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Patterns of Molecular Response to and Relapse After Combination of Sorafenib, Idarubicin, and Cytarabine in Patients With *FLT3* Mutant Acute Myeloid Leukemia

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Abstract

Background—FMS-like tyrosine kinase 3 (*FLT3*) is a class III receptor tyrosine kinase involved in hematopoietic progenitor cell development. Mutations of *FLT3* have been reported in about a third of patients with acute myeloid leukemia (AML), and inhibitors of *FLT3* are of clinical interest. Sorafenib is an orally active multikinase inhibitor with potent activity against *FLT3* and the Raf/ERK/MEK kinase pathway.

Methods—We studied the patterns of molecular response and relapse in 18 patients with mutated *FLT3* treated with the combination of sorafenib, idarubicin, and cytarabine.

Results—The median follow-up was 9 months. Sixteen patients achieved complete remission (CR), and the other 2 patients achieved CR but lacked platelet recovery for an overall response rate of 100%. Ten patients had their *FLT3*-mutated clone eradicated, with 6 patients who showed some residual *FLT3*-mutated cells, and 2 patients who showed persistent *FLT3*-mutated cells. The elimination of *FLT3*-mutated population at the time of morphologic CR, however, was not predictive of relapse. After a median follow-up of 9 months (range, 1–16 months), 10 (55%) patients had relapsed, with a median CR duration of 8.8 months (range, 1–9.5 months). By DNA sequencing, there was no evidence of an acquired *FLT3* point mutation at the time of relapse in 7 patients tested, which suggested the presence of other mechanisms of sorafenib resistance.

Conclusion—Sorafenib, combined with chemotherapy, is effective in attaining CR, but relapses still occur.

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Disclosure

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Keywords

Acute Myeloid Leukemia AML; *FLT3* mutation; Sorafenib

Introduction

Acute myeloid leukemia (AML) is the most common acute leukemia, and its effective therapy remains challenging. Induction with cytarabine and an anthracycline remains the standard of care. New regimens are incorporating target-specific agents to improve both complete remission (CR) and survival rates. These novel agents typically target protein products of mutated genes and balanced translocations, such as upregulated or constitutively activated tyrosine kinases, in leukemia cells.

FMS-like tyrosine kinase-3 (*FLT3*) is a member of class III receptor tyrosine kinase and is involved in normal hematopoietic progenitor cell development.[1–3] It is a membrane-bound receptor with an intrinsic tyrosine kinase domain.[4] Autocrine signaling by the *FLT3* ligand through wild-type (WT) *FLT3* can lead to enhanced kinase activity.[5,6] Mutations of *FLT3*, such as an internal tandem duplication (ITD) or tyrosine kinase domain (TKD) point mutations, occur in >30% of patients with AML and lead to constitutive activation of the *FLT3* kinase.[6,7] The presence of *FLT3*-ITD is associated with reduced disease-free and overall survival (OS).[7–9] Whether *FLT3*-TKD has a similar effect on the outcome has been the subject of conflicting reports.[10–13]

FLT3-ITD occurs in approximately 25% of younger patients with AML, with a variable length of duplicated DNA (between 3 and >400 base pairs in the juxtamembrane domain).[14] The juxtamembrane domain is a negative regulatory domain that inhibits the activation loop from adopting the active conformation. Several studies have documented the prognostic impact of these genetic alterations with patients with the ITD having a poor prognosis.[7–9,15,16] High mutant to WT allele burden, the number of mutants of different size in the same patient, and the size of DNA insertions, have all been linked to a worse prognosis.[17–20]

Sorafenib is an oral, small-molecule, multikinase inhibitor that has been approved for the treatment of renal and hepatocellular cancers at a dose of 400 mg twice daily. It inhibits *FLT3* and its downstream Raf/ERK/MEK pathway.[21] In preclinical studies, it caused dephosphorylation of MEK1/2 and ERK, and induced apoptosis in AML cells.[22] This effect was seen preferentially in *FLT3*-mutated cells compared with cells with WT *FLT3* by >1000 –3000-fold. Sorafenib has demonstrated clinical activity in phase I studies in patients with *FLT3*-ITD AML.[23–25]

