Preclinical and Early Clinical Evaluation of the Oral AKT Inhibitor, MK-2206, for the Treatment of Acute Myelogenous Leukemia

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Abstract

Purpose: Recent studies suggested that AKT activation might confer poor prognosis in acute myelogenous leukemia (AML), providing the rationale for therapeutic targeting of this signaling pathway. We, therefore, explored the preclinical and clinical anti-AML activity of an oral AKT inhibitor, MK-2206.

Experimental Methods: We first studied the effects of MK-2206 in human AML cell lines and primary AML specimens in vitro. Subsequently, we conducted a phase II trial of MK-2206 (200 mg weekly) in adults requiring second salvage therapy for relapsed/refractory AML, and assessed target inhibition via reverse phase protein array (RPPA).

Results: In preclinical studies, MK-2206 dose-dependently inhibited growth and induced apoptosis in AML cell lines and primary AML blasts. We then treated 19 patients with MK-2206 but, among 18 evaluable participants, observed only 1 (95% confidence interval, 0%–17%) response (complete remission with incomplete platelet count recovery), leading to early study termination. The most common grade 3/4 drug-related toxicity was a pruritic rash in 6 of 18 patients. Nevertheless, despite the use of MK-2206 at maximum tolerated doses, RPPA analyses indicated only modest decreases in Ser473 AKT (median 28%; range, 12%–45%) and limited inhibition of downstream targets.

Conclusions: Although preclinical activity of MK-2206 can be demonstrated, this inhibitor has insufficient clinical antileukemia activity when given alone at tolerated doses, and alternative approaches to block AKT signaling should be explored.

Clin Cancer Res; 20(8); 2226–35. ©2014 AACR.
Translational Relevance
Outcomes for patients with acute myelogenous leukemia (AML) remain largely unsatisfactory. Recent studies have suggested that AKT activation is common in AML and may be associated with worse survival, providing the rationale for therapeutically targeting this signaling pathway. In this report, we establish the preclinical activity of the AKT inhibitor, MK-2206, against human AML cells and describe its efficacy in patients with AML requiring second salvage treatment for relapsed/refractory disease. Our study fails to demonstrate the desired clinical benefit but reveals insufficient target modulation at maximally tolerated drug doses. These results emphasize the importance of pharmacodynamic monitoring in clinical trials of targeted agents and support investigation of more potent AKT pathway modulators in AML.

Analysis of cell viability and apoptosis. Cells were treated with various doses of MK-2206 for up to 72 hours. Cell viability and cell numbers were quantified by Trypan blue dye exclusion assay using a Vicell. To determine the mechanism of cell death, cells were washed in PBS, and resuspended in binding buffer containing Annexin V (Roche Diagnostics). Apoptotic cells were identified by positive Annexin V staining using a BD LSR II flow cytometer (BD Biosciences).

Western blot analysis. OCI-AML3, MOLM13, or primary AML blasts were sonicated in lysis buffer (62.5 mmol/L Tris (pH 8.0), 2% SDS, 10% glycerol, 100 μmol/L AEBSF, 80 nmol/L aprotinin, 5 μmol/L bestatin, 1.5 μmol/L E-64, 2 μmol/L leupeptin, 1 μmol/L pepstatin, 500 μmol/L sodium orthovanadate, 500 μmol/L glycerol phosphate, 500 μmol/L sodium pyrophosphate, and 50 μmol/L DTT), and protein (5 × 10^5 cell equivalents) was subjected to electrophoresis using 10% to 14% acrylamide/0.1% SDS gels. Proteins were transferred onto nitrocellulose, and membranes were probed with monoclonal antibodies against pAKT Thr308 and Ser473, phospho-S6, S6 (all from Cell Signaling Technology), and Tubulin (Sigma-Aldrich).

Materials and Methods
In vitro investigations

Materials. All reagents were purchased from commercial sources unless otherwise stated. MK-2206 was partially provided by Merck & Co., Inc. and partially obtained from LC Laboratories.

