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Minimal Residual Disease Eradication with Epigenetic Therapy in Core Binding Factor Acute Myeloid Leukemia

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Abstract

Recurrent translocations, t(8;21) or inv(16), in core binding factor acute myeloid leukemia (CBF-AML) are amenable to monitoring for minimal residual disease (MRD) with reverse transcriptase polymerase chain reaction (RTPCR). Despite a favorable prognosis, disease relapse remains the single cause of treatment failure in CBF-AML. Fusion products of these translocations recruit epigenetic silencing complexes resulting in hematopoietic maturation arrest. We hypothesized that maintenance therapy with hypomethylating agents (HMA), including decitabine (DAC) and azacitidine (AZA) after induction/consolidation, can be used for MRD elimination to ultimately prolong relapse free survival. Real-time quantitative (RTPCR) trends were reviewed in 23 patients (median age 53 years) with CBF-AML that received HMA therapy following induction/consolidation with fludarabine, cytarabine and G-CSF (FLAG) with low dose gemtuzumab or idarubicin (NCT00801489). Of the 23 patients evaluated, 17 had a detectable RTPCR at HMA initiation. Five patients had progressive disease and a notable increase in RTPCR values over 1 to 2 cycles of HMA therapy. Twelve patients did not fail HMA and had a median RTPCR at HMA initiation of 0.06 (range, 0.01–0.91). Unlike the HMA failure subset, 11 of these patients had a reduction in RTPCR after the first or second cycle of HMA. Our data suggests that CBF-AML patients with low levels of RTPCR (between 0.01 and 0.05) at the conclusion of induction/consolidation chemotherapy benefit most from maintenance HMA, particularly those that have a reduction in the RTPCR within the first 2 cycles of HMA therapy.

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Author Contributions

BKR, ND, FR, JC, TK, BO, GGM, MO, AF, NP, HK, and GB collected and reviewed the data and wrote the paper. All authors participated in the discussion, have reviewed the final manuscript, have added comments or suggestions, and approved the current version of the manuscript.

Keywords

Core binding factor; acute myeloid leukemia; minimal residual disease; reverse transcriptase polymerase chain reaction; hypomethylating agent

INTRODUCTION

The presence of translocation t(8;21) (q22;q22) or inversion inv(16) (p13q22)/t(16;16) characterizes core binding factor acute myeloid leukemia (CBF-AML).[1] CBF-AML, representing 15% of all acute myeloid leukemia, has a favorable prognosis when treated with intermediate to high dose cytarabine-based induction and consolidation regimens.[2–4] However, disease relapse remains as a major cause of treatment failure, despite a cure rate of >65% with chemotherapy alone.[5]

Real-time quantitative (RTPCR) techniques allow for the detection and quantification of leukemia-associated genes to measure minimal residual disease (MRD). RTPCR based MRD monitoring provides prognostic information and guides post-remission therapy including allogeneic stem cell transplantation (SCT) in CBF-AML.[5] Several prior studies have shown that routine monitoring of MRD is important in CBF-AML to monitor for relapse and provide dynamic risk stratification while on treatment, with several efforts trying to quantitatively define the MRD level that might signify overt relapse and necessitate salvage therapy.[5–13]

In addition to persistent elevation of RTPCR transcript, identification of concomitant mutations along with t(8;21) and inv(16) also portend a higher risk of relapse.[14,15] Mutations in *c-KIT* have been shown to shorten relapse free survival (RFS) and event free survival (EFS) in t(8;21) patients, reduced overall survival (OS) in inv(16) patients, and when present with persistent MRD and a high white blood cell count, shorter RFS in all CBF-AML patients.[14–16] *FLT3-ITD* mutations have been shown to shorten EFS and RFS in CBF-AML.[15] *RAS* mutations do not seem to impact prognosis but are important in the pathogenesis of CBF-AML and are possible targets for therapy with tyrosine kinase inhibitors.[17] Along with evaluation of MRD, additional mutation data was obtained for further investigation in this analysis.