Several potential mechanisms of resistance have been entertained in patients treated with *FLT3* inhibitors.[26,27] These mechanisms include plasma protein binding, bypass activation of downstream STAT5 and MAP kinase signaling, limited specificity against target *FLT3*, and secondary TKD mutations that interfere with drug activity.[28–32] In in vitro culture studies, exposure of AML cell lines to continuous *FLT3* inhibitors (including sorafenib) induces kinase domain mutations that confer resistance.[30] The objective of this report was to

determine the pattern of molecular response and relapse in patients with previously untreated AML who received induction therapy with the combination of sorafenib and chemotherapy, and to determine potential mechanisms of resistance. An earlier report of this clinical trial, including patients treated on the phase I portion of the study, has been previously published.[33] In this report, we focus only on the 18 patients with *FLT3* mutation (including 3 patients not included in the previous report) treated on the phase II portion of the study.

Patients and Methods

Patient Eligibility

Patients 18–60 years old with previously untreated AML (based on the World Health Organization [WHO] criteria) were eligible for treatment on this phase II study. Patients 61–65 years old also were eligible if they had a low probability of 8-week mortality with intensive chemotherapy based on adverse risk factors (cytogenetics, ECOG PS [Eastern Cooperative Oncology Group performance status], antecedent hematologic diseases, and organ function).[34] All the patients had to have adequate cardiac, renal, and hepatic function, with an ECOG PS of 0, 1, 2, and 3 (left ventricular ejection fraction ≥50%, creatinine ≤ 2.0 mg/dL, bilirubin ≤ 2.0 mg/dL, and liver transaminases < 3 times the institutional upper limit of normal). All the patients signed an informed consent approved by the institutional review board. Only patients with *FLT3* mutations (ITD, TKD, or both) were included in this report.

Treatment Regimen

Induction consisted of sorafenib 400 mg orally (p.o.) twice daily for 7 days combined with cytarabine 1.5 g/m^2 by continuous intravenous (I.V.) infusion daily for 4 days (patients > 60 years of age received 3 days only) in addition to idarubicin 12 mg/m^2 I.V. over 1 hour daily for 3 days. The patients who did not achieve CR after 1 course could receive another induction course. For consolidation, patients in CR received up to 5 cycles of idarubicin 8 mg/m^2 I.V. daily for 2 days with cytarabine 0.75 g/m^2 I.V. over 24 hours for 3 days in addition to sorafenib 400 mg p.o. twice daily for 28 days. The cycles were repeated every 4–6 weeks based on toxicity and recovery of counts. Patients who completed consolidation received up to 1 year of sorafenib as maintenance therapy unless they underwent stem cell transplantation. The dose of all agents could be reduced during consolidation and maintenance based on the available guidelines related to the various adverse effects.

Response Criteria

CR was defined by the presence of <5% blasts in the bone marrow (BM) with >1 × 10^9/L neutrophils and >100 × 10^9/L platelets in the peripheral blood. CR duration was calculated from the time of CR until relapse. Relapse was defined by the recurrence of > 5% blasts in BM aspirate not related to recovery or the development of extramedullary disease. OS was calculated from the time of diagnosis until death.

FLT3 Mutation Detection

FLT3-ITD and codon 835/836 TKD mutation status were determined in DNA from initial, postinduction, follow-up, and relapsed unsorted BM aspirate samples by a polymerase chain

reaction (PCR) based method, with an analytical sensitivity of 1%–2% mutation-bearing cells. *FLT3* allele burden was determined by the ratio of the area under the mutated and unmutated PCR amplicon peaks as detected software calls after capillary electrophoresis on a 3100 or 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). A manual 400-cell differential performed on smears and multicolor flow cytometry on aspirate samples was used to track the levels of residual leukemia blasts. When leukemic blasts were detected, ratios of *FLT3* mutation were normalized to blast count. Relapsed BM samples (or skin biopsy in 1 case) were analyzed for kinase domain mutations in exons 16, 17, 20, and 21 by using PCR-based Sanger sequencing of genomic DNA (sensitivity level of 20% mutation-bearing cells). In diagnostic BM samples, exon 1 and 2 *KRAS* and *NRAS* point mutations were assessed by using PCR-based pyrosequencing of genomic DNA (sensitivity level of 5% mutation-bearing cells) and *NPM1* exon 12 mutation and/or duplications were assessed by using a PCR-based capillary electrophoresis method (sensitivity level of 2% mutation-bearing cells) as previously described.[35,36]

