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Clinical and Proteomic Characterization of Acute Myeloid Leukemia with Mutated *RAS*

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Abstract

Background—Activating mutations in *RAS* are frequently present in patients with AML, but their overall prognostic impact is not clear.

Methods—A retrospective analysis was done to establish the clinical characteristics of patients with *RAS*^{mut} AML, to analyze their outcome by therapy, and to describe the proteomic profile of *RAS*^{mut} relative to *RAS*^{WT} AML.

Results—Out of 609 patients with newly diagnosed AML, 11% had mutated *RAS*. Compared with *RAS*^{WT}, patients with *RAS*^{mut} AML were younger (median 54 vs. 63 yrs, $p=0.001$), had a higher WBC (16 vs. 4.2, $p<0.001$) and marrow blast percentage (56% vs. 42%, $p=0.01$) at diagnosis, and less likely to have an antecedent hematologic disorder (36% vs. 50%, $p=0.03$). The inv(16) karyotype was overrepresented in patients with *RAS*^{mut} and -5 and/or -7 karyotype was underrepresented. *RAS* mutation had no prognostic impact on OS or DFS overall or within cytogenetic subgroups. There was a suggestion that patients with *RAS*^{mut} benefited from AraC-based therapy. Proteomic analysis revealed simultaneous upregulation of the *RAS*-Raf-MAP kinase and PI3K signaling pathways in patients with *RAS*^{mut}.

Conclusions—*RAS* mutations in AML may delineate a subset of patients who benefit from AraC-based therapy and who may be amenable to treatment with inhibitors of *RAS* and PI3K signaling pathways.

Keywords

AML; *RAS*; signal transduction

Introduction

Activating mutations in the *RAS* protooncogene are among the most common genetic alterations in human cancer.¹⁻³ They are present overall in about 40% of all malignancies, seen in up to 90% of pancreatic adenocarcinoma, 30% of non-small cell lung cancer, and

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about a third of colorectal cancers.^{1, 2} Several series have reported *RAS* mutations in 10 to 25% of cases of acute myeloid leukemia (AML)⁴⁻¹⁴ and 7 to 48% of cases of myelodysplastic syndrome (MDS).¹⁵⁻¹⁷ N-*RAS*, K-*RAS*, and H-*RAS* are the most common isoforms of *RAS* involved in human cancer. The activating point mutations most frequently occur in codons 12, 13, or 61, although less common point mutations have also been described.^{2, 18} *RAS* is a GTP-dependent second messenger protein that couples signals from receptor tyrosine kinases, cytokine receptors, and growth factor receptors to intracellular signalling networks to induce proliferation and survival (Figure 1).^{2, 3, 19, 20} Under normal conditions an upstream activation signal promotes the binding of GTP to *RAS*, facilitated by GTP exchange factors (GEF). This activation leads to the phosphorylation of downstream Raf, Mek, and the MAP-kinase Erk. Erk can then translocate to the nucleus and modulate transcription.^{2, 20} This process is self-regulated by the GTPase activity associated with *RAS*, which hydrolyzes the GTP and turns the signal off. Oncogenic mutations of *RAS* which disrupt this GTPase activity lead to aberrant constitutive signaling that is independent of the upstream stimuli.^{2, 3, 19, 20} There is also important crosstalk between the *RAS*-Mek-MAP-kinase pathway and other growth and survival pathways such as the PI3K-Akt pathway (Figure 1). In malignancies where the *RAS* pathway is constitutively active due to mutations, it is important to recognize that additional associated pathways such as the PI3K-Akt axis may provide redundant growth and survival signals. An important mechanism of resistance to therapeutic targeting of *RAS* is crosstalk and signaling through alternate pathways.²⁰ Determining the activation status of proteins downstream from *RAS* and PI3K in samples of AML patients with mutated versus wild-type *RAS*, could potentially yield additional therapeutic targets and mechanisms of resistance.

The impact of *RAS* mutations on outcome and response to therapy in various cancers is under extensive investigation.^{11, 21, 22} Recent work has shown that *RAS* mutations in AML may predict for benefit from postremission therapy with high dose AraC.¹¹ However, the overall prognostic impact of *RAS* mutations in AML is not clear. While some investigators reported that *RAS* mutations were associated with an adverse outcome^{9, 14, 17}, others have suggested a favorable impact^{7, 10}, and several have noted no significant prognostic impact^{4, 6, 12, 13}. Herein we sought to further characterize the role of *RAS* mutations in AML. We analyzed the baseline characteristics of patients with newly diagnosed AML with mutated or wildtype *RAS* and evaluated their response to different therapies and their overall outcome. We also examined their pretreatment proteomic profiles to determine any differences in the 2 cohorts.

