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Improved FLT3/ITD PCR assay predicts outcome following allogeneic transplant for AML

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Abstract

Acute myeloid leukemia (AML) patients harboring internal tandem duplication (ITD) mutations of the FMS-like tyrosine kinase 3 (*FLT3*) gene carry a poor prognosis. While allogeneic transplantation may improve outcomes, relapse occurs frequently. The *FLT3/ITD* mutation has been deemed an unsuitable minimal residual disease (MRD) marker because it is unstable and because the standard assay for the mutation is relatively insensitive. The *FLT3* mutation is undetectable by polymerase chain reaction (PCR) at pre- or post-transplant time points in many FLT3/ITD AML patients who subsequently relapse following transplant. We report the application of a new technique, tandem duplication PCR (TD-PCR), for detecting MRD in FLT3/ITD AML patients. Between October 2004 and January 2012, 54 FLT3/ITD AML patients in remission underwent transplantation at our institution. Of 37 patients with available Day 60 marrow samples, 28 (76%) were evaluable for MRD detection. In seven (25%) of the 28 patients, the *FLT3/ITD* mutation was detectable by TD-PCR, but not by standard PCR, on day 60. Six out of the seven patients (86%) with MRD by TD-PCR have relapsed to date compared with only 2 of 21 (10%) patients who were negative for MRD ($p = 0.0003$). The ability to detect MRD by this sensitive technique may provide an opportunity for early clinical intervention.

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Keywords

acute myeloid leukemia (AML); FLT3; internal tandem duplication (ITD); minimal residual disease (MRD); tandem duplication PCR (TD-PCR); bone marrow transplant

Introduction

FLT3/ITD mutations are present in approximately one quarter of adult AML cases. Patients with these mutations carry a particularly poor prognosis, with an estimated four-year overall survival of approximately 20 percent.[1–4] For this reason, a number of small molecule FLT3 inhibitors are currently being evaluated in clinical trials. It has become an increasingly common practice to pursue allogeneic bone marrow transplantation during first remission in patients with *FLT3/ITD* mutations.[2, 5–7]

Among the FLT3/ITD AML patient population, relapse following transplant occurs frequently.[8–10] As a result, there are numerous ongoing efforts to investigate the potential role of maintenance therapy with FLT3 inhibitors in the post-transplant setting. Historically, post-transplant remission in AML patients has been assessed using bone marrow morphology as well as hematologic recovery.[11] Morphologic techniques, however, are insensitive, and better methods are needed to assess minimal residual disease (MRD) in AML patients.

Because *FLT3* status can change over time, it has been suggested that *FLT3* mutational status may not be a good marker for MRD in FLT3/ITD AML.[12] The standard method of detecting the *FLT3/ITD* mutation with polymerase chain reaction (PCR) has a limit of detection of approximately 1 in 100 cells.[13] The relatively low sensitivity of this assay is, in part, due to competition between the mutant and wild type alleles,[13, 14] which we here refer to as PCR bias. The standard *FLT3* PCR amplification product is significantly longer in patients with the ITD mutation than in patients with a wild type *FLT3* gene.[15] The shorter, wild type amplification product will, therefore, be multiplied preferentially by PCR as compared to the longer, mutant product. The *FLT3* mutation is still not detectable by standard PCR assay at time points before and after transplant in many FLT3/ITD AML patients who ultimately relapse with *FLT3*-mutated disease after transplant, because their burden of disease is lower than the detection threshold of the standard assay. Thus, the change in *FLT3* status may be due, at least in part, to the low sensitivity of this assay.

The presence of pre-transplant MRD by flow cytometry is known to carry an adverse prognosis.[16] In addition, in the core binding factor leukemias and nucleophosmin (*NPM1*) mutated AML, MRD as assessed by PCR has been shown to predict relapse.[17, 18] In spite of the widely held belief that *FLT3* status is not a good marker for MRD, multiple groups have reported that identification of MRD by *FLT3* status using patient-specific PCR serves as a marker of relapse.[19–22] However, patient-specific PCR is cumbersome to perform, difficult to standardize, and is not routinely available outside of a research setting.

A better method of MRD detection in FLT3/ITD AML would be useful both for determining prognosis and for guiding therapy, such as determining whether transplantation should be

performed and whether FLT3 inhibitors should be introduced. We report here the retrospective application of a novel technique, tandem duplication PCR (TD-PCR), to a cohort of consecutive FLT3/ITD AML patients who underwent allogeneic transplant at our institution, allowing us to correlate the presence of the *FLT3/ITD* mutation with clinical outcomes. Our analysis demonstrates that the *FLT3/ITD* mutation is a useful marker for MRD and can be used to guide clinical decision making for these patients, including, potentially, the timely use of FLT3 inhibitors.

Materials and Methods

Selection of patients

We performed a chart review to evaluate all patients diagnosed with FLT3/ITD AML between October 2004 and January 2012 who underwent allogeneic bone marrow transplant at our institution while in morphologic and immunophenotypic remission. Patients who had more than 5% blasts by morphology or flow cytometry immediately prior to transplant were excluded from the analysis. Patients followed at our institution but transplanted elsewhere were also excluded.