AML cell lines and primary AML cells. OCI-AML3 cells were kindly provided by M.D. Minden (Ontario Cancer Institute, Toronto, ON, Canada). HL60, U937, and MOLM13 cells were obtained from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (Braunschweig, Germany). THP-1 and MO7e were purchased from the American Type Culture Collection. MOLM14 cells were kindly provided by Dr. Mark Levis (Johns Hopkins University, Baltimore, MD). Cells were maintained in RPMI-1640 supplemented with 5% FBS and 5% bovine calf serum at 37°C in 5% CO2. Peripheral blood specimens containing >40% blasts were obtained from patients with newly diagnosed or recurrent AML. Informed consent was obtained following institutional guidelines. Mononuclear cells were isolated by Ficoll density gradients (Sigma-Aldrich). Samples from healthy bone marrow donors were selected for CD34+ cells using a MiniMacs Separator (Miltenyi Biotec) according to the manufacturer’s instructions.

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Clinical trial

Study population. A phase II study with MK-2206 was conducted at the MD Anderson Cancer Center (MDACC; Houston, TX) and the Fred Hutchinson Cancer Research Center (FHCRC; Seattle, WA) between October 2010 and October 2012. Patients ≥18 years of age were eligible if they had persistent or relapsing AML (other than acute promyelocytic leukemia; ref. 27) requiring second salvage therapy (i.e., treatment for second or higher relapse or for primary refractory disease after failure of two prior treatment regimens) provided they had a prior complete remission (CR) duration <12 months. At MDACC, patients ≥60 years were also eligible with <2 prior regimens if they did not have favorable risk cytogenetics and were not candidates, or refused standard chemotherapy. Other inclusion criteria included: an Eastern Cooperative Oncology Group performance status of 0 to 2; total bilirubin ≤2.0 × upper limit of normal (ULN) unless elevation was due to hepatic infiltration by AML; Gilbert syndrome, or hemolysis; aspartate aminotransferase (AST)/ALT ≤2.5 × ULN unless elevation was due to hepatic infiltration by AML; serum creatinine ≤1.5 × ULN; fasting glucose ≤150 mg/dL; and HbA1c ≤9%. Exclusion criteria were: use of other investigational agents; major surgery within 4 weeks before treatment without complete recovery; uncontroled systemic infection; systemic chemotherapy within 14 days; central nervous system disease; history of clinically significant heart disease; QTc prolongation >480 ms; uncontroled hypertension; pregnancy and breast feeding; HIV infection with CD4 cells below leukemia onset ≤400 cells/mm^3 or AIDS-defining illness; and active hepatitis B or C. Cytogenetic risk-group assignment was done according to the refined National Cancer Research Institute (NCRI)/Medical Research Council (MRC) criteria (28). Treatment responses were defined according to standard criteria (2, 29). The Institutional

www.aacrjournals.org Clin Cancer Res; 20(8) April 15, 2014 2227

Published OnlineFirst February 28, 2014; DOI: 10.1158/1078-0432.CCR-13-1978
Review Boards at MDACC and FHCRC approved this study (ClinicalTrials.gov: NCT01253447), and patients gave consent in accordance with the declaration of Helsinki.

Treatment plan. Treatment consisted of MK-2206 200 mg orally once weekly, the maximum tolerated dose (MTD) in patients with solid tumors (26). The weekly dosing was recommended by CTEP based on phase I data demonstrating pAKT suppression in platelet-rich plasma up to 96 hours after dose (26). With the exception of hydroxyurea during the first month of therapy, concomitant cytotoxic medications were prohibited. Patients were assessed for response after every 28-day cycle and were allowed to continue study treatment if there was no evidence of progressive disease or clinically significant toxicities. Patients achieving either CR, CR with incomplete platelet count recovery (CRp), or partial remission (PR) after three cycles of therapy could continue study treatment for up to 12 cycles. Patients not achieving an objective response but obtaining clinical benefit from the trial drug could also remain on study beyond 3 months if no significant toxicities occurred.