Patients, Materials, and Methods

Study Group

The charts of patients with newly diagnosed AML seen at MD Anderson Cancer Center (MDACC) between 2003 and 2007 were analyzed. This included 609 patients in whom *RAS* mutational analysis was performed at diagnosis. A total of 228 patients, which were representative of the entire group, had banked material for proteomic analysis. All patients underwent a bone marrow examination at diagnosis. Informed consent was appropriately obtained from all patients, according to the institutional guidelines and in compliance with the Declaration of Helsinki (Lab 01-473, Lab 05-0654). AML was diagnosed according to the WHO criteria²³, by the presence of at least 20% myeloid blasts in the blood and/or bone marrow.

Laboratory Data

Bone marrow cytogenetics were performed by conventional chromosomal banding and/or fluorescent in situ hybridization (FISH) when needed. The cytogenetics were classified into

3 prognostic subgroups: favorable (t(8;21), inv(16), t(16;16), or t(15;17)); adverse (-5, -7, 11q23 abnormality, or complex karyotype [≥ 3 cytogenetic abnormalities]); or intermediate (neither adverse nor favorable).

RAS mutation analysis was performed on unsorted cells from diagnostic bone marrow aspirate samples (with a median blast percentage of 44) for exon 1 (codons 12 and 13) and exon 2 (codon 61) for both *NRAS* and *KRAS* using PCR-based DNA sequencing methods. *FLT3* mutation analysis was performed on the same samples using PCR-based DNA sequencing methods.

Reverse Phase Protein Array

Proteomic analysis of matched, banked pretreatment patient blood or bone marrow samples was performed using an automated, high-throughput reverse phase protein array (RPPA) as previously described.^{24, 25} Briefly, protein lysates from patient samples were printed onto methylcellulose-coated slides in 5 serial dilutions onto slides along with normalization and expression controls. Each slide was probed with a distinct validated anti-human antibody for the protein or phosphoprotein in question and a secondary antibody to amplify the signal. Finally, a stable dye is precipitated.²⁶ The stained slides were analyzed using Microvigene® software (Vigene Tech, Carlisle, MA) to produce quantified data. A total of 176 validated antibodies were used. Supercurve algorithms were used to generate a single value from the 5 serial dilutions.²⁷ Loading control²⁸ and topographical normalization procedures accounted for protein concentration and background staining variations. Association between protein expression levels and *RAS* mutation status were assessed in R using standard t-tests.

Treatment

Patients received several different treatment regimens depending on the available clinical trials, age, and performance status. For the purposes of this analysis, induction therapy could be divided into three groups: (1) High dose AraC based (≥ 1000 mg/m²/day, HiAraC), (2) Conventional dose AraC based (100 mg/m²/day to 500 mg/m²/day, ConvAraC), and (3) non-AraC based (nonAraC). Treatments within the nonAraC cohort included hypomethylating agents (azacytidine, decitabine), other nucleoside analogues such as clofarabine, and small molecule targeted therapies among others (Table 1).

Criteria for response and definitions

Complete remission (CR) is defined by the presence of $< 5\%$ blasts in the bone marrow, absence of extramedullary leukemia, and peripheral blood count recovery with a neutrophil count of at least 1×10^9 / L and platelet count of at least 100×10^9 / L.²⁹ Treatment failure was defined by the absence of a documented CR after therapy, including induction death or refractory disease. Relapse was defined by an excess of 10% leukemic blasts in a marrow aspirate that is not related to normal hematopoietic recovery or the development of new extramedullary leukemia. Disease free survival was calculated from the time of first documented CR to relapse or death in CR. CR duration was calculated from the time of first documented CR to relapse. Overall survival was calculated from the time of diagnosis of AML to death from any cause.

