**Title**

Phase II Study of the Oral MEK Inhibitor Selumetinib in Advanced Acute Myelogenous Leukemia: A University of Chicago Phase II Consortium Trial

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# Phase II Study of the Oral Mek Inhibitor Selumetinib in Advanced Acute Myeloid Leukemia (AML): A University of Chicago Phase II Consortium Trial

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## Abstract

**Purpose**—The clinical relevance of targeting RAS/RAF/MEK/ERK pathway, activated in 70-80% of acute myeloid leukemia (AML) patients, is unknown.

**Experimental Design**—Selumetinib is an oral small molecule inhibitor of MEK1/2 kinase. Forty-seven patients with relapsed/refractory AML or ≥60 years old with untreated AML were enrolled on a phase II study. Patients were stratified by *FLT3* ITD mutation status. The primary endpoint was response rate (complete, partial and minor). Leukemia cells were analyzed for ERK and mTOR phosphorylation.

**Results**—Common drug-related toxicities were grade I-II diarrhea, fatigue, nausea, vomiting and skin rash. In the *FLT3* wild type cohort, 6/36 (17%) patients had a response [1 partial response, 3 minor responses, 2 unconfirmed minor responses (uMR)]. No patient with *FLT3* ITD responded. *NRAS* and *KRAS* mutations were detected in 7% and 2% patients, respectively. The sole patient with *KRAS* mutation had uMR with hematologic improvement in platelets. Baseline p-ERK

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activation was observed in 85% of patients analyzed but did not correlate with a response. A single nucleotide polymorphism (SNP) rs3733542 in exon 18 of *KIT* gene was detected in significantly higher number of patients with response/stable disease compared with non-responders (60% vs 23%; p=0.027).

**Conclusion—**Selumetinib is associated with modest single agent antileukemic activity in advanced AML. However, given its favorable toxicity profile, combination with drugs that target other signaling pathways in AML should be considered. The potential association of SNP rs3733542 in exon 18 of *KIT* gene with antileukemic activity of selumetinib is intriguing, but will require validation in larger trials.

## Introduction

The outcome for relapsed/refractory acute myeloid leukemia (AML) is poor, and effective treatment options are limited.(1) The explosion of information on the molecular pathogenesis of AML has afforded new opportunities for the development of novel molecularly-targeted agents.(2)

The RAS/RAF/MEK/ERK signaling pathway plays a central role in the regulation of many cellular processes and in growth factor receptors signaling.(3) Therefore, it is not unexpected that it is one of the most dysregulated pathways in human cancers.(3-7) In AML, the RAS pathway is activated by mutations in upstream receptor tyrosine kinases such as FLT3 and c-KIT or mutations or overexpression of components of downstream effector pathways. In a recent report, the mutation frequency for *FLT3*, *NRAS* and *KRAS* genes in AML were 37%, 10% and 2%, respectively.(8) In addition, constitutive activation of ERK1/2 is seen in 70-80% of AML cell lines and primary AML samples, and MEK inhibition in vitro has resulted in growth arrest of primary AML cells.(9, 10)

Selumetinib (AZD6244, ARRY-142886) is a potent, selective, orally bioavailable, and non-ATP competitive small molecule inhibitor of MEK1/2.(11, 12) Selumetinib inhibits ERK1 and ERK2 phosphorylation in a variety of cancer cell lines and in xenograft models.(11, 13) Selumetinib is particularly potent in inhibiting viability of cell lines with *BRAF* or *KRAS* mutation.(13) In a prior phase I study, doses ranging from 50mg orally twice daily to 300mg orally twice daily were explored in a variety of advanced solid tumors.(12) Skin rash was the most frequent toxicity and DLT and the dose of 100mg twice daily was selected as the recommended phase II dose. We hypothesized that the use of selumetinib in AML patients would lead to inhibition of RAS-mediated signal transduction with subsequent antileukemic effect. We also hypothesized that such an effect would be most pronounced in patients who have evidence of constitutive activation of the pathway at baseline through a mutation in the *RAS, FLT3* or *KIT* genes.

We report here the results of a phase II multicenter study of single-agent selumetinib in advanced AML patients. The primary objective of the study was to determine the response rate to selumetinib. Secondary objectives of this study were to determine the relationship between baseline p-ERK activation and clinical outcome, and to correlate the outcomes with the presence of mutated *RAS, FLT3*, or *KIT*.