Statistical Analysis

The main objective of this trial was to provide an assessment of the efficacy of adding sorafenib to combination chemotherapy. Survival was calculated by the Kaplan-Meier method, and different categories were compared by the log-rank test. Differences in subgroups by different covariates were evaluated by using the χ^2 test for nominal values (high vs. low *FLT3* mutation burden) and the Mann-Whitney *U* test and Fisher exact test for continuous variables (*FLT3* burden and outcome).

Results

Patient Characteristics

From October 2007 to September 2009, 64 patients with AML were enrolled into this phase II clinical study; 18 had mutated *FLT3* and are subjects of this report (Table 1). These patients are the patients who participated in the phase II portion of the study previously reported, including 3 patients not reported in the previous publication.[33] Their median age was 54 years (range, 20–65 years); 10 were women. Fifteen patients had *FLT3*-ITD, 2 had *FLT3*-TKD, and one had mutations at both sites. Five patients had a low *FLT3* mutation burden (ie, blast-normalized mutation ratio of <25%) consistent with presence in only a subset of the blast population. Two patients also had *RAS* mutations (both were *NRAS* exon 12), whereas 5 others had *NPM1* mutations. Most patients (83%) had a PS of 0 and 1 compared with only 3 (17%) patients with a PS of 2 and 3. Ten (55%) of 18 patients had diploid cytogenetics, and 8 had various abnormalities (deletions or additions but no translocations). The most common FAB subtype was M1 in 8 (44%) patients and M4 in 4 (22%) of them.

Response and Outcome

All 18 (100%) patients achieved CR or CR but lacked platelet recovery (CRp) (16 CR and 2 CRp). All but 2 patients responded after one cycle of induction; 2 patients achieved CRp after 2 cycles of induction. After a median follow-up of 9 months (range, 1–16 months), 10 (55%) patients had relapsed, with a median CR duration of 8.8 months (range, 1–9.5

months) The CR duration was independent of *FLT3* mutation burden at diagnosis ($P = .63$) (Figures 1, 2). Twelve (67%) patients proceeded to allogeneic stem cell transplantation; 5 in CR1, and 7 after relapse. Four patients died (3 of whom had matched unrelated allogeneic donor stem cell transplantation in either CR1 [n = 1] or CR2 [n = 2]), with a median OS of 14.8 months (range, 1–16 months). Survival duration was independent of *FLT3* mutation burden at diagnosis ($P = .9$) (Figures 3, 4). Three of the 4 deceased patients had a high mutant allele burden.

FLT3 Mutation Levels After Initial Therapy

Samples from all the patients after induction (approximately day 30) were collected and analyzed for molecular response (Figure 5). Ten (56%) patients showed no evidence of the mutated *FLT3* clone by PCR analysis, whereas 6 (33%) showed partial regression of the *FLT3* mutated clone, and 2 (11%) patients had persistent molecular evidence of the disease, with no preferential regression of the mutated clone. The degree of regression of the mutated *FLT3* clone did not predict relapse (the relapse rate in patients with complete regression was 6/10 [60%] vs. 5/8 [63%] in the rest). We also compared the regression of *FLT3* mutated clone in the study group with 67 patients with *FLT3-ITD* treated at our institution with standard anthracycline-cytarabine regimens without sorafenib. Overall, partial or complete *FLT3* regression after the first cycle of therapy was evident in 89% of the study patients compared with 6% among patients treated with regimens that lacked sorafenib ($P < .001$, χ^2 test).

Pattern of *FLT3* Mutation After Relapse

Nine of the 10 patients who relapsed had recurrence of *FLT3* mutated clone, although one had no evidence of mutated *FLT3* in the sample obtained at relapse. The relapse rate was independent of the level of *FLT3* mutation at diagnosis ($P = .63$). In 7 patients at relapse, sequencing of the TKD domain (which covers exons 16, 17, 20, and 21) revealed no additional mutation in *FLT3* in BM specimens (n = 6) or skin biopsy (n = 1).