Polymerase Chain Reaction (PCR)

The conventional PCR for detecting an ITD mutation was performed as previously described.[13]

Tandem Duplication PCR (TD-PCR)

TD-PCR was modified from an original proof-of-principle version.[23] TD-PCR employs a pair of primers for amplification, but oriented in the opposite direction from standard PCR. This technique allows exponential amplification only when the targeted region is duplicated, in a manner analogous to inverse PCR, thus eliminating the background wild-type amplicons obtained with standard approaches and improving the limit of detection to a single molecule. In our previous design, only ITD mutants with large duplications extending into intron 14 could be amplified. We revised the design to improve the clinical applicability of TD-PCR for MRD detection. We found that overlapping primers pointing in opposite directions sufficed for TD-PCR, particularly when a few bases were altered in each primer so that primer-primer annealing is disfavored. This design improved the capability of TD-PCR to detect shorter ITD mutants, although duplications under 30–40 bases remained too small to anneal both primers. With this approach, we constructed a series of 7 primer pairs that cover exon 14 and proximal intron 14. The redesigned TD-PCR has a limit of detection of a single ITD molecule. In a cohort of 58 newly diagnosed or relapsed FLT3/ITD AML patients, we found that up to 70% of ITD mutants can be amplified by 1 or more of the 7 primer pairs (*manuscript in preparation*).

For the current study, the initial diagnostic specimen of each patient was first tested with all 7 primer pairs to determine informativeness. One or two informative primer pairs were subsequently used to test pre-transplant specimens and day 60 post-transplant bone marrow specimens in quadruplicate (total 1 μg DNA examined, equivalent to approximately 160,000

cells). PCR products were subjected to capillary electrophoresis for analysis as described previously.[23]

Of note, day 60 TD-PCR results were not available to clinicians and, therefore, did not impact clinical decision-making in the patient population studied.

Statistics

The logrank test was used to assess differences in relapse free survival and overall survival between patients with positive TD-PCR and negative TD-PCR results.

Results

In order to assess whether it is possible to detect MRD at pre- and post-transplant time points in FLT3/ITD AML patients, we identified 54 patients who were diagnosed between October 2004 and January 2012 with FLT3/ITD AML, subsequently underwent allogeneic bone marrow transplant while in morphologic remission, survived the transplant, and were confirmed to be in complete remission (CR) with a bone marrow biopsy obtained 60 days post-transplant. This group represents a consecutive series of such patients from our institution. The demographics of these patients are reported in Table 1. Median duration of follow-up was 35.1 months.

All patients receiving myeloablative preparative regimens received busulfan and cyclophosphamide, except for one patient who received a myeloablative conditioning regimen consisting of busulfan and fludarabine. All patients receiving nonmyeloablative regimens were treated with fludarabine, cyclophosphamide, and total body irradiation (TBI). For graft versus host disease (GVHD) prophylaxis, all patients received post-transplant cyclophosphamide. For myeloablative matched related and unrelated donor transplants, no further prophylaxis was administered. For myeloablative haploidentical transplants, patients received tacrolimus through day +180. For nonmyeloablative transplants, patients received tacrolimus through day +180 and mycophenolate mofetil through day +35.

Of the 54 patients studied, 23 patients (43%) were alive and in remission at the time of data analysis, and 13 (24%) had died of transplant-related toxicity. One patient (2%) died of an unknown cause, thought likely related to relapse of an underlying myeloproliferative neoplasm. 17 patients (31%) had relapsed following transplant (six of whom were still alive at the time of data analysis). Of these 17 relapsed patients, 16 (94%) harbored the identical *FLT3/ITD* mutation present at diagnosis, as detected by the standard clinical PCR assay for *FLT3* mutations. DNA samples prepared from bone marrow collected at Day 60 were available for 37 of the 54 patients (69%). Because of the lengths of patients' ITDs, the *FLT3* TD-PCR assay was informative for 28 of the 37 (76%) patients with available samples. *FLT3* TD-PCR results for these 28 patients are shown in Table 2. Outcomes and MRD results for the 28 evaluable patients are shown in Figure 1. At the time of transplantation, 24 of the 28 evaluable patients were in CR. Three patients were in CR with incomplete platelet recovery (CRp). One patient was in CR with incomplete hematologic recovery (CRi).

Among the 28 patients, seven (25%) were positive for MRD by *FLT3* TD-PCR on their day 60 bone marrow specimens. The standard clinical PCR for *FLT3* mutation was performed and was negative in all seven cases. Of these seven patients, six (86%) have relapsed to date, while one (14%) remains in remission 1855 days following transplant. The remaining 21 evaluable patients (75%) were negative for MRD by TD-PCR on their day 60 bone marrow specimens. Of these 21 patients, only two (10%) have relapsed.

In the total population of 54 transplanted patients, median estimated relapse-free survival is 15.0 months (Figure 2A). Median overall survival is 27.7 months (Figure 2B). Among the 28 patients with evaluable results, evidence of MRD by *FLT3* TD-PCR was highly prognostic ($p = 0.0003$) of relapse following allogeneic transplant (Figure 3A). Overall survival by TD-PCR status is shown in Figure 3B.

The results shown in Figure 3 indicate that *FLT3/ITD* mutations can be used as important markers of MRD. In interpreting our findings, we noted that patients could be divided by MRD status (as determined by *FLT3* TD-PCR) into three groups, each with unique characteristics.

Group 1: Patients Negative for Pre-Transplant MRD and Negative for Post-Transplant MRD

Of the seven patients who were negative for MRD both prior to transplant and at day 60, none (0%) have relapsed. One patient (Patient 19) died due to complications of suspected cytomegalovirus pneumonitis approximately 10 months following transplant. No patients with a negative pre-transplant MRD assay became positive for MRD following transplant.