## Materials and Methods

### Patients and Eligibility Criteria

Patients were eligible for the study if they had histologically confirmed relapsed or refractory AML, secondary AML, including therapy-related AML or AML arising from an antecedent hematologic disorder, or newly diagnosed AML if 60 years of age or older and not a candidate, or who had refused standard chemotherapy for AML. Other inclusion criteria included age 18 years of age, ECOG performance status of 2, relatively normal organ function at baseline, and ability to give informed consent.

There was no limitation on the number of prior chemotherapy regimens received, including prior autologous or allogeneic stem cell transplantation. Hydroxyurea was permitted for the first 7 days. The protocol was reviewed and approved at each institution's Institutional Review Board, and all subjects enrolled gave written informed consent.

### Treatment and Response Evaluation

The study was a National Cancer Institute (NCI)/Cancer Therapy Evaluation Program (CTEP) –sponsored open label, multicenter phase II trial conducted through the University of Chicago Phase II Consortium. Selumetinib was provided by NCI/CTEP in collaboration with AstraZeneca Pharmaceuticals (Macclesfield, UK).

Based on the results of a prior phase I study, (12) selumetinib was administered orally (freebase formulation) at 100mg twice daily for a 28-day cycle, without interruption. Baseline evaluation included bone marrow aspiration and biopsy for morphology, immunophenotyping, cytogenetics, and molecular analyses.

To assess response to therapy, a CBC was performed weekly for the first 8 weeks, then every other week for subsequent cycles. Bone marrow aspiration and biopsy were performed after 1, 2 and 4 cycles of therapy and thereafter as clinically indicated. Responses [CR, complete remission with incomplete blood count recovery (CRi) and partial remission (PR)] were defined using standard criteria developed by an International Working Group.(14, 15) In addition, given the anticipated cytostatic nature of this agent,(10) the following additional criteria were proposed to capture any evidence of antileukemic activity in this early phase trial: Minor response (MR) was defined as 50% decrease in blasts in peripheral blood and/or bone marrow (similar to a prior definition used to describe antileukemic activity observed in other early phase trials with kinase inhibitors in AML),(16, 17) maintained for 4 weeks. Unconfirmed minor response (uMR) was defined as 50% decline in marrow blasts without a follow-up confirmatory marrow evaluation. Hematologic improvement (HI), stable disease (SD) and disease progression (PD) criteria were adapted from and applied as previously defined for myelodysplastic syndrome (MDS).(18) Treatment could continue indefinitely in the absence of disease progression or unacceptable adverse events. Toxicities were graded according to the NCI-Common Terminology Criteria for Adverse Events version 3.0.

### Correlative Studies

Bone marrow aspirate and peripheral blood samples were collected at baseline for the correlative studies outlined below.

### Mutational Analysis of *FLT3*, *NRAS*, *KRAS* and *KIT* Genes

***FLT3* mutation analysis—**Evaluation for the presence of *FLT3* ITD and activation loop mutations (Asp835) was performed by the institutional molecular diagnostic laboratories. This analysis was performed in real-time and the results were utilized to stratify patients at the time of enrollment into the *FLT3* wild type or *FLT3* mutant cohort.

***NRAS* and *KRAS* mutation analysis—**Genomic DNA was extracted from cryopreserved bone marrow or peripheral blood mononuclear cells using the Gentra Puregene kit (Qiagen Inc, Valencia, CA). *NRAS* mutation analysis at codons 12, 13, and 61 was performed using the hybridization probes method described by Nakao and colleagues, (19) and the results were confirmed by direct sequencing. Samples were analyzed for *KRAS* mutations at codons 12, 13, and 61 by direct sequencing. The primer sequences and PCR conditions were the same as those used by Liang and colleagues.(20)

***KIT* mutation analysis—**DNA was prepared from whole blood or marrow aspirate using the QIA amp DNA blood mini kit (Qiagen). DNA purity and concentration were determined by UV spectrophotometry. Using 50 ng DNA per reaction, KIT exons 8, 9, 11, 13, 17 and 18 were each separately amplified by PCR with intronic primers (supplementary table 1). The amplicons were sequenced using the BigDye Terminator v1.1 Cycle sequencing kit (Applied Biosystems) and run on an ABI_3100 capillary electrophoresis analyzer. DNA sequences were analyzed with both SeqScape v2.1.1 (Applied Biosystems) and Mutation Surveyor (Softgenetics), using NM_000222 as the reference sequence.