Discussion

Patients with *FLT3*-mutated AML have a worse prognosis than those with WT *FLT3*. Predictors of outcome such as the length of the tandem duplication mutation as well as the ratio of WT to mutant allele have been reported.[17–20,37] Several novel agents targeted against *FLT3* kinase have been evaluated in clinical trials.[33,38–44] An improved response in patients with *FLT3*-mutated disease has been reported in these studies, but further studies are needed to demonstrate any effect on survival. Combining these agents with chemotherapy is preferred because a synergistic effect has been shown in in vitro studies.[45] This inhibitory effect is present in both WT- and mutant-*FLT3* cells in vitro but varies with dependence on *FLT3* signaling.[46]

In this study, we observed an excellent response, with all patients achieving CR or CRp, which suggests a beneficial on-target effect. However, the majority of the patients relapsed suggesting the limited efficacy of the regimen to produce long-term remissions. Sorafenib was administered in induction and consolidation, and for maintenance for about 1 year. We

were unable to identify any secondary mutations that, in 6 of 10 patients who relapsed, would confer resistance to sorafenib as has previously been reported and as has been demonstrated for BCR-ABL inhibitors,[26,29,30,32] which suggests that other mechanisms of resistance may exist, although there are several limitations associated with this conclusion. For instance, when looking for secondary mutations, we only sequenced 4 exons and not the whole *FLT3* gene. In addition, no pharmacokinetic data were collected in this phase II trial to demonstrate that adequate serum sorafenib levels were achieved. Finally, the small number of patients whose samples were sequenced further limits the validity of any conclusions.

The lack of correlation of molecular response with relapse makes any conclusions on the role of sorafenib and other kinase inhibitors in this disease difficult. For instance, the presence of minimal residual disease by PCR did not predict for relapse. Two patients relapsed with WT-*FLT3* AML, which raises the possibility of clonal evolution (vs. selective inhibition of the mutated clone and the appearance of less competing clones). Achieving maximal or partial regression of the mutated clone did not predict relapse because 3 of 7 patients who achieved partial regression continued to be disease free until the date of this article. Monocytic phenotype (M4, M5) did not show any worse outcome compared with other FAB subtypes (3/5 patients were disease free) that did not support the report by Koh et al,[15] which suggests clinical significance of *FLT3* in monocytic subtype only.

In conclusion, induction therapy with sorafenib, idarubicin, and cytarabine is effective in attaining remission and in reducing the mutated clone in patients with mutated *FLT3* but does not universally eradicate it. Continuous therapy with sorafenib in induction may be beneficial in further reducing the leukemic clone; however, the extent of suppression of the clone is not predictive of relapse. No secondary *FLT3* mutations were found in the evaluated patients at relapse, which suggests that factors other than mutations that confer resistance to sorafenib may be important.

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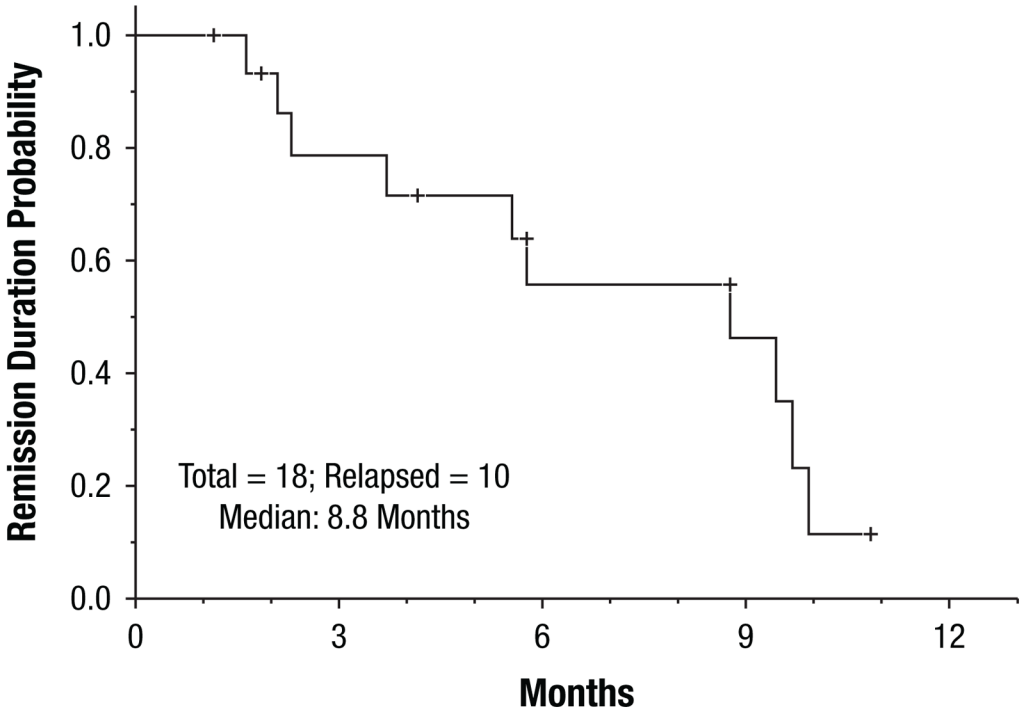