Group 2: Patients Positive for Pre-Transplant MRD and Negative for Post-Transplant MRD

Unlike the patients who were negative for MRD before transplant, some of the patients with positive pre-transplant MRD assays underwent conversion of their MRD status post-transplant. Of the 20 patients with positive pre-transplant MRD assays, 13 (65%) were negative for MRD by *FLT3* TD-PCR at day 60. Of these 13 patients, only one (Patient 24 in Table 2) has relapsed. This patient had an ITD allele burden of less than 5% in the initial diagnostic specimen and relapsed approximately 10 months following transplant. Interestingly, this patient relapsed without the ITD mutation by either the standard assay or TD-PCR. Of the other 12 patients who converted from positive MRD status pre-transplant to negative MRD status post-transplant, 11 are alive and in remission, and one died of sepsis four months following transplant.

One patient (Patient 27) did not have a pre-transplant bone marrow sample available. This patient had a negative post-transplant MRD assay at day 60, but a marrow sample collected 12 months after transplant was positive by TD-PCR for the *FLT3/ITD* mutation. This patient relapsed (with the *FLT3/ITD* mutation) 28 months following transplant, suggesting that the presence of this MRD marker heralds relapse.

Group 3: Patients Positive for Pre-Transplant MRD and Positive for Post-Transplant MRD

Seven of the 20 patients (35%) who were positive for pre-transplant MRD continued to be positive for MRD by *FLT3* mutational status at day 60 following transplant. Six (86%) of

these patients have relapsed. One patient (Patient 28) who was positive for the *FLT3/ITD* mutation at pre- and post-transplant time points has not relapsed. This patient developed cutaneous GVHD and has now been in remission for longer than four years. The ITD mutation was not detected by TD-PCR in bone marrow specimens from 1-year and 3-year post-transplant time points, potentially indicative of a graft-versus-leukemia effect.

Five of the six patients with post-transplant MRD by TD-PCR who ultimately relapsed underwent bone marrow aspirate and biopsy at the time of relapse. Among these five patients, four had metaphase cytogenetics identical to the metaphase cytogenetics seen at diagnosis. One patient with normal cytogenetics at diagnosis had an additional copy of chromosome 13 at relapse.

Having established that TD-PCR represents a sensitive, convenient assay for MRD in FLT3/ITD patients, the question arises as to what type of clinical intervention could be performed when MRD is detected. FLT3 inhibitors represented an obvious possibility. We therefore examined our dataset for patients who received FLT3 inhibitors in the peri-transplant setting.

Patients who Received FLT3 Inhibitors in the Peri-Transplant Setting

Three patients received small molecule FLT3 inhibitors in the peri-transplant setting. Patient 2, who was positive for MRD both pre- and post-transplant, received sorafenib as post-transplant maintenance therapy on a clinical trial from four months until 12 months following transplantation. Sorafenib was discontinued due to intolerability. During the period in which the patient was on sorafenib, retrospective testing revealed that the patient had been positive for the *FLT3/ITD* mutation by TD-PCR, despite being in clinical/morphologic remission and despite the standard clinical assay for *FLT3* remaining negative. The patient relapsed with FLT3/ITD AML one month after discontinuing sorafenib (Figure 4).

Patient 21, who was positive for MRD by *FLT3* TD-PCR both before and after transplant, received FLT3 inhibitors on two occasions. The patient received the FLT3 inhibitor quizartinib as a single agent on a clinical trial, ultimately achieving a morphologic remission on this drug prior to transplant. The patient was initiated on off-label sorafenib approximately three months following transplant as maintenance therapy. The patient self-discontinued sorafenib after approximately six weeks on this medication. A bone marrow biopsy three weeks after stopping sorafenib revealed AML in early relapse, with morphologic evidence of disease (approximately 4% of cells were leukemic blasts) and with the presence of the *FLT3/ITD* mutation detectable by standard PCR.

Patient 25 was positive for MRD both by standard PCR and by *FLT3* TD-PCR prior to transplant. As a precaution to prevent relapse prior to transplant, this patient received sorafenib for approximately one month prior to the start of the transplant preparative regimen. The patient was negative for MRD by TD-PCR at day 60 following transplant. The patient did not receive a FLT3 inhibitor following transplant but experienced grade 2 GVHD and remains in remission approximately 30 months following transplant.

Discussion

Given the poor prognosis of patients with FLT3/ITD AML and recent reports of some success with allogeneic bone marrow transplant, transplant has become the standard of care for this disease.[2, 5–7] However, there remains a significant relapse rate following transplant for FLT3/ITD AML. Thus, it would be useful to have a marker for the presence of MRD in patients after transplant. Such a prognostic biomarker might help guide early intervention with a strategy such as FLT3 inhibition and/or alteration in GVHD prophylaxis in patients who are at high risk for relapse.