### Phospho-flow Analysis

**p-ERK1/2 and p-mTOR analysis—**For flow cytometry, cells were collected and cryopreserved immediately at the sites (University of Chicago and City of Hope) with that capability prior to shipment. For sites that did not have that capability, samples were shipped overnight and immediately cryopreserved on arrival. Cryopreserved samples were batched for phospho-flow analysis and were thawed, permeabilized and fixed prior to shipment for that analysis. Whenever possible, samples were also collected at 4 hours post-dose and at 4 weeks after initiation of therapy. The phospho-flow assay has been described and validated elsewhere.(21, 22) Briefly, primary antibodies (p-ERK #9101 from Cell Signaling Technology and p-mTOR #44-1125G from Invitrogen) were added at optimized concentration in FACS buffer and incubated at room temperature for 1 hour. Samples were washed once with FACS buffer. Secondary and directly conjugated surface marker antibodies were added at optimized concentrations and incubated in the dark at room temperature for 30 minutes. Samples were washed once with PBS and analyzed on a Becton Dickinson LSR II. Data were collected using DIVA software (Becton Dickinson) and analyzed using FLOWJO (Tree Star) and Cytobank. Peripheral blood mononuclear cells obtained from healthy donors were used as normal controls. Activation of the pathway was defined as mean fluorescent intensity (MFI) ratio of patient sample vs. control >1.

### Statistical Design and Analysis

The primary analysis was to be based on cohort A, consisting of patients without a *FLT3* mutation. In this cohort, a Simon two-stage design was employed. Enrollment to this cohort was to be stopped for lack of efficacy if no responses (CR, CRi, PR or MR) were observed in the first 12 evaluable patients. Otherwise, an additional 23 patients would be enrolled for a total of 35 patients and, if 6 or more responses were observed, the treatment would be considered sufficiently active to warrant further study. This sample size was calculated to yield a 0.90 probability of a positive result if the true response rate is greater than or equal to 30%. This yields a 0.90 probability of a negative result if the true response rate is 10% (null hypothesis) with 0.65 probability of early negative stopping.

A second smaller cohort (cohort B, maximum number =10) with *FLT3* ITD mutations was also enrolled and their responses analyzed separately. Wilcoxon nonparametric rank test was utilized to assess serial changes in p-ERK and p-mTOR levels. In the course of the analysis for KIT mutations, a previously described single nucleotide polymorphism (SNP) rs3733542, with a known population frequency of 20%, was identified in exon 18 of the *KIT* gene in several patients. Chi-square test was utilized to assess the difference in the incidence of this SNP between the responders and stable disease patients versus the non-responders.

## Results

### Patient Characteristics

Forty-seven patients were enrolled between January 2008 and June 2009, including 36 patients in cohort A and 10 patients in cohort B. One patient had unknown *FLT3* ITD status. Accrual exceeded 35 patients in cohort A due to the 36th patient on cohort A having signed consent prior to notification of study closure. Pretreatment patient characteristics are summarized in Table 1. Median age was 69 years (range, 26-83). Fifty-one percent of patients had AML arising from MDS or myeloproliferative neoplasm (MPN), or had therapy-related AML. Fifty-two percent of patients had poor risk cytogenetics.(23)

### Treatment Toxicity

Selumetinib was relatively well tolerated. The most common drug related toxicities included diarrhea (45%), fatigue (43%), nausea (36%), vomiting (24%) and skin rash (21%) (Figure 1). Most toxicities were mild (grade 1-2). Grade 3 toxicities included nausea (6%), diarrhea (4%), anorexia (4%), fatigue (2%), vomiting (2%), rash (2%), and dry mouth (2%). One patient experienced grade 4 toxicity (fatigue).

### Drug Delivery

A total of 107 treatment cycles were delivered. The median number of cycles administered was 1 (range, 1-21). The most common reason for treatment discontinuation was disease progression or lack of benefit. Two patients discontinued therapy due to adverse events- in 1 of these patients, this was due to grade 3 gastrointestinal toxicity (nausea, vomiting, diarrhea) occurring in the second cycle of therapy, and the other patient discontinued therapy after 1 cycle due to an infection occurring in the setting of neutropenia (patient was also neutropenic at baseline).

