Use of Alefacept for Preconditioning in Multiply Transfused Pediatric Patients with Nonmalignant Diseases

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INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only curative therapy for many pediatric patients with life-threatening nonmalignant diseases (NMDs) [1-14]. Rejection remains an impediment to successful transplantation in many of these disorders, especially in patients with nonmalignant diseases (NMDs).
the setting of alternative donor graft combined with reduced-intensity conditioning [15-18]. Because most of these patients require multiple erythrocyte and/or platelet transfusions as part of their routine medical care before HSCT, transfusion-related alloimmunization to minor and major histocompatibility antigens likely accounts for part of their risk for graft rejection [19-23]. Biologically, memory T cells have been shown to underlie this sensitization to donor antigens and to drive graft rejection [22-24]. Natural killer (NK) cells have also been shown to induce potent transplant rejection in murine models and patients [25-27], especially in the setting of limiting stem cell doses, with the NK cell immunoglobulin-like receptor ligand group correlating with risk of rejection [28,29].

Alefacept (Amevive; Astellas, Inc., Northbrook, IL, USA) is a recombinant leukocyte function antigen-3 (LFA-3)/IgG1 fusion protein that targets CD2, thereby depleting CD2-expressing cells via antibody-dependent cellular cytotoxicity (ADCC) [30-36]. Alefacept is well tolerated and has been previously demonstrated to produce a gradual and dose-dependent reduction in memory T cells (that highly express CD2), which plateaus 5 to 6 weeks after drug administration [36]. The phase III study of alefacept in psoriasis used a mid-range dose of 7.5 mg (.15 mg/kg for a 50-kg adult) weekly for 12 weeks. The mid-range dosing strategy was chosen for psoriasis patients due to concerns regarding the potential infectious risks associated with the more significant CD4 depletion that occurred with higher doses, and this was the dose ultimately approved by the US Food and Drug Administration in 2003. Given the link between memory T cell-mediated alloimmunization and HSCT rejection and the ability of alefacept to selectively deplete these cells, we hypothesized that alefacept administration before the start of conditioning might safely and effectively counter the risk of rejection in multiply transfused patients with NMDs. Based on the dose-dependent reduction in memory T cells demonstrated in the dose-finding study in psoriasis [36], we additionally reasoned that dose escalation beyond the highest dose in the previous trials might maximize the benefit of alefacept preconditioning in a patient population in which post-transplant reconstitution with donor T cells would mitigate any long-term risks of alefacept-induced T cell depletion.

To begin to test these hypotheses, we launched a pilot study of alefacept given at a dose of 0.5 mg/kg/dose weekly for 5 weeks during transplant preconditioning in multiply transfused children receiving reduced-intensity conditioning and unrelated donor transplant for nonmalignant hematologic disorders. Herein we report our results, both clinical and mechanistic, in the first 3 patients we enrolled on this study. Unfortunately, the manufacturer terminated production of alefacept for fiscal reasons during the conduct of this study, thus prompting premature interruption of the trial. Results in the first 3 patients demonstrate that full donor engraftment was achieved in all cases, and flow cytometry demonstrated depletion of both memory T cells and NK cells during alefacept treatment. These results suggest biologic activity of alefacept in pediatric HSCT patients and argue for renewed development of CD2-targeted agents for HSCT patients at high risk of transplant rejection.

**METHODS**

**Study Design and Patients**

This was a single-arm pilot study with an accrual goal of 5 patients. Patients were enrolled at the Aflac Cancer and Blood Disorders Center within Children’s Healthcare of Atlanta (CHOA). The trial was registered at clinicaltrials.gov (NCT01319851) and approved by the Institutional Review Board at CHOA and Emory University. Investigational New Drug exemption was obtained for the administration of alefacept. Eligibility criteria included signed informed consent, age 0 to 21 years, NMD requiring at least 5 transfusions (platelet, erythrocyte, or granulocyte units), adequate organ function, ≤7/8 matched related or unrelated bone marrow donor (HLA-A, -B, -C, and -DR; antigen or allele mismatch was permitted) or ≤5/6 cord blood unit (HLA-A, -B, and -DRB1; allele mismatch permitted except at -DRB1) with a recommended dose of 5 × 10^7 total nucleated cells (TNCs)/kg recipient weight, and an HLA antibody screen negative for disparate HLA molecules (in patients with partially HLA-matched allografts). The study was closed to accrual in 2012 after Astellas discontinued production of alefacept and the drug became unavailable.