Figure 1.
Complete Remission Duration

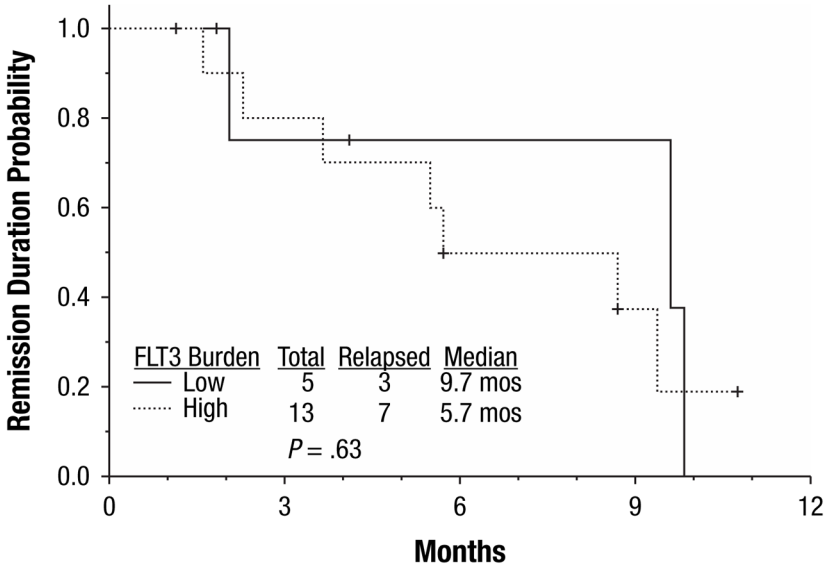

Figure 2.
Complete Remission Duration for Patients with High- and Low-*FLT3* Mutation Burden