**Treatment Outcome**

In cohort A (*FLT3* wild type), 6 out of 36 patients (17%) had a response (1 PR, 3 MR, 2 uMR) that lasted a median of 58 days (range, 33-273 days) (Table 2). Among the first 35 patients enrolled in cohort A, there were 5 responders; therefore the trial did not reach the pre-specified criteria for a positive result. Four additional patients in this cohort had stable disease, with no evidence of disease progression for >8 weeks (median 16 weeks, range 9-85 weeks). The patient with a partial response was a 53-year-old woman with AML who had received multiple prior therapies for AML including matched-unrelated donor stem cell transplantation. After treatment initiation, her bone marrow blast percentage decreased from 44% at baseline to 10% at 1 month and 9% at 4 months. Absolute neutrophil count (ANC) increased from 700/μL at baseline to >1000/μL on day 7 and hemoglobin improved from 8.3 g/dL at baseline to >10 g/dL on day 49 (without transfusions). Overall, the response lasted 6.7 months. The patient received 8 cycles of treatment and was taken off protocol due to disease progression. No patient with *FLT3* ITD (n=10) responded. One patient had unknown *FLT3* status and did not respond.

***FLT3*, *NRAS*, *KRAS* and *KIT* analysis**

To determine whether mutations in RAS or upstream receptor tyrosine kinases are associated with clinical activity of selumetinib, baseline bone marrow and/or peripheral blood samples were analyzed for mutations in *FLT3*, *NRAS*, *KRAS* and *KIT*. The results for *FLT3* mutations are described above.

Of the 41 patients with samples available for analysis, *NRAS* mutation was detected in 3 (7%) patients and *KRAS* mutation in 1 (2%). One patient with a *NRAS* mutation had a concomitant *FLT3* ITD detected. None of the 3 patients with *NRAS* mutation had a response. The sole patient with a *KRAS* mutation had an unconfirmed minor response with hematologic improvement in platelets. The patient was a 75-year-old man with AML (*FLT3* wild type and monosomy 5 chromosomal abnormality) arising from prior MDS. His baseline bone marrow blast percentage was 25% and the percentage of blasts remained relatively unchanged after cycles 1 and 2, but had declined to 7% by cycle 4. No follow-up bone marrow was done thereafter. The platelet count increased from 75,000/μL at baseline to 156,000/μL at 1-month to 208,000/μL at 4 months. He received a total of 6 cycles of treatment and was taken off protocol due to a protocol violation (use of amiodarone, which was prohibited in this study).

*KIT* mutation analysis revealed the presence of a SNP rs3733542 (known population frequency of 20%), in exon 18 of the KIT gene in 6 of the 10 (60%) patients assessed as having a response or disease stabilization to selumetinib. In contrast this SNP was detected in only 7 of 31 (23%) patients without a response or disease stabilization (p=0.027).

**Phospho-flow analysis**

Baseline samples were available for p-ERK analysis in 20 patients (cohort A, n= 16; cohort B, n= 4). The raw phospho-flow data is provided as supplementary files. Analysis of p-ERK showed baseline activation in 17/20 (85%) patients, but no statistically significant difference in baseline levels in the responders (n=6) vs. non-responders (n=14) (p=0.15) (Figure 2). In

addition, there was no significant difference in the baseline p-mTOR levels between responders and non-responders (p=0.30) (Figure 2).

Only four patients had serial samples (baseline, at 4 hours and at 4 weeks) available for analysis. All four had baseline p-ERK activation, and in 2 of these patients (#3 and #5), there was evidence of inhibition of p-ERK at the 4 week time-point (supplementary figure 1). Only 1 of these patients (#5) had any evidence of a response.

## Discussion

This phase II study of single agent selumetinib in advanced AML demonstrates that administration of this MEK inhibitor in this patient population is feasible and is associated with modest antileukemic activity. Although dysregulation of the RAS/RAF/MEK/ERK pathway has been shown to be important in leukemogenesis, and pharmacologic inhibition of MEK has also been shown to inhibit the growth of leukemic cell lines and primary AML samples in vitro,(10, 24, 25) this is the first study to attempt to document, *in vivo*, the potential clinical relevance of targeting this pathway in relapsed/refractory AML.