One unanswered question is what strategy to adopt when a patient is in morphological remission but has persistent molecular MRD or in patients who had truncated high dose consolidation because of adverse events. Hypermethylation of certain genes while patients are in remission has been associated with an increased likelihood of relapse in AML.[5,18,19] Promoter hypermethylation of tumor suppressors is also frequently seen in CBF-AML.[20,21] Hypomethylating agents including decitabine (DAC) and azacitidine (AZA) can potentially reverse such epigenetic silencing.[22,23] Several studies have recently explored HMA maintenance therapy in AML.[24,25] Though significant survival improvement has not been shown with this strategy, some clinical benefit has been observed, and the use of MRD directed HMA maintenance has not been extensively explored.[25] We hypothesized that maintenance therapy with hypomethylating agents, such as DAC and AZA, can be used to

target residual low-level PCR positivity and eliminate minimal residual disease (MRD) to ultimately prolong relapse free survival in CBF-AML.

METHODS

An analysis of 23 patients with CBF-AML who received HMA after fludarabine, cytarabine and G-CSF (FLAG) with low dose gemtuzumab or idarubicin induction/consolidation (NCT00801489) was performed between August 2008 and April 2015. Serial RTPCR from peripheral blood or bone marrow was obtained approximately every 3 months per protocol. The methodology of our RTPCR analysis panel for CBF-AML has been previously published and is in line with the Europe Against Cancer (EAC) Program.[26,27] The sensitivity of detection for transcript RTPCR at the time of this analysis was between 1 in 10,000 and 1 in 100,000. Patient characteristics and outcomes were obtained from chart review and departmental database. Mutations in *KIT, FLT3ITD, FLT3D835* and *RAS* genes were tested at baseline. In addition, most patients were evaluated for *NPM1, TP53, IDH1* and *IDH2* mutations at baseline.

Objectives

The primary objectives of this analysis were to monitor MRD status by serial RTPCR response to HMA therapy after the completion of induction/consolidation and to determine RTPCR baseline values and trends that predict for a higher likelihood of achieving durable remission on HMA maintenance.

Patient Selection

All patients ≥18 years old with a diagnosis of AML with t(8;21), inv(16), or t(16;16), with or without additional cytogenetic abnormalities, who received at least 1 cycle of HMA maintenance therapy following completion of FLAG-based induction or consolidation were included in this analysis. No patients were in morphologic relapse at time of enrollment.

Treatment Regimen

The induction regimen included fludarabine (FL) 30 mg/m^2 on Days 1–5, cytarabine (A) 2 g/m^2 IV on Days 1–5, gemtuzumab ozogamicin (GO) 3 mg/m^2 on Day 1 or idarubicin (Ida) 6 mg/m2 on days 3–4, and G-CSF (G) 5 mcg/kg on Day −1 until neutrophil recovery. FLAG for 3 days with GO on day 1 in Cycle 2/3 and 5/6 or with Ida (Dose: 6 mg/m^2 on Days 2 and 3 in one post remission cycle) was the consolidation regimen utilized, and the target was to complete approximately 6 cycles.[28] Patients transitioned to HMA therapy received monthly maintenance therapy with DAC 20 mg/m^2 on Days 1–5 or AZA 75 mg/m^2 on Days 1–5 repeated every 4–5 weeks based on count recovery and toxicity.

Mutation Analysis

Mutation analysis was carried out in exons 8 and 17 in *KIT* gene, in codons 12, 14, and 61 in *NRAS* and *KRAS* genes using PCR-based DNA sequencing methods, and for internal tandem duplications (ITD) or D835 mutations in *FLT3* gene according to published methods.[29]

Statistical Analysis

Median RTPCR values between HMA responders and HMA failures were compared using the non-parametric Mann-Whitney test. OS was calculated using Kaplan-Meier estimates, and survival estimates were compared by using the log-rank test.