Statistical methods

Patient and disease characteristics were compared using Chi squared tests. These characteristics are listed in Table 2 and include age, sex, WBC count, platelet count, beta-2 microglobulin level, blast percentage, FAB morphology, cytogenetic category, *FLT3*-ITD positivity, and history of antecedent hematologic disorder. Estimates of survival curves and CR duration were calculated according to the Kaplan-Meier product limit method and

compared by means of the log-rank test. The Cox proportional hazards regression model was used to assess the relationship between patients characteristics and survival. Predictive variables with p values of less than 0.1 by univariate analysis were included in a multivariate analysis. All reported p values are 2-sided, and those <0.05 were considered significant. A multivariate logistic regression analysis was conducted assessing complete response to treatment as a dichotomous variable (response vs. no response). Independent predictors included in the model were: adverse cytogenetics, AHD, RAS mutation, performance status, use of Ara-C therapy, and diagnosis of therapy related AML. Two way interactions between the variables in the model were explored and reported and the model Goodness-of-fit was verified by using the Hosmer-Lemeshow Chi-square test. We used $p < .05$ as the level of statistical significance. The statistical analyses were accomplished by using IBM SPSS Statistics version 19.0 for Windows.

Results

Patient Characteristics

From January 2003 to December 2007, 609 patients with newly diagnosed AML were identified in whom *RAS* mutational analysis was performed. Of these, 66 patients (11%) were found to have *RAS* mutations: 48 (73%) with an *N-RAS* mutation, 16 (24%) with a *K-RAS* mutation and 2 patients with both. Patient characteristics of the *RAS*-mutated (*RAS*^{mut}) cohort and *RAS*-wild-type (*RAS*^{WT}) cohort are summarized in Table 2. The median age for patients with *RAS*^{mut} was 54 years vs. 63 years for those with *RAS*^{WT} ($p=0.001$). In addition, patients with *RAS*^{mut} had a significantly higher WBC count (median 16 vs. 4.2, $p<0.001$); peripheral blast percentage (median 22 vs. 10, $p=0.007$); bone marrow blast percentage (median 56 vs. 42, $p=0.01$); and beta-2-microglobulin (median 3.6 vs. 2.7, $p=0.002$) at diagnosis. Patients with *RAS*^{mut} were less likely than those in the wild-type cohort to have a history of an antecedent hematologic disorder (AHD, 36% vs. 50%, $p=0.03$). A higher percentage of patients in the wild-type cohort had FLT3-ITD, but this did not reach statistical significance. The prevalence of AML with *RAS*^{mut} among different FAB morphologic subtypes and different karyotypes is depicted in Figure 2a and 2b. There was a significantly higher prevalence of mutated *RAS* among patients with M4 (acute myelomonocytic leukemia; 24%, $p<0.001$) and M5 (acute monocytic leukemia; 23%, $p=0.004$) histologies. *RAS* mutations were significantly overrepresented in inv(16), found in 39% of patients with this karyotype ($p < 0.001$). Conversely, *RAS* mutations were significantly underrepresented in -5/-7 and t(15;17) karyotypes ($p=0.03$).

Outcome

This cohort of patients with newly diagnosed AML was treated with a variety of different therapies according to their age, performance status, karyotype, and availability of specific clinical trials (Table 1). Since these are among the covariates important in predicting outcome in patients with AML, we first performed a multivariate analysis of predictive factors for achievement of CR (Table 3). Independent predictors for achieving CR in the entire cohort were age, cytogenetics, history of AHD, and treatment with AraC-based therapy. Notably, the presence of a Ras mutation and performance status were not significant. We then analyzed the overall survival and remission duration for the entire cohort of patients by *RAS* mutation status, regardless of treatment or cytogenetics. There was no significant difference in overall survival (OS) or CR duration (CRd) between AML patients with *RAS*^{mut} or *RAS*^{WT} (Figure 3). Among patients with inv(16) karyotype, there was no significant difference in overall survival or CRd between patients with mutated or wild-type *RAS* (Figure 4a). Among patients with intermediate risk karyotype, there was no significant difference in OS or CRd between the mutated and wild-type *RAS* patients (Figure 4b). In patients with adverse risk karyotype, there was a trend for worse OS and

CRd in patients with RAS^{mut} , but this did not reach statistical significance (Figure 4c). Among patients with RAS^{mut} , there was no significant difference in OS between those who had an $N-RAS$ vs. $K-RAS$ mutation (median 12.9 vs. 14.5 months, $p=0.78$; Supplemental Figure 1). Similarly, there was no significant difference in CR rate between those with N - or $K-RAS$ (56% vs. 63%, $p=0.66$)