In AML, this pathway is activated both by mutations occurring in *RAS*, as well mutations and/or over expression of upstream receptor tyrosine kinases such as *FLT3*.(8, 10) Given the fact that *FLT3* mutations occur in approximately one-third of patients with AML, we implemented a study design that included a cohort of ten patients with *FLT3* mutations, in an attempt to enrich for this subset. Contrary to our underlying hypothesis however, there were no responders in this cohort. These results differ from *in vitro* data, where inhibition of MEK induced apoptosis in cells expressing mutant FLT3-ITD.(26-28)

Although the study did not meet its primary endpoint, which required demonstrating 6 responses (CR, CRi, PR or MR) in the first 35 patients enrolled, we did observe a modest antileukemic effect in 10 of 36 patients in the *FLT3* wild-type cohort (Table 2). It has been postulated that higher baseline level of p-ERK predicts sensitivity to MEK inhibition in AML cell lines and primary AML samples.(25) Therefore, we analyzed p-ERK at baseline, and confirmed p-ERK activation in the majority of patients (85%) evaluated at baseline, but there was no correlation with treatment outcome. Larger studies are needed to confirm this finding, although our experience is similar to that of Bekaii-Saab and colleagues, who also reported no correlation between p-ERK activation and clinical outcomes in patients with metastatic biliary cancer treated with selumetinib.(29) In the biliary cancer study, 28 patients with advanced stage disease were treated with single-agent selumetinib 100mg twice daily. Overall 12% of patients had an objective response with an additional 68% patients with stable disease. The activation of p-ERK, assessed by immunohistochemistry on biopsy specimens, did not correlate with progression-free survival (PFS) or overall survival (OS), though the investigators were able to show that total lack of p-ERK was associated with an inferior OS. In another study with single-agent selumetinib in advanced hepatocellular cancer patients (n=17), although the investigators were able to demonstrate inhibition of p-ERK on serial tumor biopsies by western blot analysis, they could not demonstrate a correlation with response.(30)

Since KIT is also upstream of the RAS/RAF/MEK/ERK signaling pathway, and *KIT* mutations have been described in AML, we evaluated for evidence of *KIT* mutations (n=41). None of these patients had activating mutations in KIT. A polymorphism in exon 18 of KIT (SNP rs3733542), was detected in 13 of 41 patients (32%). This synonymous SNP has a known frequency of approximately 20% in the general population. Interestingly however, this SNP was detected in 60% of patients with a response or disease stabilization. In contrast only 23% of patients without any evidence of an antileukemic effect harbored this SNP. This SNP in exon 18 is located within a crucial region of the miR-146a and miR-146b domain of the *KIT* gene (*KIT* is a known target of these miRs). In papillary thyroid cancer (PTC), the presence of this SNP has been associated with low levels of *KIT*.(31) Low levels of *KIT* expression as demonstrated in association with the presence of this SNP in the PTC experience, would not in general be predicted to lead to significant activation of the RAS/RAF/MEK/ERK signaling pathway. It is possible however that low levels of KIT, may be associated with low levels of the putative target MEK, which may in turn confer enhanced susceptibility to inhibition of the pathway, but this is purely speculative at this point. The biological significance of this finding remains unclear at this time, and requires validation, since it is plausible that this finding could be an artifact of the relatively small sample size.

Selumetinib has also been evaluated as single-agent in many solid tumors including biliary cancers,(29) melanoma,(32) hepatocellular cancer,(30) non-small cell lung cancer,(33) papillary thyroid cancer,(34) and colorectal cancer(35) with modest objective response rates. Mutations in components of the RAS/RAF/MEK/ERK signaling pathway such as BRAF or KRAS have been shown to increase sensitivity to MEK inhibition. This has been demonstrated in many solid tumor cell lines.(36, 37) Early clinical data also support this observation with presence of the *BRAF* mutation in melanoma patients and *KRAS* mutation in non-small cell lung cancer (NSCLC) patients.(32, 38-40) Improved PFS has also been observed in NSCLC patients with *KRAS* mutation who received treatment with selumetinib plus docetaxel compared to docetaxel alone.(40) Addition of selumetinib to dacarbazine has also been shown to improve PFS in patients with previously untreated BRAF-mutant melanoma.(41) In our study, 41 patients, including all the responding patients were evaluated for *NRAS* and *KRAS* mutations, and 4 (approximately 10%) had mutated *NRAS* (n=3) or *KRAS* (n=1). This is in keeping with the known mutational frequency of the *RAS* oncogene in AML.(8) Interestingly, the sole patient with *KRAS* mutation in our study had a minor response. Although this finding raises the possibility that the presence of *RAS* mutations may confer sensitivity to this class of agents, the numbers of *RAS* mutant patients in this study are too small to draw any definitive conclusions. Preliminary results in a *RAS* mutated cohort enrolled on a separate, ongoing clinical trial with another MEK inhibitor in relapsed/refractory AML patients, however, lends some credence to this hypothesis.(42)