RESULTS

Patient Characteristics

Between August 2008 and April 2015, a total of 23 patients [t(8;21)=8 and inv(16)=15] received maintenance HMA. Patient characteristics are summarized in Table 1. The reason for transition to HMA was persistent PCR positivity in 13 patients (57%), prolonged myelosuppression precluding chemotherapy based consolidation in 9 patients (39%), and molecular relapse after SCT in 1 patient (4%). Three of these patients received salvage clofarabine, cytarabine and idarubicin (CIA) for relapsed CBF-AML after front-line FLAG based therapy, achieved morphologic remission, and then went on HMA maintenance for persistent molecular MRD. Decision to pursue HMA therapy was at the discretion of the treating physician. There were patients with persistent PCR positivity and prolonged myelosuppression (N=4). In these cases, the reason for pursuing HMA reported depicts what was documented in the patient's chart by the treating physician. Last follow up was in April 2015.

Response

Patients received a median of 6 cycles of FLAG based induction/consolidation (range, 1–7) prior to transitioning to HMA. The median number of HMA cycles received was 6 (range, 1–17). Seventeen patients had detectable MRD (RTPCR ≥ 0.01) at initiation of HMA maintenance with a median RTPCR of 0.06 (range, 0.01–3.84). All 17 patients were in morphologic complete remission (CR) when HMA was initiated. FISH was performed at HMA initiation for 7 patients and was negative in all cases. Responses for these patients are summarized in Table 2.

Of these 17 patients, 5 patients (29%) had progressive disease post HMA initiation, including 1/7 with t(8:21) and 4/10 with inv(16). The RTPCR values preceding HMA in these 5 patients were 3.84, 0.06, 0.08, 0.01, and 0.01, respectively (median=0.06). All 5 patients had a log increase in the RTPCR value after one to two cycles of HMA as noted on sequential RTPCR done after first or second cycle of HMA. RTPCRs for these 5 patients following the first or second cycle of HMA were 51.67, >100, 77.48, 1.33, and 0.17, respectively (shown in Figure 1). RTPCR transcript levels measured after first or second cycle of HMA maintenance in the HMA failure group were significantly greater than transcript values in the HMA responders (p=0.01) based on the Mann-Whitney test. All five patients failing HMA proceeded to SCT, and 3 are alive and disease free post-SCT with a median follow up of 11 months (range 3–13 months). Two of the 3 surviving HMA failure patients had undetectable MRD following SCT. As of last follow up, 1 surviving patient from the HMA failure group had a detectable RTPCR of 0.02 following SCT, and re-initiation of maintenance therapy with AZA was planned.

Of the 23 patients reviewed in this analysis only 2 deaths occurred between August 2008 and April 2015. These 2 deaths occurred in the HMA failure group, 1 from leukemia relapse, and 1 from SCT complications. OS difference between two groups is shown in Supplemental Figure S1 and S2.

For the 12 patients who did not fail HMA, the median RTPCR at HMA initiation was also 0.06 (range, 0.01–0.91). Unlike the HMA failure subset described above, 11 of these patients had a reduction in RTPCR after the first or second cycle of HMA. One patient was in early follow up. These 12 patients remained in morphologic and cytogenetic complete remission as of last follow up. RTPCR values and trends over time are shown in Figure 2.

As of last follow up, all 6 patients who started HMA with undetectable MRD remained MRD negative (RTPCR < 0.01) and in remission, and 8 out of the total 23 patients continued on maintenance therapy. Median follow up was 11.3 months (range, 2.9–67.7).

Mutational Status

Of the 5 patients failing HMA, 4 [inv(16)=3 and t(8;21)=1] had a *RAS* mutation detected, with the t(8;21) patient also having a concomitant *KIT* mutation, and 1 inv(16) patient with a co-existing *FLT3D835* mutation. Of the 12 patients who responded to HMA, 4 [inv(16)=2 and t(8;21)=2] had a *RAS* mutation, one t(8;21) had a co-existing *FLT3ITD*, 3 [inv(16)=1 and t(8;21)=2] had a sole *KIT* mutation, and 1 inv(16) patient had a *FLT3D835* mutation. Of the 6 patients who proceeded to HMA maintenance without detectable MRD, only 1 inv(16) patient had a co-existing *FLT3D835* mutation. All other mutations (*NPM1*=20 evaluated, *TP53*=13 evaluated, *IDH1* and *IDH2*=16 evaluated) were negative at diagnosis.