Investigators have questioned whether *FLT3* mutational status is an adequate marker for MRD in FLT3/ITD AML patients receiving chemotherapy and/or undergoing allogeneic transplant. For instance, it has been shown in multiple studies that the *FLT3/ITD* mutational status of AML patients can be different at relapse than at diagnosis.[24–26] Not only can FLT3/ITD patients relapse without the ITD mutation, but AML patients without the mutation can relapse with FLT3/ITD AML. This phenomenon is not unique to FLT3/ITD AML and also applies to other mutations occurring in leukemia, including N-ras and p53 mutations.[24] It has been argued that, because of this "instability" of FLT3 mutations, *FLT3/ITD* mutational status is not a valuable indicator of MRD or predictor of relapse.[12]

In spite of the potential for an AML patient's *FLT3/ITD* mutational status to change over time, such alterations are not common. Among patients who are *FLT3/ITD* negative at diagnosis, approximately 10% acquire the mutation at relapse.[12] When relapse occurs in patients harboring the *FLT3/ITD* mutation at diagnosis, the mutation is present in the large majority of cases. In one study, 13 of 16 (81%) relapsing FLT3/ITD AML patients relapsed with the ITD.[12] In another report, 16 of 17 (94%) FLT3/ITD AML patients who relapsed had the ITD mutation at relapse,[26] which is identical to what we found in the present study.

Our findings are similar to those of prior studies using patient-specific PCR for detection of *FLT3/ITD* mutational status.[19–22] A significant advantage of *FLT3* TD-PCR, however, is that it uses standardized primers rather than patient specific primers, so that it can be performed routinely in a clinical laboratory. Our results confirm that post-transplant MRD by *FLT3* mutational status predicts relapse in FLT3/ITD AML patients. Since all seven patients who were negative for MRD before transplant remained negative at day 60 following transplant and did not relapse, a negative pre-transplant MRD assay appears to be quite meaningful.

Of the 21 patients who were negative for MRD at day 60, the two who ultimately relapsed both had informative courses. Patient 24 relapsed with *FLT3* wild type AML. Given that this patient had an ITD allelic burden of less than 5% at diagnosis, it is likely that the *FLT3/ITD* mutation represented a non-dominant sub-clone in this patient. While this patient's case might be interpreted as corroborating the warnings of those who are wary of using *FLT3* mutational status as a test for MRD, the changing of a patient's *FLT3* status appears to interfere with MRD detection in only a small proportion of patients. The "instability" of *FLT3* mutations therefore represents a relatively uncommon limitation of using *FLT3* status to determine MRD. Patient 27, the other patient who was negative for MRD at day 60 but

relapsed, had converted to positive by the 12 month mark and ultimately relapsed with FLT3/ITD AML. This patient's relapse at 28 months was the latest relapse among our patients. Thus, based on a limited sample, it appears that a negative day 60 post-transplant MRD assay suggests that relapse is unlikely. Moreover, the case of Patient 27 suggests that it may be useful to continue monitoring for MRD after day 60, since it is possible that this patient would have benefitted from therapy with a FLT3 inhibitor at the time of conversion from MRD negative to MRD positive.

There are also instructive cases of patients who were positive for the *FLT3/ITD* mutation on day 60. Patient 28 had a positive day 60 MRD assay and remains in a durable remission. This individual's case provides evidence that a graft versus leukemia effect can occur after day 60. Patient 2 also was positive for the *FLT3/ITD* mutation at day 60. While treated with sorafenib, this patient remained in remission until approximately 12 months following transplant and relapsed quickly upon discontinuation of this drug, suggesting that FLT3 inhibition may have a role in maintenance therapy for some patients with FLT3/ITD AML following allogeneic transplant, much in the same way that BCR-ABL inhibition is used in Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ ALL).

Sustained remissions have been reported with the FLT3 inhibitor sorafenib administered as monotherapy following transplant.[27] However, using sorafenib following post-transplant relapse does not always appear to be an effective strategy.[28] Currently, investigators are working to identify patients early who may be at high risk for relapse and likely to benefit from posttransplant FLT3 inhibition. Studies have suggested that sorafenib, in combination with the allogeneic immune effects of transplant, may induce durable remissions in some patients.[27] It is possible that MRD by *FLT3/ITD* mutational status will identify patients who will benefit from post-transplant therapy such as FLT3 inhibition, withdrawal of GVHD prevention, or donor lymphocyte infusion. We are currently examining the use of TD-PCR prospectively in an ongoing clinical trial using post-transplant sorafenib in FLT3/ITD AML patients.

One significant limitation of the TD-PCR technique is the inability of the assay to be informative in FLT3/ITD AML patient with shorter ITDs, which account for approximately 25% of FLT3/ITD AML cases. Moreover, in our study, bone marrow DNA specimens were not available for 17 patients in our cohort of FLT3/ITD AML patients undergoing transplant. Furthermore, the TD-PCR technique does not overcome instability of some patients' *FLT3* mutational status, which remains an important challenge to finding an optimal MRD marker. In spite of these limitations, our findings reveal that *FLT3* mutational status can be useful in detecting MRD. In addition, to the authors' knowledge, this study represents the largest consecutive series of FLT3/ITD AML patients undergoing transplant at a single institution.

In summary, our results support the use of *FLT3/ITD* mutational status by TD-PCR as a marker for MRD. The *FLT3* TD-PCR technique, with defined primer sets, would be relatively simple to adopt as a standard assay in clinical laboratories. Given that the present study has been performed using bone marrow samples, one future direction of this work will be to determine whether the TD-PCR assay is predictive using peripheral blood, which

would render the assay more convenient to perform. Prospective studies will likely be necessary to validate MRD assessed by *FLT3* TD-PCR as a prognostic instrument and as a means of guiding therapy.

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- A new technique, TD-PCR, can detect the FLT3/ITD mutation with high sensitivity.
- Using TD-PCR, *FLT3* mutational status can be used as a marker of MRD.
- TD-PCR predicts relapse in FLT3/ITD AML patients undergoing allogeneic transplant.
- Median relapse-free survival in transplanted FLT3/ITD AML patients was 15.0 months.
- Median overall survival in transplanted FLT3/ITD AML patients was 27.7 months.