In summary, administration of selumetinib is feasible in AML, but is associated with limited antileukemic activity as a single agent in this patient population with several poor risk characteristics. One of the weaknesses of the present study is that we had very limited number of paired pre- and post-selumetinib samples (supplementary figure 1) and given the small sample size, the analysis was not informative. Thus, we are unable to report on the

extent of pathway inhibition. A new formulation of selumetinib with hydrogen sulfate salt that gives twice the exposure of the freebase drug tested in the present study has recently been developed and it is plausible that the newer formulation may be associated with higher efficacy.(43)

Given modest single agent activity of selumetinib in general, combinations evaluating selumetinib plus existing chemotherapy agents or other novel agents are already underway in a number of tumor types and the early results have been encouraging.(40, 41) Recent evidence suggests that resistance to MEK inhibition may be related to over compensatory upstream hyperactivation of RAS/RAF or activation of the parallel PI3K/PTEN/AKT/ mTOR pathway.(25, 44-47) Mutations in genes not directly involved in cytokine signaling as well as copy number variation of genes in signaling pathways may also mediate resistance to MEK inhibition.(48) Our group and others have shown *in vitro*, synergistic induction of apoptosis when MEK inhibitors are combined with inhibitors of PI3K/Akt pathway.(49) Based on emerging experience with MEK inhibitors in *RAS* mutated AML, it is reasonable to hypothesize that this subset of patients may be most sensitive to such combination approaches. Given the favorable toxicity profile of selumetinib based on our experience and that of others,(29, 30) combination therapy with inhibitors of PI3K/AKT pathway should be considered, especially for *RAS* mutated AML.(50)

## Acknowledgments

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## Statement of Translational Relevance