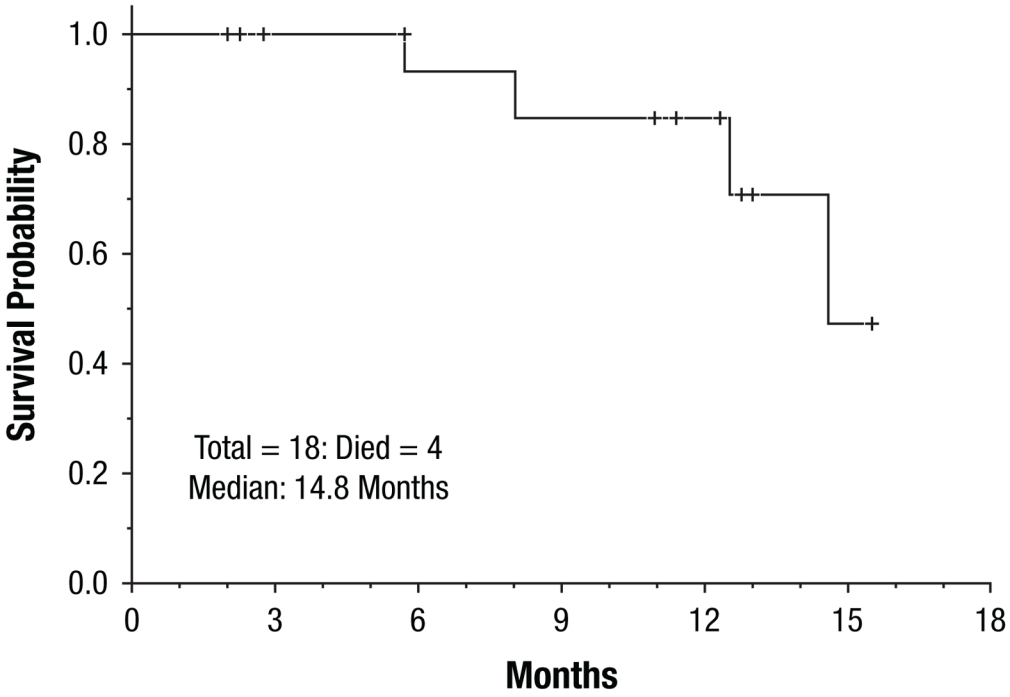

Figure 3.
Overall Survival

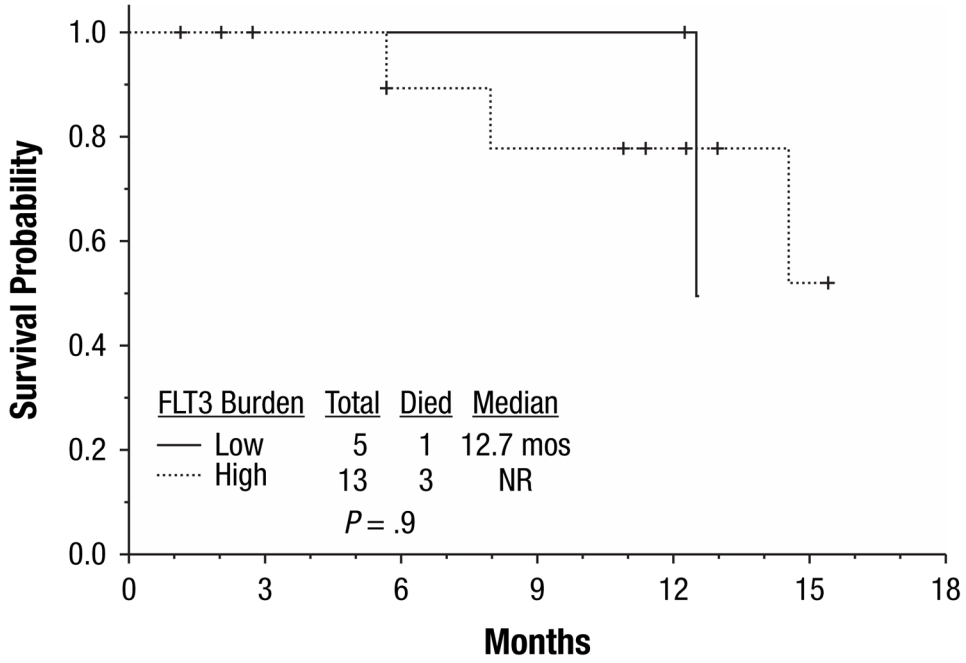

Figure 4.
Overall Survival for Patients with High and Low FLT3 Mutation Burden

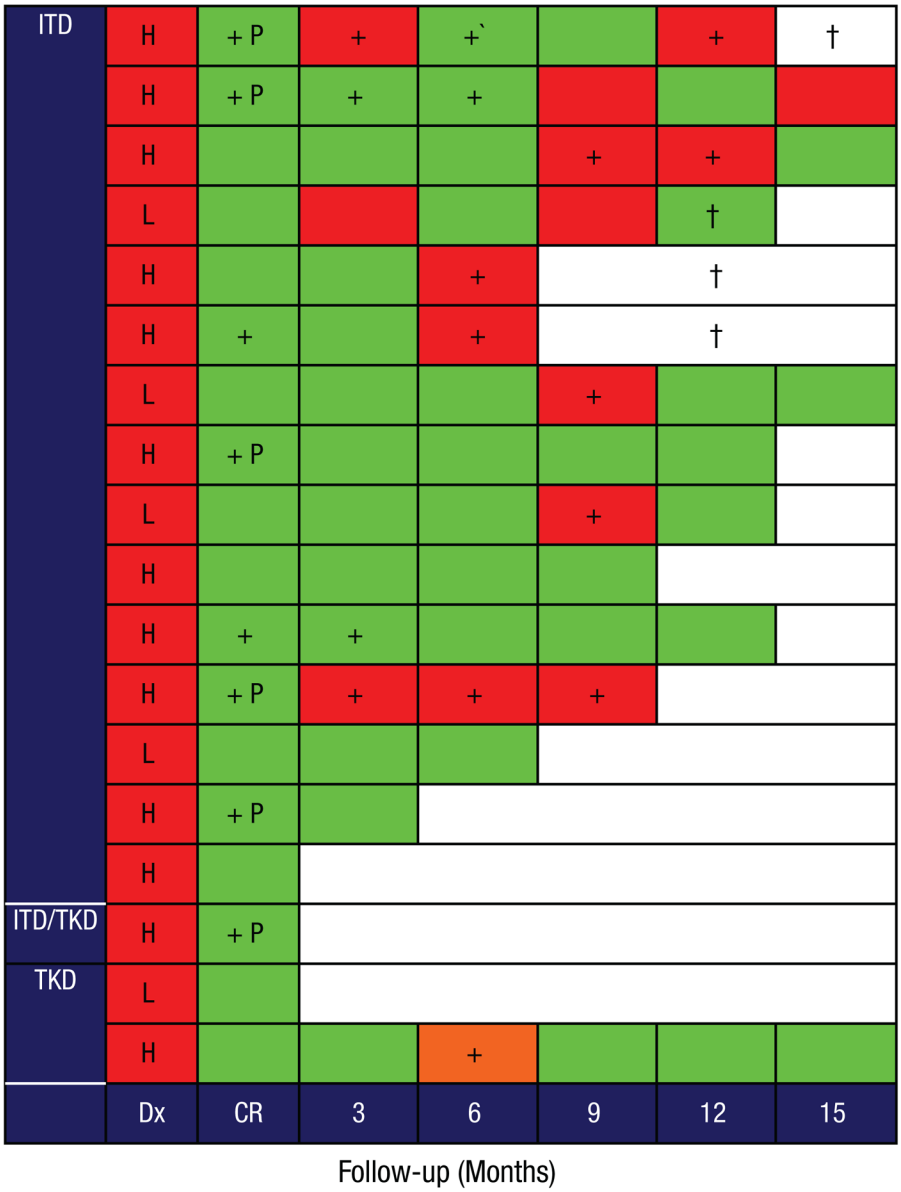

Figure 5.

Follow-up, Morphologic and Molecular Relapse

Red Color = Positive for Leukemia by Flow Cytometry (FCM) and/or Morphology; Green Color = Negative for Leukemia by FCM and/or Morphology; Orange Color = Extramedullary Acute Myeloid Leukemia (AML); H = High Mutant *FLT3* Burden, L = Low Mutant *FLT3* Burden, † = Deceased, + = Positive Minimal Residual Disease by Polymerase Chain Reaction for Mutant *FLT3*; P = Partial Regression of Mutant *FLT3* clone

Table 1

Patient Characteristics

Patient Characteristics	
Median (Range) Age (y)	54 (20–65)
***FLT3* Mutation Type**	
ITD	15 (83%)
TKD	2 (11%)
ITD/TKD	1 (6%)
***FLT3* Mutation Burden**	
High[a]	13 (72%)
Low	5 (28%)
Eastern Cooperative Oncology Group Performance Status	
0	5 (28%)
1	10 (55%)
2	2 (11%)
3	1 (6%)
Median (Range) White Blood Cell Count ($\times 10^9$/L)	18 (1.4–196)
Median (Range) Hemoglobin (g/dL)	8.5 (6.9–10.8)
Median (Range) Platelet ($\times 10^9$/L)	51.5 (18–189)
Cytogenetics	
Diploid	10 (55%)
Other	8 (45%)

[a] ≥25% blast-normalized mutation ratio (see Methods section).