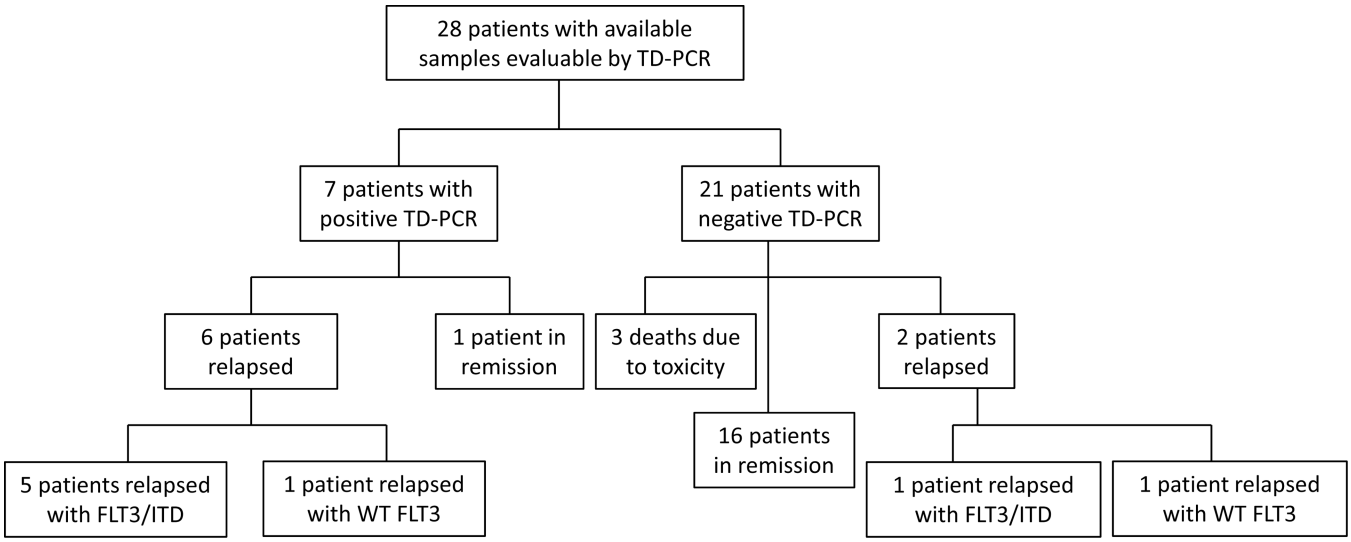
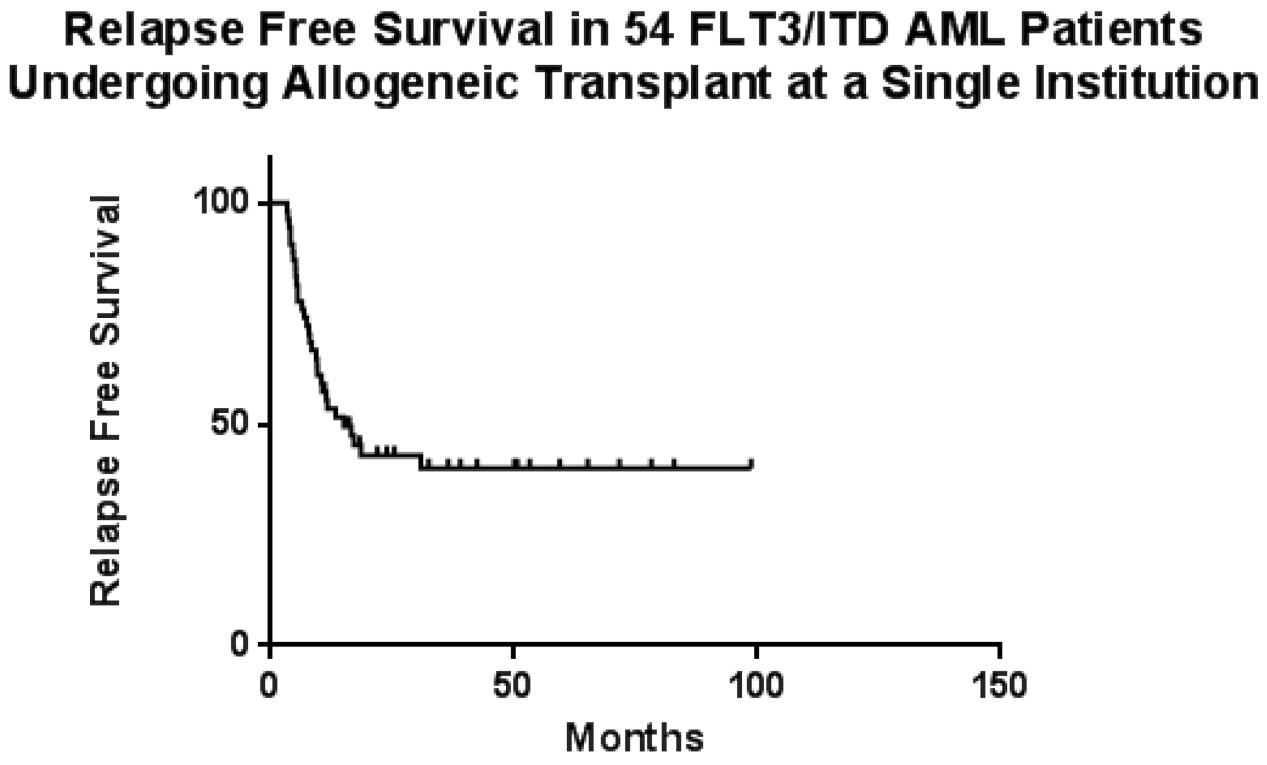