The RAS/RAF/MEK/ERK pathway is activated in majority of acute myeloid leukemia (AML) patients. Selumetinib is an oral small molecule inhibitor of MEK1/2 kinase, and this study was based on the hypothesis that MEK kinase inhibition in AML would result in anti-proliferative effects and inhibition of the leukemia clone. This is the first study to document, *in vivo*, the potential clinical relevance of targeting this pathway in relapsed/refractory AML. We demonstrate that administration of selumetinib is safe and is associated with modest anti-leukemic activity. Baseline p-ERK activation seen in 85% of patients analyzed did not correlate with response. A potential association of SNP rs3733542 in exon 18 of the *KIT* gene with antileukemic activity of selumetinib was observed, and deserves validation in larger trials. Given the cross-talk between dysregulated signal transduction pathways in AML, combination studies with MEK inhibitors and other agents that target relevant signaling pathways in AML are warranted.
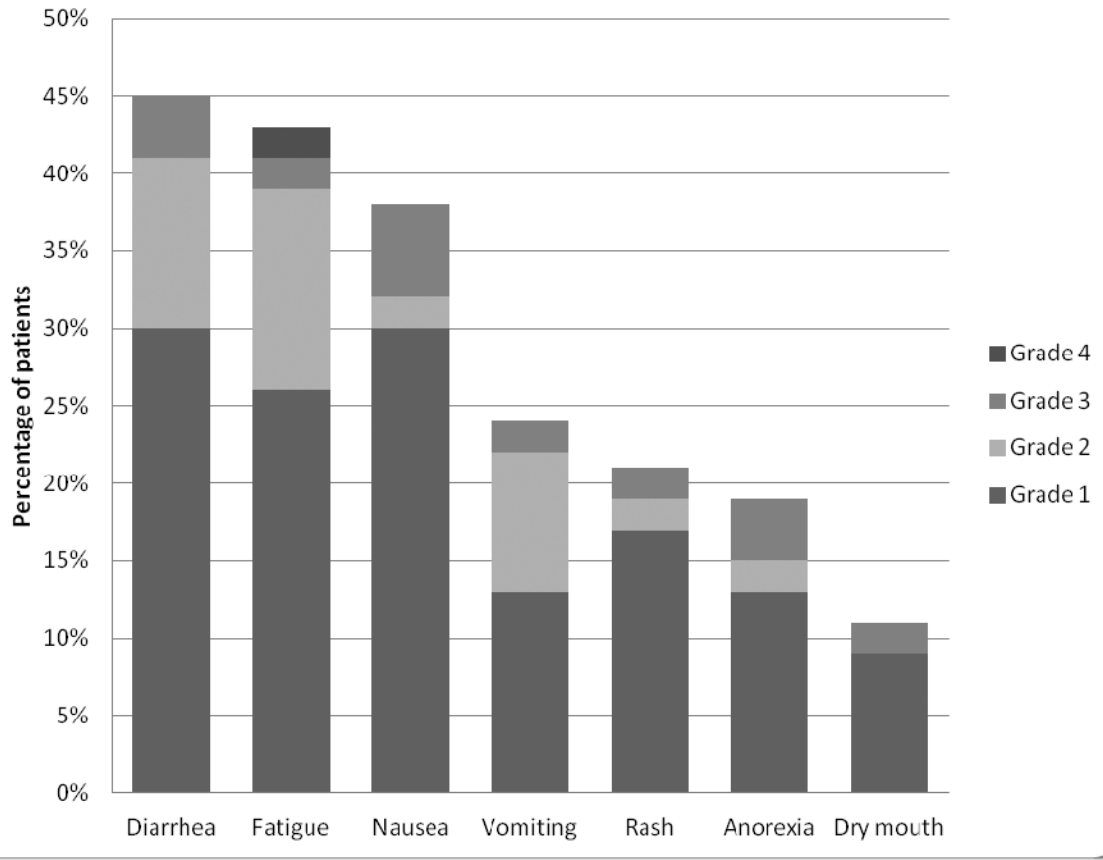
**Figure 1. Common (>10%) non-hematological treatment toxicities**
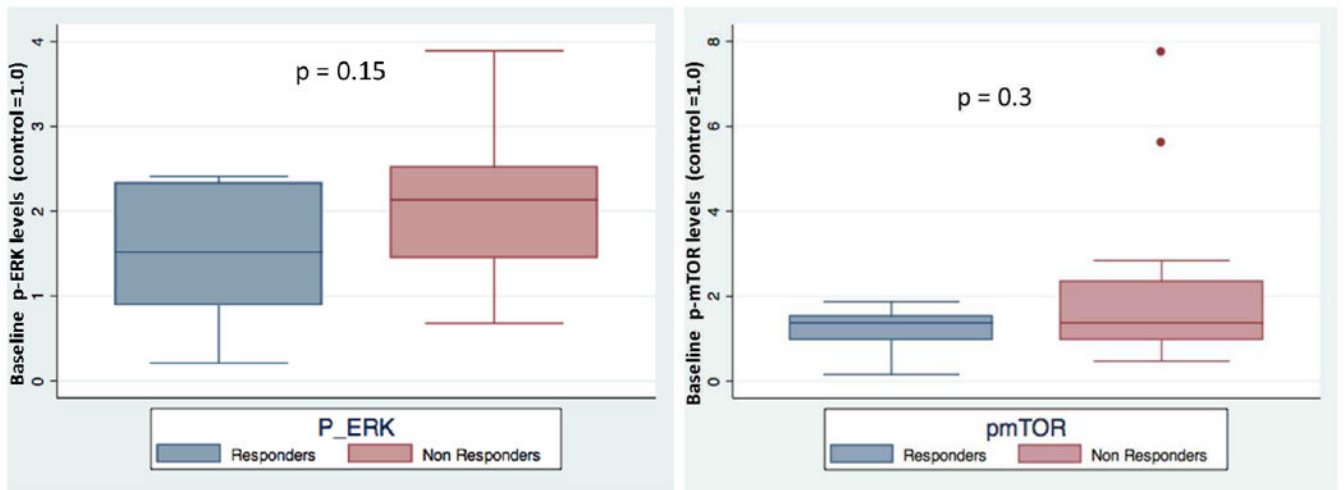
**Figure 2.**
Box-plot of baseline p-ERK and p-mTOR in responding (n=6) and non-responding (n=14) patients. Values expressed as ratio of mean fluorescent intensity of patient sample to normal controls. There was no significant difference in baseline levels between responders and non-responders.