DISCUSSION

Despite a favorable prognosis, relapse occurs in 20–25% of the patients with CBF and is usually preceded by a period of increased RTPCR transcripts. Strategies to intervene if a patient does not achieve appropriate reduction in RTPCR levels or shows rising trends post completion of planned induction/consolidation may avert full-blown relapses. SCT is one such option, but donor availability and patient related factors including age, performance status, and organ dysfunction may be potential barriers to timely and safe SCT.[30]

The importance of MRD monitoring in CBF-AML has been well defined.[5,7,9,13] What has not been clearly defined, however, is what to do when MRD positivity beyond a threshold level is detected, particularly when SCT is not an option or when MRD levels are positive but overt relapse is not yet recognized. Beyond re-induction followed by SCT, other strategies for preventing relapse in CBF-AML patients with detectable MRD have not been explored extensively. Recently, results of a phase II study (Cancer and Leukemia Group B Study 10503) of maintenance DAC in AML patients younger than 60 years old were published.[24] Though preliminary data and case reports of using DAC maintenance in CBF-AML were promising, maintenance DAC following complete remission after induction therapy did not provide clinical benefit in either the non CBF-AML or CBF-AML cohorts; the authors reported the study was not powered to identify small differences in survival, and minimal residual disease was not addressed.[24] Boumber and colleagues explored DAC

maintenance versus conventional care in AML patients in complete remission and found that DAC maintenance was safe, feasible, and led to fewer relapses; however, the trial was not powered to provide more significant conclusions.[25] In Boumber's study, MRD was assessed and associated with EFS and OS in a multivariate Cox regression model. Only 1 patient had CBF-AML, and that patient received conventional care and went on to relapse.[25] With these studies in mind, using MRD to guide HMA maintenance might provide improved outcomes for CBF-AML patients. Our analysis reveals that HMA maintenance is effective at controlling MRD and prolonging remission in patients with CBF-AML with detectable or rising RTPCR at the end of induction/consolidation.

Though we have shown that HMA maintenance therapy is effective at prolonging remission in patients with low level MRD, determining the specific MRD cut-off that would benefit most from the HMA maintenance strategy and deciding when to abort such therapy and proceed to SCT remain as important concerns. Our data suggests that patients with low levels of RTPCR (between 0.01 and 0.05) residual disease following induction/consolidation chemotherapy might benefit most from maintenance HMA, particularly those that have a reduction in the RTPCR within one to two cycles following initiation of hypomethylating therapy. A structured introduction of HMAs at a pre-defined cut-off for MRD positivity post induction/consolidation in a larger cohort of CBF-AML will allow for further exploration of these conclusions and further understanding of the impact of baseline mutation status on this population. Such an effort is underway.

Co-existing mutations were identified in patients including *RAS*, *KIT*, and *FLT3*. In the HMA failure group, 80% of patients had a co-existing mutation. For those who responded to HMA maintenance, 67% carried a concomitant mutation. Co-existing *RAS* mutations were identified in highest frequency, followed by *KIT*, then *FLT3* mutations. This is concordant with the typical distribution of co-existing mutations in CBF-AML.[31] With the small number of patients in this analysis, the impact of co-existing mutations on the efficacy of HMA maintenance therapy cannot be adequately determined. In future studies, it will be important to monitor for co-existing mutations as a majority of patients with persistent MRD in our analysis had concomitant mutations. Only 1 of 6 patients (17%) who proceeded to HMA maintenance without detectable MRD had a co-existing mutation, suggesting that mutation burden might contribute to persistent MRD in CBF-AML.

There are many limitations to this analysis. The small sample size and exploratory nature of this retrospective analysis limit the conclusions that can be made. An important limitation in this analysis is that levels of MRD required for initiation of HMA therapy were not pre-defined. Subsequently, observations regarding appropriate levels of MRD that would warrant HMA maintenance can be made, but a well-designed clinical trial with pre-defined MRD levels will provide improved guidance in utilizing maintenance therapy in CBF-AML. Another possible limitation is that peripheral blood and bone marrow were used in MRD assessments for patients in this analysis. The study was not powered to recognize differences between the two sample types, but others studies suggest that either can be used for MRD assessment.[32] An additional important limitation identified is that this analysis includes patients who proceeded to maintenance therapy without detectable MRD, and although these

patients have successfully remained in remission, it is difficult to attribute the success completely to HMA maintenance.