Statistical considerations. The primary objective of the study was to determine the proportion of patients achieving CR, CRp, or PR as best response within three cycles of therapy; secondary objectives included the estimation of disease-free survival of patients who achieved CR/CRp, and the establishment of the toxicity profile of MK-2206. A Simon two-stage optimum design (30) was used to test the null hypothesis that the response rate (i.e., CR, CRp, or PR) at week 12 was ≤10% versus the alternative that the response rate is ≥25% at an α level of 0.05 with 80% power. Patients who were removed from study therapy for reasons other than disease progression (e.g., noncompliance, fatal infections) before completion of three cycles of therapy were not included in the efficacy assessment, and additional patients were enrolled. With three or more responses in the first 18 patients, the trial would continue to accrue up to 43 patients; otherwise, the trial would stop early and the drug would be rejected for further study. With ≥8 responses among 43 patients enrolled, the drug would be considered effective. With this design, the probability of early termination was 73% if the true response rate was only 10%. With the concern of treatment-related toxicity, the nonhematologic toxicity (≥grade 3, with the exception of hyperglycemia) would also be continuously monitored during the study using established Bayesian-based methods (31). The stopping rule was set such that the trial would be halted if, at any time during the study, there was a >90% chance that the toxicity rate exceeded 30%.

Biomarker analyses. Biomarkers studies were optional and only performed in samples from patients consenting to these studies. Peripheral blood samples were collected before and 24 hours after the first administration of MK-2206 during the initial treatment cycle, and bone marrow samples were collected at baseline and before the second treatment cycle. Mononuclear cells were separated by Ficoll gradients, and cell lysates were subjected to reverse phase protein array (RPPA) analysis following previously described and validated methods (32–34). Slides were probed with 157 primary antibodies, including two recognizing pAKT (Thr308 and Ser473; both Cell Signaling Technology), a secondary antibody for signal amplification, and a stable dye for precipitation (35). The slides were scanned, and images quantified using Microvigene Version 2.9 software (Vigene Tech). Raw signal intensity data were processed with SuperCurve to estimate relative protein concentrations, with further data normalization to adjust loading bias by median centering each marker and median centering each sample (36, 37). We used paired t tests using the Benjamini–Hochberg method for adjustment to control the false discovery rate (38) to detect differential protein expression or phosphorylation of each protein in the following three subsets: (i) the target itself (pAKT Ser473 and Thr308); (ii) selected proteins associated with AKT inhibition (4E-BP1-pS65, 4E-BP1-pT37-T46, Bim, cyclin D1, FOXO3a pS318-S321, GSK3α-β pS21-S9, IRS1, Mc1, mTOR pS2448, NF-kB-p65 pS536, p70S6K-pT389, PRAS40-pT246, PTEN, S6 pS235-S236, S6 pS240-S244, Survivin, and XIAP); and (iii) all 157 proteins. In subsets (i) and (ii), we used one-sided tests because we could specify the expected directions of change a priori. Heatmaps of selected markers and samples were plotted in Supplementary Fig. S4, in which the data were median centered for each marker.

Results

In vitro investigations of MK-2206 in human AML cells

In line with previous investigations in solid tumor cancer as well as ALL cells (18, 20, 39–41), treatment of human AML cell lines (OCI-AML3 and U937) with MK-2206 dose-dependently reduced Ser473 AKT phosphorylation but left total AKT levels unchanged (Fig. 1A). Reflective of loss of AKT activity, both cell lines exhibited reduced phosphorylation of PRAS40 and the mTOR targets, S6 kinase (at Ser240), and 4EBP1 (Thr37/45) in response to MK-2206, albeit inhibition of these downstream targets required higher concentrations of MK-2206. Similar findings were seen in FLT3-mutated MOLM13 and MOLM14 cells (Supplementary Fig. S1). Consistent with these cell line data, treatment with MK-2206 suppressed Ser473 AKT phosphorylation, pPRAS40, and p4EBP1 (Thr37/46) in three primary AML samples tested (Fig. 1B).