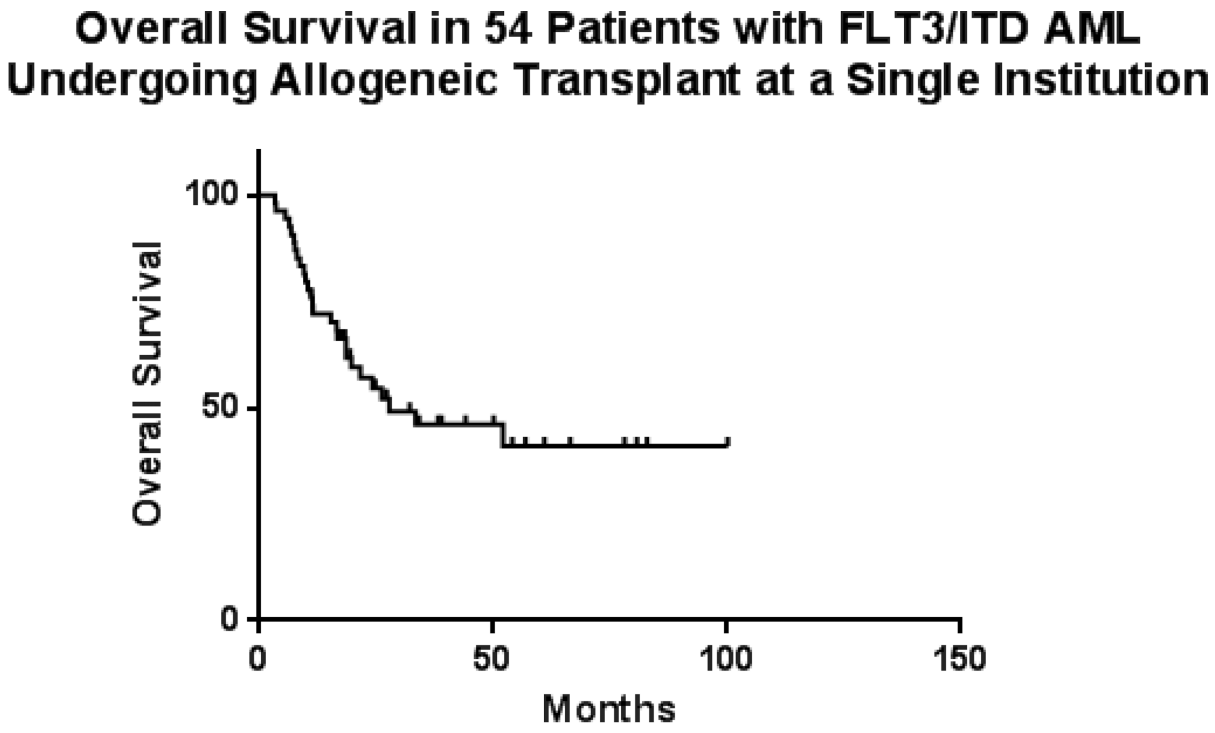