Despite the limitations of this analysis, CBF-AML patients with low levels of RTPCR (between 0.01 and 0.05) at the conclusion of induction/consolidation chemotherapy derived the most benefit from maintenance HMA, particularly those with a reduction in the RTPCR within the first 2 cycles of HMA therapy. We propose this population be explored further in future studies of HMA maintenance in CBF-AML.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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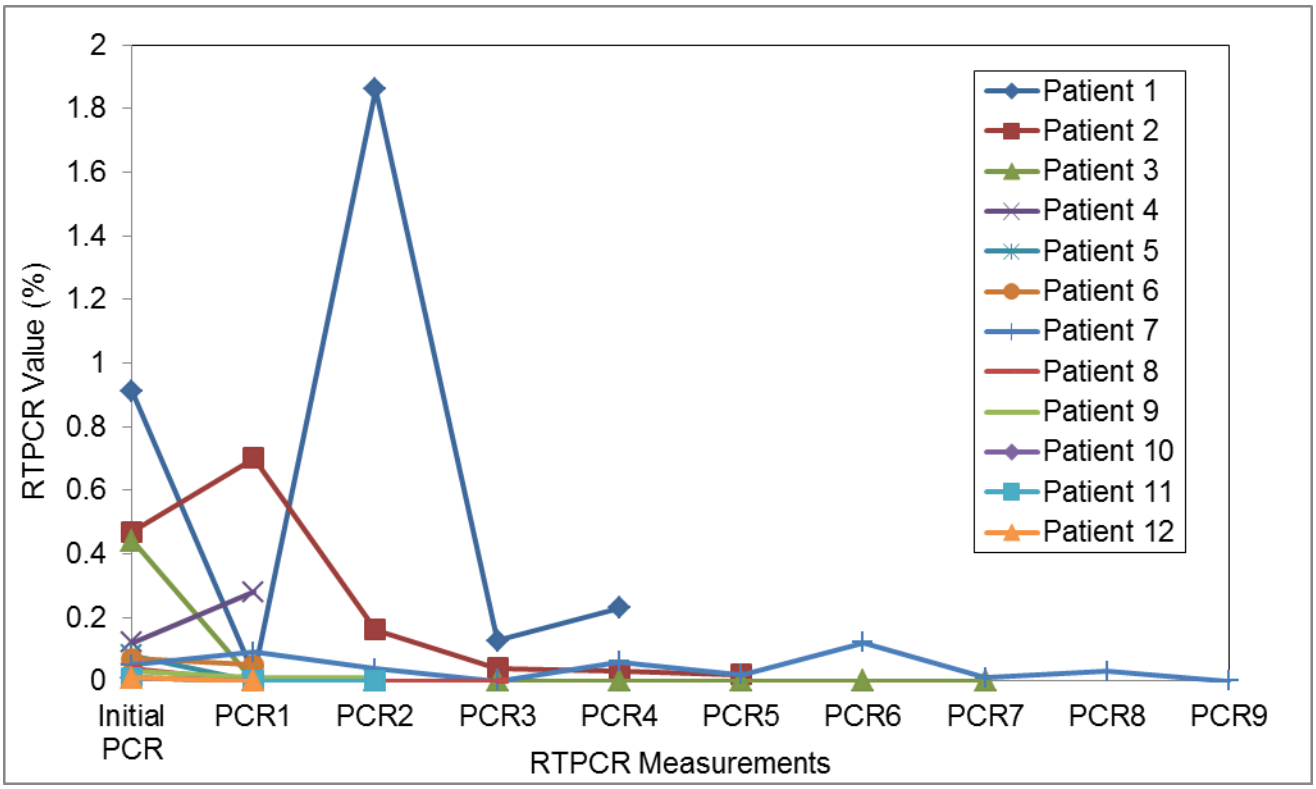

Figure 1.
RTPCR values over serial measurements for patients with HMA failure.