Having confirmed target inhibition with MK-2206, we then investigated the effects of the drug on viability of various human AML cell lines. As shown in Table 1 and Supplementary Fig. S2A, MK-2206 at 1 μmol/L effectively suppressed cell growth in all cell lines except Mo7e cells after 72 hours, with OCI-AML3, MV4;11, MOLM14, THP1 but not U937 or MOLM13 cells exhibiting growth inhibition with as little as 0.1 to 0.2 μmol/L of MK-2206. In turn, induction of apoptosis was only seen at higher doses (5–10 μmol/L) of MK-2206 (Supplementary Fig. S2A). Cell-cycle analyses in OCI-AML3 cells indicated that MK-2206 induced G1 arrest, particularly at higher drug concentrations (Supplementary Fig. S2B). Finally, we assessed the effects of MK-2206 in two primary AML...
specimens harboring FLT3 mutations and found blockade of AKT signaling, as indicated by inhibition of 4EBP1 phosphorylation and S6 (Fig. 2). Furthermore, as shown in Fig. 2, MK-2206 induced apoptosis in both samples after 48 hours. Expression of AKT signaling proteins was analyzed by immunoblotting (left) and quantified by densitometry (right). PT#1, refractory AML, N-Ras mutated, del(12p); PT#2, refractory AML, diploid; and PT#3, newly diagnosed AML arising from antecedent MDS, N-Ras mutated, del(5q) and -7.

Clinical trial
Given the above findings, we conducted a phase II trial of MK-2206 in adults with poor prognosis AML to begin testing its clinical efficacy and tolerability in this disease. Although initial clinical studies explored alternate dosing schedules (23), preclinical data indicated that weekly dosing would be at least as efficacious. Thus, later clinical studies investigated MK-2206 given once weekly (26).
Building upon this experience, we administered MK-2006 at 200 mg weekly, the MTD established in patients with solid tumors (26).

Over a period of 2 years, 19 adults with a median age of 70 years were enrolled in this study and received at least one dose of MK-2206 (Table 2). The median number of cycles administered was two, with 4 patients completing ≥3 cycles. Eighteen patients were eligible for efficacy and toxicity assessments. One patient was not included in these analyses because of study removal after one cycle due to noncompliance; at that time, however, this patient had stable disease and no evidence of drug-associated toxicities. Among the 18 eligible patients, only one CRp was observed (5.6%; exact 95% confidence interval, 0%–17%). This patient was a 77-year-old male with normal karyotype secondary AML without FLT3, KIT, or RAS mutations that arose from antecedent chronic myelomonocytic leukemia. He initially achieved CR with clofarabine/low-dose cytarabine but then relapsed and failed a first salvage therapy with twice-daily fludarabine and cytarabine. He achieved a CRp after the first cycle of MK-2206 with a decrease in bone marrow blasts from 13% to <5%. Subsequently, his leukemia burden fluctuated over the next several months of therapy between 4% and 15%, and he was removed from study after completion of 11 treatment cycles. In contrast, the 17 nonresponders were removed from study for disease progression after one cycle (n = 7), two cycles (n = 8), three cycles (n = 1), or four cycles (n = 1), respectively.

