
Hyperuricemia	1 (6)			
Acute Kidney Injury		2 (13)		
Febrile Neutropenia				1 (6)
Oral Pain	1 (6)			
Anemia		1 (6)		1 (6)
Decreased Platelet Count	1 (6)			
Decreased WBC Count	1 (6)	1 (6)		
Weight Loss	1 (6)			
Dysgeusia	1 (6)			
Infection			1 (6)	
ALT/AST Increase		1 (6)		
Gastric Hemorrhage				1 (6)

Table 4.

Adverse events for dose level 2, N=7.

	Grade 1	Grade 2	Grade 3
	N (%)	N (%)	N (%)
Maculopapular Rash		3 (43)	1 (14)
Mucositis	1 (14)		1 (14)
Diarrhea	2 (29)		1 (14)
Nausea	1 (14)		1 (14)
Vomiting			1 (14)
Abdominal Pain	1 (14)		
Anorexia	1 (14)		
Atrial Fibrillation	1 (14)		
Malaise	1 (14)		
Colitis			1 (14)
Dizziness	1 (14)		
Headache		1 (14)	