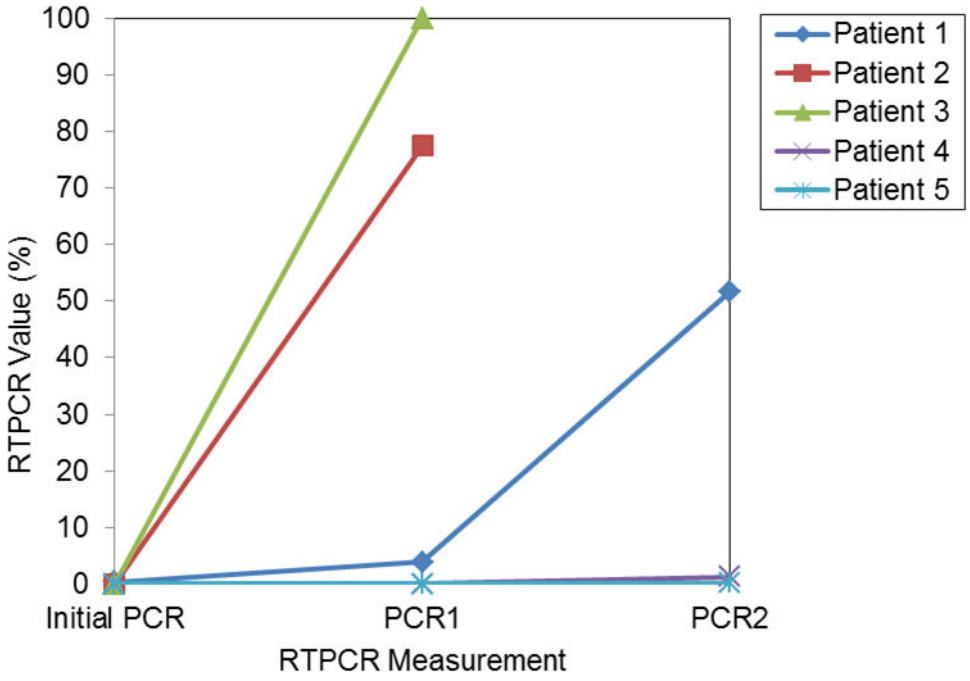

Figure 2.
RTPCR values over serial measurements for HMA responders.

Table 1

Baseline characteristics, N=23.

Characteristic	N (%)/Median [Range]
Age	53 [23–70]
Gender	
Male	12 (52)
Cytogenetics	
Inv(16)	15 (65)
t(8;21)	8 (35)
WBC (K/μL) × 10^9/L at start of HMA	2.8 [0.9–7.3]
Platelets × 10^9/L at start of HMA	67 [9–227]
BM Blasts at start of HMA	1% [0%–2%]
Cycles of FLAG-Based Induction/Consolidation	6 [1–7]
Reason for Transition to HMA Therapy	
Prolonged myelosuppression	9 (39)
Persistent PCR positivity	13 (57)
Molecular relapse after transplant	1 (4)
Hypomethylating Maintenance Therapy	
DAC	21 (91)
AZA	2 (9)
Detectable PCR at start of HMA	
PCR ≥ 0.01	17 (74)
PCR < 0.01	6 (26)
Median PCR at start of HMA	0.06 [0–0.3.84]
Cycles of HMA Maintenance	6 [1–17]
Median Follow-Up	11.3 [2.9–67.7]

Abbreviations: WBC = white blood count; BM = bone marrow; HMA = hypomethylating agents; PCR = polymerase chain reaction; DAC = decitabine; AZA = azacitidine.

Table 2

Current status of MRD positive patients, N=17.

	Continued Remission on HMA		HMA Failure	
	N=12	**(%)**	**N=5**	**(%)**
Morphologic Complete Remission	12	100%	5	100%
Cytogenetic Complete Remission	12	100%	5	100%
RTPCR at Initiation, median (range)	0.06 (0.01–0.91)		0.06 (0.01–3.84)	
RTPCR Increase after initial 2 cycles HMA	1	8%	5	100%
RTPCR Decrease after initial 2 cycles HMA	11	92%	0	0%