In general, MK-2206 monotherapy was reasonably well tolerated. However, as the most common grade 3/4 adverse event related to study drug, a pruritic rash occurred in 6 of 18 evaluable patients (33.3%); this side effect led to dose

| Table 1. Effects of MK-2206 on cell growth and survival in human AML cell lines |
|-----------------|-----------------|----------------|
| Cell line      | Characteristics | IC50 (μmol/L) |
| MOLM13         | FLT3-Mutant     | 1.16           |
| OCI-AML3       | NPM1-Mutant     | 1              |
| U937           | PTEN-mutant     | 1.48           |
| THP1           | N-Ras-mutant    | 4.46           |
| Mo7e           | N-Ras-mutant    | >5             |
| MV4;11         | t(4;11), MLL-rearranged | 3.92 |
| MOLM14         | FLT3-Mutant     | 0.42           |

NOTE: AML cells were cultured with increasing concentrations of MK-2206 (from 40 nmol/L up to 10 μmol/L, in triplicates) for 48 hours, after which effects on cell growth were determined by viable cell counts. IC50 values were calculated using CalcuSyn software. Abbreviation: MLL, myeloid/lymphoid, or mixed lineages leukemia.
Table 2. Characteristics of study cohort

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n = 19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (range), y</td>
<td>70 (31–86)</td>
</tr>
<tr>
<td>Sex (male/female), n</td>
<td>12/7</td>
</tr>
<tr>
<td>Disease stage, n (%)</td>
<td></td>
</tr>
<tr>
<td>First relapse</td>
<td>10 (52.6%)</td>
</tr>
<tr>
<td>Second relapse</td>
<td>9 (47.4%)</td>
</tr>
<tr>
<td>Disease manifestation, n (%)</td>
<td></td>
</tr>
<tr>
<td>Bone marrow relapse</td>
<td>18 (94.7%)</td>
</tr>
<tr>
<td>Isolated extramedullary relapse</td>
<td>1 (5.3%)</td>
</tr>
<tr>
<td>Performance status, n (%)</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>17 (89.5%)</td>
</tr>
<tr>
<td>2</td>
<td>2 (10.5%)</td>
</tr>
<tr>
<td>Cytogenetic risk-group, n (%)a</td>
<td></td>
</tr>
<tr>
<td>Favorable</td>
<td>0</td>
</tr>
<tr>
<td>Intermediate</td>
<td>13</td>
</tr>
<tr>
<td>Unfavorable</td>
<td>6</td>
</tr>
<tr>
<td>Secondary AML, n (%)</td>
<td>9 (47.4%)</td>
</tr>
<tr>
<td>Laboratory findings at baseline, median (range)</td>
<td></td>
</tr>
<tr>
<td>WBC (×10^3/μL)</td>
<td>1.7 (0.5–11.5)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>9.6 (8.4–13.2)</td>
</tr>
<tr>
<td>Platelets (×10^3/μL)</td>
<td>39 (11–1,887)</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>455 (153–1,222)</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.91 (0.6–1.69)</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>0.6 (0.3–1.3)</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>22 (11–42)</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>27 (12–64)</td>
</tr>
</tbody>
</table>

aOn the basis of revised MRC prognostic classification (28).

duration in 3 patients (135 mg/wk) and discontinuation of drug in 1. Besides milder (grade 1/2) rashes in another 4 patients (22.2%), other adverse events were hyperglycemia [n = 12 (66.7%), all grade 1/2 except for grade 4 in one patient], and grade 1 QTc prolongation [n = 4 (22.2%)], respectively (Table 3). Ultimately, the study was terminated early after inclusion of 18 eligible patients because of insufficient drug efficacy, as per predefined stopping rules.