Figure 1.
MRD results and outcomes for 28 evaluable patients.



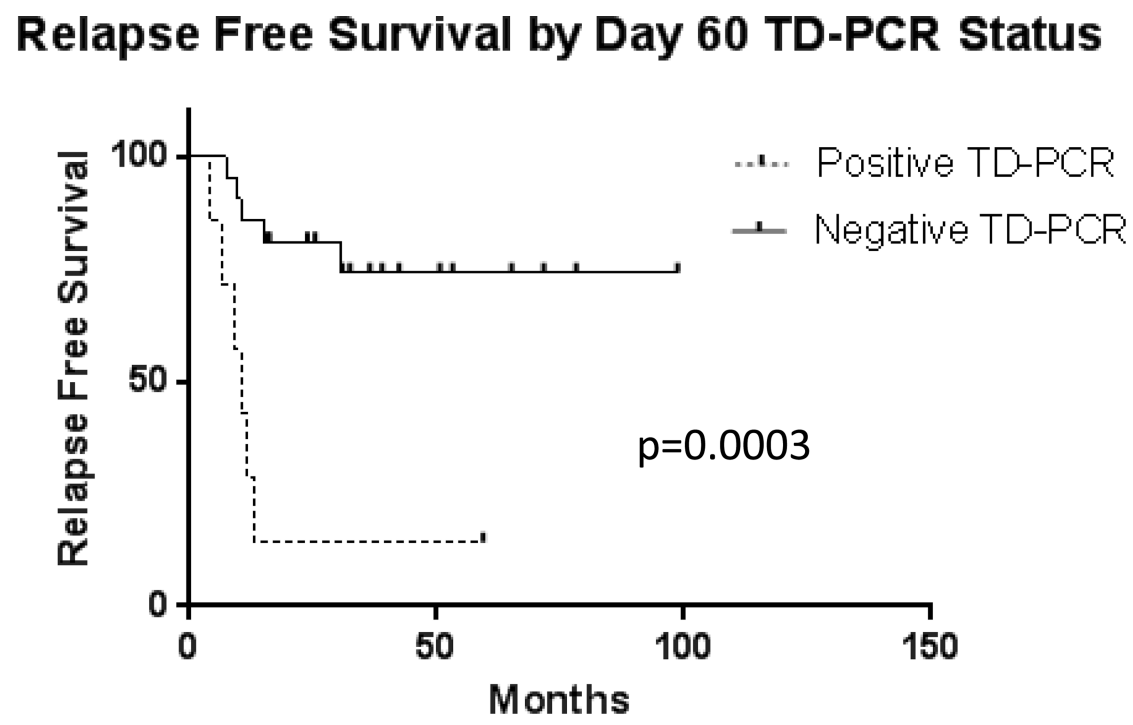
A



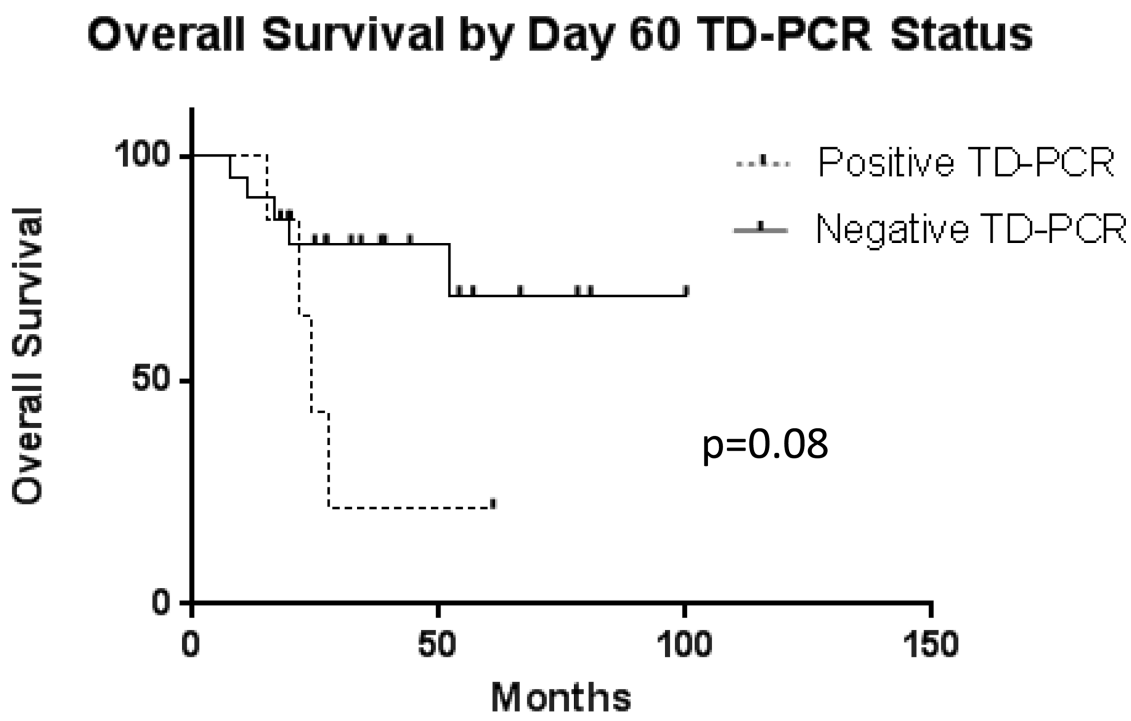
B

Figure 2.

A) Relapse Free Survival in 54 FLT3/ITD AML Patients Undergoing Transplant at a Single Institution. B) Overall Survival in 54 FLT3/ITD AML Patients Undergoing Transplant at a Single Institution.



A



B

Figure 3.

A) Relapse Free Survival by Day 60 TD-PCR Status. B) Overall Survival by Day 60 TD-PCR Status.