The response rates by treatment in the 66 patients with RAS^{mut} are outlined in Table 4 and compared with patients with RAS^{WT} . There was a significantly higher rate of CR in patients who received any AraC versus those who received no AraC (70%, 70%, and 0% for Hi-AraC, Conv-AraC, and No-AraC, respectively; $p<0.001$). Since clinical factors such as age and history of AHD may influence the decision to treat with AraC or non AraC-based therapy, we next looked at the outcome of patients with RAS^{mut} by age (<60 vs. 60), diagnosis of secondary AML (vs. primary or *de novo* AML), and whether or not they had received AraC-based therapy. (Figure 5) All of the 39 patients less than 60 years of age in this cohort received AraC-based therapy and had the best outcome (median OS: 23.5 months). Among the older patients with RAS^{mut} , those who received AraC had a significantly improved OS compared to those who did not (median 12 vs. 2.5 months). When the patients were analyzed by their diagnosis of primary vs. secondary AML, those patients who had primary AML and received AraC-based therapy had the longest OS (median: 26.5 months), compared to those who had either secondary AML (treated with or without AraC) or those who had primary AML and were treated with non AraC-based therapy. Indeed, by multivariate analysis, the factors that independently predicted for overall survival in patients with RAS^{mut} were adverse cytogenetics ($p=0.016$) and a history of AHD ($p=0.001$). (Table 5).

Proteomic analysis

Of the 609 cases analyzed, 228 patients (37%) had banked material available for proteomic analysis. The baseline characteristics of these patients were representative of the entire cohort (Table 6). High throughput analysis of these 228 samples was done using a panel of 176 validated antibodies. Of the 176 proteins tested, 39 (22%) were differentially expressed between baseline AML samples with wild-type and mutated RAS (Table 7). With a false discovery rate of 5%, the expectation would be to find 8.8 proteins being significantly different, so this frequency was significantly higher than expected. Twenty proteins were expressed at relatively higher levels in samples with RAS^{mut} and 19 were relatively higher in samples with RAS^{WT} (Table 7). Among the proteins that were expressed at relatively higher levels in RAS^{mut} were a substantial number of downstream targets and effectors of both the RAS -Raf-MAP kinase pathway, as well as the PI3 kinase-Akt pathway such as total (p=0.003) and phospho-MEK (p=0.04), total (p=0.004) and phospho-p38 (p=0.1), phospho-Akt (p=0.01), phospho-GSK (p=0.002), phospho-ERK (p=0.1), and total (p=0.05) and phospho-PDK1 (p=0.009) (Table 7).

Discussion

In order to further characterize the significance of RAS^{mut} in patients with newly diagnosed AML, we performed a retrospective, single-institution, clinical and proteomic analysis on a large cohort of patients who were heterogeneously treated. We aimed to describe the clinical characteristics of these patients, to analyze the effect of different treatments on outcome, to assess the prognostic impact of RAS^{mut} in AML, and to examine the differential protein expression in leukemic cells of patients with mutated and wild-type RAS . We found several pretreatment clinical characteristics that were significantly different between patients with mutated and wild-type RAS . Patients with RAS^{mut} in our cohort tended to be younger than those with RAS^{WT} and less likely to have AHD. Similar to other reported series^{9, 13}, patients with RAS^{mut} had a higher median WBC at presentation. In contrast to other reports^{10, 11, 13},

however, our study showed that patients with RAS^{mut} had higher peripheral and bone marrow blast percentage at presentation.

Confirming observations from other large studies of AML patients, RAS mutations were significantly overrepresented in patients with the inv(16) karyotype and underrepresented in patients with t(15;17) and -5/-7 karyotypes.^{4, 6} This strong association with inv(16) suggests a cooperative interaction between the 2 genetic abnormalities. Activation of the RAS pathway in patients with inv(16) may be an important mechanism of pathogenesis and should also be further investigated in patients with inv(16) without RAS mutations for other routes of pathway activation. If this signaling pathway is, indeed, mechanistically important in inv(16) AML, targeting this pathway could offer therapeutic opportunities. Conversely, one explanation for the relative underrepresentation of RAS mutations in patients with t(15;17) could be the presence of alternate genetic abnormalities that can activate constitutive signaling through the RAS -MAPK and related pathways. For example, FLT3-ITD mutations, which are present in about 40% of cases³⁰⁻³² with t(15;17) are upstream of and exploit the RAS -MAPK and PI3K-Akt signaling pathways. Indeed, we and others^{6, 9, 13} have shown that RAS mutations and FLT-ITD rarely coexist in the same AML patient. This suggests deregulation of common final signaling pathways resulting from different aberrantly activated upstream molecules.¹⁹ If this is confirmed, specifically inhibiting a limited number of signaling pathways could be broadly applicable in AML.