Finally, we conducted proteomic analyses to investigate the degree of target inhibition with MK-2206 using RPPA in paired peripheral blood specimens—obtained before and 24 hours after the first dose of MK-2206—in 8 patients, as well as in paired bone marrow specimens—obtained before and 28 days after the first dose of MK-2206—in 5 patients, respectively. Two patients (#1 and #17, the latter with extramedullary disease only) who had low pAKT levels in the baseline samples (less than −4.0 on log, scale) were excluded from this analysis and, unfortunately, the sole responding patient did not consent to these optional biomarker studies. Considering AKT alone, the decrease in Ser473 AKT was significant (P = 0.018). However, the larger sets (i.e., testing direct AKT targets or the entire 157 proteins) showed no significant changes after adjusting for multiple testing. Of note, a decrease in Ser473 AKT (median 28%; range, 12%–45%) was seen in five of seven peripheral blood and three of four bone marrow specimens, whereas a reduction in Thr308 AKT phosphorylation was found in five of seven peripheral blood as well as three of four bone marrow samples, respectively (Fig. 3A and B). In turn, changes in AKT targets were less evident (downregulation of pFOX3A in 3/7, pPRA40 in 2/7, pS6K in 4/7, and p4EBP1 in 2/7 peripheral blood samples), possibly due to incomplete AKT inhibition. In samples with sufficient amounts of protein available, confirmatory immunoblotting was performed, which showed findings consistent with RPPA. Specifically, ≥50% downregulation of pAKT-473 was seen in peripheral blood samples from subjects #2, 3, 7 and in bone marrow sample #6, with lesser decreases in the mTOR targets, pS6 and p4EBP1 (Fig. 4A and B), with changes in pAKT being highly concordant between the two techniques (P = 0.01; Supplementary Fig. S3). With regard to downstream targets or other proteins, including phosphorylated mitogen-activated protein kinase (MAPK), no significant changes were found after adjustment for multiple testing by RPPA (Fig. 3A, last column). We further analyzed a subset of proteins recently reported to be upregulated in a compensatory fashion upon PI3K/AKT blockade. RPPA analysis indeed demonstrated upregulation of a subset of these proteins, such as Bcl-2 (P = 0.036), Smad3 (P = 0.028), HER2 (P = 0.064), p70S6k Stat-3 (P = 0.033), p38 MAPK (P = 0.039) and MEK1 (P = 0.01), although these changes did not remain significant after accounting for multiple comparisons (Supplementary Fig. S4).

Discussion

The in vitro data presented in this study showing that AKT inhibition impairs the survival of AML cells provide further rationale for selecting this signaling pathway as pharmacologic target in AML. However, our findings also indicate that the oral AKT inhibitor, MK-2206, has insufficient activity as a single agent in patients with relapsed/refractory AML at the chosen dose. Although initial studies with MK-2206 were conducted using every-other-day drug dosing, a weekly
dosing schedule was explored because of the long half-life of the drug (60–80 hours) and an attempt to ameliorate feedback activation of alternative signaling pathways in response to continuous AKT inhibition. Despite the fact that MK-2206 was reasonably well tolerated in our study cohort, the frequent occurrence of grade 3/4 rash suggests that dose escalation at the weekly schedule may not be feasible. Skin toxicity was also a prominent toxicity in patients with solid tumor who received MK-2206 at doses higher than 60 mg every-other-day (23), a finding that disabled escalation of drug dosing in those patients.

In our study population, an objective response was documented in only one patient, indicating suboptimal activity of MK-2206. Failure of targeted anticancer agents has been attributed to multiple causes, including (i) use in the “wrong” patient population, for example, because tumor cells do not depend on the specific pathway; (ii) rapid activation of the compensatory survival pathways; and (iii) incomplete target inhibition. In AML, activation of the AKT pathway is generally attributed to mutation(s) and hyperactivation of the upstream receptor tyrosine kinases (RTK) such as KIT, FLT3, or RAS, without activating mutations in PI3K or AKT itself. If activating mutations in AKT are present, cells may become hypersensitive to MK-2206 (42). Because of the present study did not include patients with such mutations, potential activity of MK-2206 in selected patient populations may have been missed.