**Table 1**
**Patient Demographics and Baseline Characteristics**

| Characteristic | No. patients (%) |
|---|---|
| Age (y) | |
| Median (range) | 69 (26-83) |
| 60 | 40 (85) |
| Sex | |
| Male | 27 (57) |
| Female | 20 (43) |
| Performance Status | |
| 0 | 12 (26) |
| 1 | 27 (57) |
| 2 | 8 (17) |
| AML Diagnosis | |
| De novo | 23 (49) |
| Antecedent hematologic disorder | 21 (45) |
| Prior MDS | 17 |
| Prior CMML | 1 |
| Prior PV | 2 |
| Prior ET | 1 |
| Therapy-related AML | 3 (6) |
| Bone marrow cytogenetics (n=46) | |
| Good[#] | 2 (4) |
| Intermediate | 20 (44) |
| Normal | 14 (30) |
| Poor | 24 (52) |
| FLT3 ITD status | |
| Wild type | 36 (77) |
| Mutant | 10 (21) |
| Unknown | 1 (2) |
| Stage of disease | |

| Characteristic | No. patients (%) |
|---|---|
| Primary refractory | 14 (30) |
| First relapse | 8 (17) |
| Beyond first relapse | 12 (25) |
| Previously untreated and >60 years* | 13 (28) |
| Prior therapy | |
| Cytarabine | 24 |
| High-dose cytarabine | 17 |
| Allogeneic SCT | 6 |
| Autologous SCT | 1 |

#Both patients had t(8;21). One patient was an 83-year-old gentleman with primary refractory AML that had evolved from MDS. The other patient was a 69-year-old gentleman with relapsed/refractory AML, who had received more than 3 prior chemotherapy regimens.

*Of the 13 previously untreated patients in the study (all 60 years old), 11 had an antecedent hematologic disorder (10 MDS, 1 CMML). Only 2 patients (ages 79 and 80 years, one with complex karyotype) had untreated de novo AML and neither of these responded.

AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; CMML: Chronic myelomonocytic leukemia; PV: polycythemia vera; ET: essential thrombocythemia; SCT: stem cell transplant

**Table 2**

**Characteristics of the treatment responders and patients with stable disease**

| Patient ID | Disease stage | Age (years) | Prior therapies | Cytogenetic risk group | FLT3 ITD | NRAS | KRAS | No. of cycles | Response |
|---|---|---|---|---|---|---|---|---|---|
| 47[#] | Beyond first relapse | 53 | SCT, 7+3, HiDAC | Not available | wt | wt | wt | 8 | PR |
| 4 | Beyond first relapse | 68 | 7+3, tipifarnib, decitabine + vorinostat | Other | wt | wt | wt | 3 | MR (BM) |
| 5[#] | Beyond first relapse | 70 | Cytarabine, daunorubicin and oblimersen sodium, HiDAC, azacitidine + belinostat | Poor | wt | wt | wt | 3 | MR (PB) |
| 6 | Untreated AML (prior MDS) | 66 | Prior MDS treatment: azacitidine | Poor | wt | wt | wt | 2 | MR (PB) |
| 10[#] | Untreated AML (prior MDS) | 75 | Prior MDS treatment: Thalidomide + Dexamethasone, azacitidine, lenalidomide | Poor | wt | wt | MUT | 6 | uMR + HI-P |
| 8[#] | Beyond first relapse | 66 | 7+3, HiDAC, Gemtuzumab ozogamicin, zosuquidar | Other | wt | wt | wt | 2 | uMR |
| 27[#] | Untreated AML (prior MDS) | 70 | Prior MDS treatment: azacitidine, decitabine + valproic acid | Intermediate | wt | wt | wt | 2 | SD |
| 46[#] | First Relapse | 64 | Decitabine + vorinostat | Intermediate | wt | wt | wt | 4 | SD |
| 37 | Primary refractory | 62 | Decitabine + valproic acid | Poor | wt | wt | wt | 4 | SD |
| 28 | Primary refractory | 55 | 7+3, HiDAC, MEC, troxacitabine | Other | wt | wt | wt | 21 | SD |

[#]Polymorphism for SNP rs3733542 in exon 18 of the KIT gene

SCT: stem cell transplant; 7+3: cytarabine/daunorubicin; HiDAC: high-dose cytarabine; wt: wild-type; PR: partial response; MR (BM): minor response (peripheral blood); AML: acute myeloid leukemia; MDS: myelodysplastic syndrome; MUT: mutated; uMR: unconfirmed minor response; HI-P: hematologic improvement-platelet; SD: stable disease; MEC: mitoxantrone/etoposide/cytarabine