In our study, RAS^{mut} had no clear prognostic significance in AML overall or within the different cytogenetic subgroups in terms of overall survival or duration of remission. This is in-line with most other large series.^{4, 6, 12} Since our cohort had been treated with a variety of different therapies, we were able to assess the effect of varying doses of AraC and no-AraC on outcome in patients with mutated RAS . Recently, investigators from the CALGB reported that patients with AML with mutated RAS derived greater benefit from post remission therapy with high dose AraC compared those with wild-type RAS and those who did not receive HiDAC consolidation.¹¹ This clinical data confirmed previous *in vitro* work by Koo et. al.^{33, 34} which demonstrated that RAS^{mut} AML cell lines were more sensitive to the cytotoxic effects of AraC than those with RAS^{WT} . The investigators showed that cells harboring the RAS mutation had a compromised S-phase checkpoint in response to chemotherapy-induced DNA damage.³³ Unlike cells with RAS^{WT} , which arrested in S-phase in response to AraC, cells with RAS^{mut} progressed through S-phase, incorporating AraC and continuing on to apoptosis.³³ This led to a cytotoxic response versus a cytostatic response to AraC. Clinical results from our study support both the preclinical and previous clinical observations highlighting the sensitivity of RAS -mutated AML to AraC.^{8, 11, 33, 34}

All patients in our study who received any AraC as part of their induction received HiDAC as their consolidation. Patients with RAS^{mut} who were not treated with AraC had a lower CR rate and shorter OS than those who received AraC as part of their induction. This OS difference was also observed in the subgroup of older patients with RAS^{mut} , and, although not statistically significant, in those with AHD and RAS^{mut} . Notably patients with RAS^{mut} and history of AHD had the poorest OS, regardless of their therapy. By multivariate analysis, the only factors that were associated with overall survival in patients with RAS^{mut} were history of AHD and cytogenetics. This may suggest a degree of selection bias in our study cohort, as older patients or those with predetermined adverse prognosis not able to tolerate intense chemotherapy were offered clinical trials not containing AraC, accounting for the diminished effect of treatment on the overall prognosis by multivariate analysis.

Exploring the differential protein expression signature of AML patients with RAS^{mut} may give some insight into the drivers of the disease and potentially highlight new therapeutic targets in this subset. Using a high-throughput proteomics array, we were able to compare

the pretreatment protein expression signature from samples of patients with AML and *RAS*^{mut}, relative to those with *RAS*^{WT}. We found a significant number of proteins that were differentially expressed between AML samples containing mutated and wild-type *RAS*. As hypothesized, several downstream targets and effectors of the *RAS*-Raf-MAP kinase pathway as well as the PI3-kinase-Akt pathway were expressed at relatively higher levels in samples with *RAS*^{mut}. Although this needs to be validated further, this suggests a greater activation of both signaling pathways in AML with mutated *RAS*, relative to wild-type *RAS*, with the implication that they may respond to small molecule inhibitors of kinases along these pathways. Ilmer et. al. conducted a similar investigation on the activation status of the *RAS* pathway in AML patients. Using western blotting to detect activated *RAS* (as defined by Raf-bound *RAS*), the investigators found an activated *RAS* pathway in about 25% of all AML cases. Interestingly, only 22% of the *RAS*-mutated cases were found to have increased *RAS* activity by their method. The study did not, however, simultaneously look at the activation of associated signaling pathways such as the PI3K-Akt pathway which may have been relatively upregulated in the AML samples with mutated *RAS*. To our knowledge, our analysis is the first to simultaneously assess the activation status of protein signaling networks in patients with AML and *RAS*^{mut}.