Figure 3. Modulation of protein expression in AML cells by MK-2206 in peripheral blood (PB; A) or bone marrow (BM; B). PB or BM mononuclear cells were collected before treatment (“1”) or at selected time points after initiation of MK-2206 (“2”), which was 24 hours for PB samples and at the completion of the first cycle of therapy for BM samples. Protein lysates were subjected to RPPA analysis. Of note, y-axis, normalized log2-transformed relative values of protein expression for the indicated proteins.
To assess the response to AKT inhibition and the potential for compensatory activation of other pathways, we interrogated intracellular signaling in AML blasts using RPPA. Recent evidence suggests that the acquired resistance to PI3K/AKT inhibition stems from the upregulation and activation of RTKs through mechanisms that involve FOXO-regulated transcription (43) and of other cellular survival proteins, including antiapoptotic proteins (Bcl-2, XIAP) and transcription factors (p-STAT3, p-STAT6, p-c-Jun, p-SMAD3), in part through cap-independent translation (44). RPPA analysis indeed demonstrated upregulation of a subset of prosurvival proteins, including Bcl-2, Smad3, pY705 Stat-3, p38 MAPK and MEK1. However, MK-2206 failed to substantially downregulate p-FOXO3A and did not
significantly modulate other FOXO targets, indicating FOXO-independent upregulation of these proteins. Further studies with MK-2206 or other PI3K/AKT inhibitors may help characterize AML-specific compensatory pathways induced in response to AKT blockade, paving the way for further combination strategies. Our correlative studies indicate that the main clinical limitation of MK-2206 may be incomplete target inhibition, with <50% decrease in pAKT expression at 24 hours. Although the degree of AKT inhibition was likely higher at earlier time points, we purposefully chose a 24-hour time point to estimate sustained AKT inhibition, consistent with prior pharmacodynamics assessments (23, 26). Not surprisingly, nonsustained AKT blockade translated into insubstantial inhibition of known AKT downstream targets. Our in vitro studies indicated that MK-2206 could inhibit AKT phosphorylation at low concentrations (of 40–200 nmol/L). However, higher doses (1–5 μmol/L) were required for complete inhibition of downstream targets, and only such higher doses translated into AML growth suppression. Although our study did not include pharmacokinetic assessments, prior phase I data (26) showed $C_{\text{max}}$ concentrations ranging from approximately 150 to 400 nmol/L at 200 mg weekly dosing. Combined, these data indicate below-target exposures in vivo at tolerable drug doses, indicating limited potential of further development of MK-2206 as a single agent. It is interesting to speculate whether a different dosing schedule (e.g., one that is optimized on the basis of pharmacokinetic/dynamic measurements) could provide better results. Although such a strategy might improve target inhibition, it will need to be approached carefully as the clinical experience obtained with MK-2206, thus far, indicates significant toxicities, which may worsen as target inhibition is improved. Alternatively, our findings might suggest that other (e.g., combinatorial) approaches to block AKT signaling should be explored in AML. As such, additive or synergistic antitumor effects were observed upon combination of MK-2206 with standard chemotherapeutic agents and targeted agents (17, 18, 43, 45–48).

In summary, although our preclinical data support further development of AKT targeting agents in AML, this first AML trial with an allosteric AKT inhibitor failed to show clinical benefit at tolerated doses. Evaluation of MK-2206 together with other agents affecting the AKT signaling pathway and/or other AKT inhibitors alone or in combination, coupled with detailed biomarker analyses, may be necessary to define the potential utility of such small-molecule inhibitors in the treatment armamentarium for AML.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Authors’ Contributions**

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**Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):** M.Y. Konopleva, R.B. Walter, S.H. Faderl, G. Borthahrk, T.M. Kadia, J.B. Felu, H. Lu, J.A. Burger, S.M. Kornblau, E.H. Estey.


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**Study supervision:** L.A. Doyle.

**Acknowledgments**

The authors thank the provision of MK-2206 by NCI/CTEP.

**Grant Support**

This work was supported by grants R21-CA159285 (to M.Y. Konopleva), P30-CA015704–3556 (to R.B. Walter), and MDACC Core grant CA16672 from the National Cancer Institute/NIH (Bethesda, MD). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received July 18, 2013; revised January 21, 2014; accepted February 4, 2014; published OnlineFirst February 28, 2014.

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