SUPPLEMENTAL FIGURES

Supplemental Figure 1. Effects of MK-2206 on AKT signaling in FLT3-mutant AML cells. MOLM13 and MOLM14 cells were exposed to the indicated concentrations of MK-2206 for 6 hours. Whole cell lysates were subjected to immunoblot analyses as described under Methods. Relative protein levels were quantified by densitometry.

Supplemental Figure 2
Effects of MK-2206 on cell growth and survival of human AML cell lines. (A) OCI-AML3, U937, MOLM13 and MOLM14 cells were cultured with indicated concentrations of MK-2206 for 48 hours, after which effects on cell growth (left) and apoptosis (right) were determined by viable cell counts and annexin V staining, respectively. (B) MK-2206 promotes accumulation of OCI-AML3 cells in the G1 phase of the cell cycle. To determine the effect of MK-2206 on OCI-AML3 cell cycle, cells were treated with 1, 5, or 10 µM MK-2206 for 24 hours and then stained with propidium iodide. Analytical flow cytometry was performed and FlowJo software utilized to identify G1, S, and G2 phases of the cell cycle.

Supplemental Figure 3
Correlation between RPPA and immunoblotting in samples from the patients treated on MK-2206 trial. Correlation of changes in relative signal intensities for pAKT by immunoblotting (X-axis) and RPPA (Y-axis) were analyzed using Spearman correlation coefficient test (R=0.817, p=0.01). Data from changes in both, PB (Days 2 and 1, n=5) and BM samples (n=4, days 28 and day 1) were combined for the purpose of the analysis.

Supplemental Figure 4
The subtraction heat maps of selected markers in paired PB samples: left, AKT targets and regulators; right, markers reported to be associated with resistance to AKT inhibitors. The data were median-centered for each marker.