In conclusion, we found that AML with *RAS*^{mut} has distinct clinical features and association with certain cytogenetic and morphologic subgroups. Although we did not find a prognostic effect of *RAS* mutations overall in AML, we did find an interaction between AraC-based treatment or history of AHD and overall outcome. Comparative proteomic profiling of *RAS* mutated AML suggests an upregulation and activation of proteins involved in the *RAS*-Raf-MAPK and PI3K-Akt signaling networks that is relatively higher than in AML without mutated *RAS*. This may form the basis of future studies investigating the use of specific inhibitors and their combinations in this subset of patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Simplified diagram depicting Ras signaling and crosstalk with PI3 kinase- Akt pathway in leukemia.

Prevalence of Mutated Ras Among Different Morphologies

Prevalence of Mutated Ras Among Different Karyotypes

Figure 2.
Prevalence of Mutated Ras among (A) FAB subtypes, and (B) different karyotypes.

Figure 3. Overall Survival and Remission duration in patients with AML, comparing those with mutated Ras to those with wild-type Ras.

Figure 4.
Overall Survival and Remission duration in patients with AML with mutated and unmutated Ras, separated by cytogenetic category.

Figure 5.

(A) Overall Survival in patients with AML with mutated Ras, separated by age and treatment. (B) Overall Survival in patients with AML with mutated Ras, separated by primary vs. secondary AML and treatment. (AraC: cytarabine; Y: patients who received AraC; N: patients who did not receive AraC-based therapy; mo: months; Prim: primary AML; Sec: secondary AML)

Table 1
Treatments for AML Patients

Category	Treatment	No. of Patients (%)
High-dose AraC based	Ida + HiDAC	209 (34)
	Flud + HiDAC	60 (10)
	Flud + HiDAC + Ida	1 (0)
	Dauno + HiDAC	2 (0)
	Misc HiDAC	4 (1)
	Clofarabine + HiDAC	38 (6)
Conventional-dose AraC based	Misc ConvAraC	101 (17)
Non-AraC based	Clofarabine	34 (6)
	DAC-based	44 (7)
	5-AZA-based	32 (5)
	Cloretazine	41 (7)
	FLT3-inhibitor	12 (2)
	Arsenic+ATRA (APL)	11 (2)
	HDACi	7 (1)
	Misc No AraC, Other	13 (2)

AML: acute myeloid leukemia; No.: number; AraC: cytarabine; HiDAC: high dose cytarabine; Ida: idarubicin; Flud: fludarabine; dauno: daunorubicin; Misc: miscellaneous; ConvAraC: conventional dose cytarabine; DAC: decitabine; 5-AZA: 5-azacytidine; FLT3: fms-related tyrosine kinase 3; ATRA: all trans retinoic acid; APL: acute promyelocytic leukemia; HDACi: histone deacetylase inhibitor;

Table 2
Baseline Patient Characteristics

Characteristic	Mutated Ras, N=66 (11%)	Wild-Type Ras, N= 543 (89%)	p-value
Median age, years (range)	54 (25-88)	63 (17-88)	0.001
Male sex, N (%)	34 (52)	284 (52)	0.9
Median WBC count (range)	16 (0.8 - 111.7)	4.2 (0.3 - 433)	< 0.001
Median platelets (range)	43 (7 - 357)	50 (2 - 676)	0.14
Median PB blast % (range)	22 (0-95)	10 (0-97)	0.007
Median BM blast % (range)	56 (20 - 93)	42 (0 -98)	0.014
FAB Morphology, N (%)			
M0	0 (0)	23 (4)	<0.001
M1	4 (6)	55 (10)	
M2	15 (23)	113 (21)	
M3	0 (0)	11 (2)	
M4	25 (38)	80 (15)	
M5	11 (17)	36 (7)	
M6	0 (0)	29 (5)	
M7	0 (0)	1 (0)	
Cytogenetics, N (%)			
inv (16)	10 (15)	16 (3)	<0.001
t(8;21)	3 (5)	16 (3)	
t(15;17)	0 (0)	11 (2)	
-5 and/or -7	8 (12)	132 (24)	
11q abnormality	3 (5)	16 (3)	
other complex karyotype	6 (9)	50 (9)	
diploid, -Y	27 (41)	233 (43)	
other	9 (14)	67 (13)	
History of AHD, N (%)	24 (36)	274 (50)	0.03
FLT3 ITD Positive, N (%)	3 (5)	64 (12)	0.08
Median β 2 microglobulin	3.6 (1.2 - 12.9)	2.7 (1.1 - 20.8)	0.002