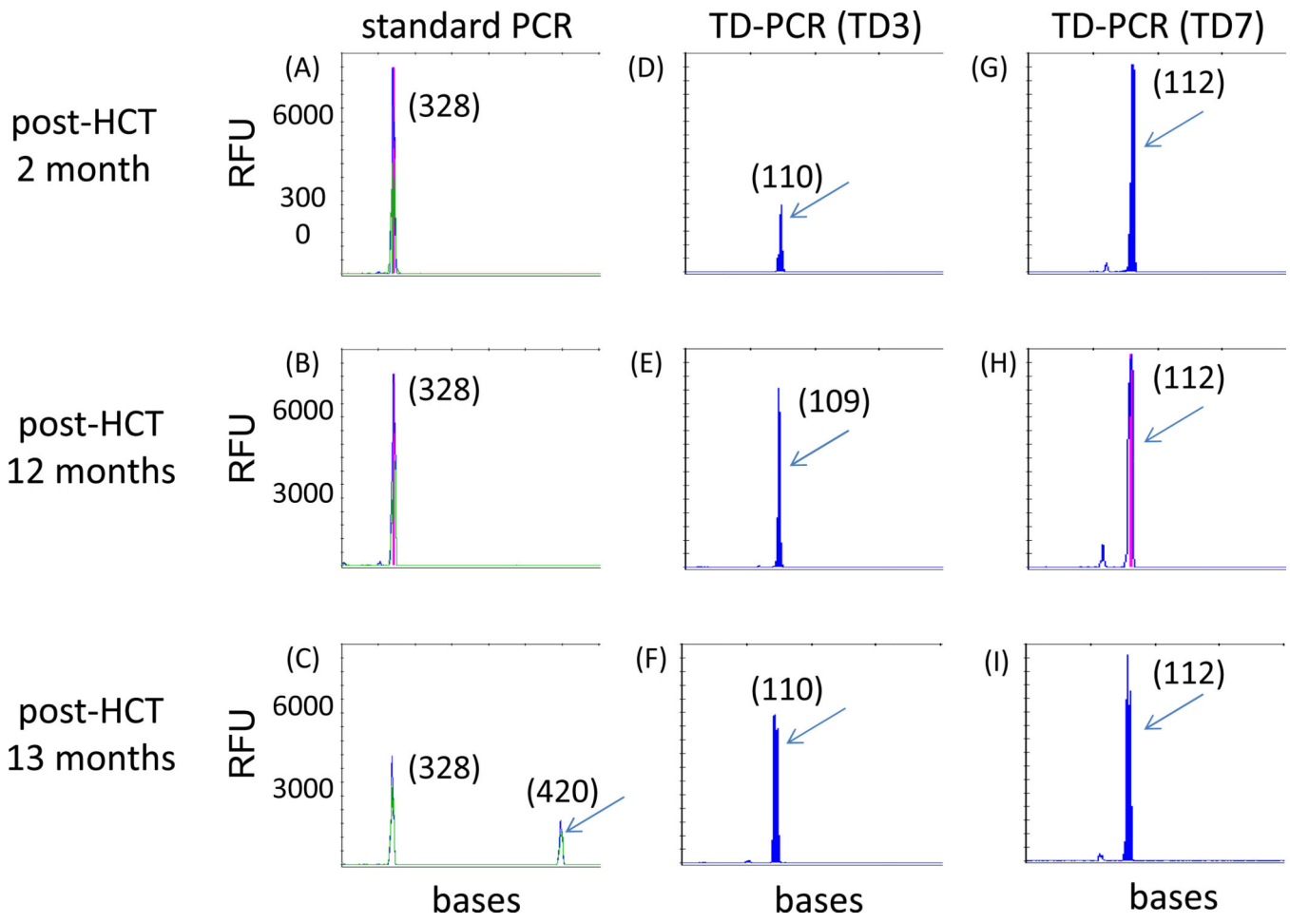


Figure 4. TD-PCR detected MRD at 2 months post-transplant and predicted relapse

In the case of patient 2, standard PCR showed relapse of the original ITD mutant migrating at 420 bases at 13 months post-transplant (C), but not at 2 months (A) or 12 months (B) post-transplant. TD-PCR using primer pairs TD3 and TD7, however, detected the ITD mutant at 2 (D and G), 12 (E and H) and 13 months (F and I) post-transplant. TD-PCR products from the relapse specimen at 13 months were diluted 30 fold before electrophoresis. The red vertical lines indicate off-scale peaks. Arrows indicate amplicons from the same ITD mutant (with sizes in nucleotides provided in parentheses). RFU, relative fluorescence units.

Table 1

Patient Demographics

Number of Patients	54
Mean Age in Years (Standard Deviation)	46 (13)
Sex (Number of Male Patients)	20 (37%)
Relapsed/refractory AML prior to transplant	11 (20%)
Cytogenetics*	
Favorable Cytogenetics	1 (2%)
Intermediate Cytogenetics	49 (91%)
Unfavorable Cytogenetics	4 (7%)
Normal Cytogenetics	36 (67%)
NPM1 Mutational Status	
Positive	17 (31%)
Negative	7 (13%)
Unknown	30 (56%)
Preparatory Regimen	
Myeloablative	44 (81%)
Nonmyeloablative	10 (19%)
Type of Transplant	
Matched Related	19 (35%)
Matched Unrelated	15 (28%)
Mismatched Unrelated	1 (2%)
Haploidentical	19 (35%)

*Cytogenetic risk was determined according to the classification scheme described in Blood 1998; 92:2322–2333.

Table 2

FLT3 TD-PCR Results for 28 Evaluable Patients

Patient Number	Pre-Transplant TD-PCR Result	Post-Transplant TD-PCR Result (Day 60)	Duration of Follow-Up (Days)	Outcome
1	−	−	1185+	Alive, in remission
2	+	+	589+	Alive, but relapsed
3	+	−	225	Death due to toxicity
4	+	−	2376+	Alive, in remission
5	+	−	1732+	Alive, in remission
6	+	−	821+	Alive, in remission
7	−	−	542+	Alive, in remission
8	+	−	751+	Alive, in remission
9	+	+	844	Death due to AML
10	+	−	2024+	Alive, in remission
11	+	+	656	Death due to AML
12	−	−	3048+	Alive, in remission
13	+	−	1645+	Alive, in remission
14	+	−	541+	Alive, in remission
15	+	−	826+	Alive, in remission
16	+	−	979+	Alive, in remission
17	−	−	343	Death due to toxicity
18	+	−	1341+	Alive, in remission
19	−	−	505	Death due to toxicity
20	−	−	2456+	Alive, in remission
21	+	+	735	Death due to AML
22	−	−	1162+	Alive, in remission
23	+	+	602+	Alive, but relapsed
24	+	−	597	Death due to AML
25	+	−	1039+	Alive, in remission
26	+	+	463	Death due to AML
27	no sample	−	1585	Death due to AML
28	+	+	1855+	Alive, in remission