N: number of patients; WBC: white blood cell; PB: peripheral blood; BM: bone marrow; FAB: French-American British classification; complex karyotype: 3 chromosomal abnormalities; AHD: antecedent hematologic disorder

Table 3
Multivariate analysis for complete response in all patients with AML

Characteristic	p-value
Age (continuous variable)	<0.001
Cytogenetics (Adverse)	<0.001
History of AHD (yes/no)	<0.001
Treatment (AraC vs. No AraC)	<0.001
Prior Chemo or XRT (yes/no)	NS
Ras mutation (present/absent)	NS
ECOG PS (continuous)	NS

ECOG: Eastern Cooperative Oncology Group; PS: performance status; AHD: antecedent hematologic disorder; chemo: chemotherapy; XRT: radiation therapy; AraC: cytarabine

Table 4
Response Rates by Treatment in Patients with AML Ras mutation status

Treatment	Mutated Ras (N=66)		Wild-Type Ras (N=543)	
	N (%)	CR*(%)	N (%)	CR (%)
High-dose AraC	44 (67)	31 (70)	269 (50)	167 (62)
Standard-dose AraC	10 (15)	7 (70)	91 (17)	56(62)
No AraC	12 (18)	0 (0)	182 (34)	63 (35)

AML: acute myeloid leukemia; N: number of patients; CR: complete remission rate; AraC: cytarabine;(*p < 0.001

Table 5
Multivariate analysis for overall survival in patients with AML and mutated Ras

Characteristic	p-value
Age (continuous variable)	0.095
Cytogenetics (Adverse)	0.016
History of AHD	0.001
Treatment (AraC vs. No AraC)	NS
Prior Chemo or XRT	NS
WBC count (continuous)	0.07
BM Blast % (continuous)	0.08

WBC: white blood cell; BM: bone marrow; AraC: cytarabine; AHD: antecedent hematologic disorder; chemo: chemotherapy; XRT: radiation therapy

Table 6
Baseline Characteristics of Patients with banked samples for proteomic analysis

Characteristic	Mutated Ras, N=30 (13%)	Wild-Type Ras, N= 198 (87%)
Median age, years (range)	57 (28-84)	69 (17-87)
Male sex, N (%)	16 (53)	114 (58)
Median WBC count (range)	17	6
Median PB blast % (range)	29	13
FAB Morphology, N (%)		
M0	0 (0)	8 (4)
M1	3 (10)	25 (13)
M2	9 (30)	79 (40)
M3	0 (0)	4 (2)
M4	8 (27)	34 (17)
M5	5 (17)	18 (9)
M6	3 (10)	14 (7)
M7	0 (0)	1 (1)
Cytogenetics, N (%)		
Favorable	7 (23)	15 (8)
Intermediate	13 (43)	91 (46)
Adverse	10 (33)	92 (47)
History of AHD, N (%)	7 (23)	82 (41)
FLT3 ITD Positive, N (%)	3 (10)	36 (18)

N: number of patients; WBC: white blood cell; PB: peripheral blood; BM: bone marrow; FAB: French-American British classification; AHD: antecedent hematologic disorder

Table 7
Proteins differentially expressed in AML samples with mutated and wild-type Ras

Proteins higher in mutated Ras		Proteins higher in wild-type Ras	
Protein	p-value	Protein	p-value
AKTp473	0.01	14.3.3Zeta	0.01
Bax	0.009	BCL-XL	0.047
E1F4e	0.02	Casp3cleaved	0.02
EBP1pser65	0.007	Casp7cleaved	0.02
ebp1pser70	0.07	EGFRp992	0.06
GAB2	0.04	FAK	0.006
GSK3	0.04	Fibronectin	0.003
GSKpser21	0.002	FOXO3A	0.007
MEK	0.003	HSP70	0.009
MEKp	0.04	IntegrinB3	0.003
P16	0.01	JunB	0.02
P38	0.004	Myc	0.007
P38phos	0.1	P53	0.05
PKD1	0.048	Parp	0.02
PKD1phos	0.009	ParpCleaved	0.0001
pERK	0.13	SRC	0.02
S6RP	0.03	SRCp416	0.03
SHIP	0.01	Stat1p701	0.04
SHIP2	0.03	ZNF342	0.005
TSC2	0.002		