**Treatment Protocol**

**Alefacept preconditioning**

All patients received alefacept 0.5 mg/kg/dose i.v., with the first dose split on days −40 and −39 and the remaining doses given on days −33, −26, −19, and −12 (eg, weekly for 5 doses). Alefacept was diluted in sterile water (2 mL total volume) and administered via i.v. push followed by a normal saline flush per the package insert.

**Table 1**

Clinical Characteristics and Outcomes

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)/Sex</th>
<th>Disease</th>
<th>Erythrocyte Transfusions</th>
<th>Platelet Transfusions</th>
<th>Previous alloimmunization (Y/N)</th>
<th>HLA Antibody Screen (+/−)</th>
<th>HLA Matching (Mismatch)</th>
<th>CMV Status (R/D)</th>
<th>Graft Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2/F</td>
<td>Dyskeratosis congenita with bone marrow failure</td>
<td>17</td>
<td>42</td>
<td>N</td>
<td>−</td>
<td>5/6 (+)</td>
<td>−/−</td>
<td>CB</td>
</tr>
<tr>
<td>2</td>
<td>10/M</td>
<td>Fanconi anemia with bone marrow failure</td>
<td>16</td>
<td>12</td>
<td>Y</td>
<td>(+)</td>
<td>5/6 (+)</td>
<td>−/−</td>
<td>CB</td>
</tr>
<tr>
<td>3</td>
<td>9/F</td>
<td>Idiopathic SAA</td>
<td>15</td>
<td>27</td>
<td>N</td>
<td>−</td>
<td>(B antigen)</td>
<td>8/8</td>
<td>BM</td>
</tr>
</tbody>
</table>

CMV indicates cytomegalovirus; CB, cord blood.

* Approximate, before transplantation.

† All regimen-related toxicities were determined to be unlikely attributable to the study drug.

‡ Pulmonary aspergillosis by computed tomography diagnosis only, day ±10. CMV viremia diagnosed day ±27, initially treated with ganciclovir, recurrent viremia (ganciclovir resistant) resolved with foscarnet. Bacterial sinusitis, diagnosed day +41.

§ Panel reactive antibody positive at class I (25%) with specificity for A30 and A31 (not donor directed).

¶ aGVHD of the skin, diagnosed on day +20, maximal grade II.

**Table 2**

Disease Erythrocyte Transfusions Platelet Transfusions Previous alloimmunization (Y/N) HLA Antibody Screen (+/−) HLA Matching (Mismatch) CMV Status (R/D) Graft Source

<table>
<thead>
<tr>
<th>Patient No.</th>
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<td>N</td>
<td>−</td>
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<td>8/8</td>
<td>BM</td>
</tr>
</tbody>
</table>
Patients received fludarabine 25 mg/m² on days −10 to −5 and cyclophosphamide 10 mg/kg on days −5 to −2. All other patients received fludarabine 25 mg/m² on days −6 to −1, cyclophosphamide 50 mg/kg on day −2, and 200 cGy total body irradiation on day −1. All patients received cyclosporine and mycophenolate mofetil for graft-versus-host disease (GVHD) prophylaxis beginning on day −2. Cyclosporine was adjusted to maintain an infusional or trough level of 200 to 300 ng/mL and was continued through at least day +180. Mycophenolate mofetil was administered at 15 mg/kg/dose (p.o. or i.v.) 3 times daily through at least day +45.

Clinical Endpoints

The primary objective of this study was to determine the feasibility of administering the 6 doses of alefacept to multiply transfused pediatric patients with NMD undergoing HSCT. In addition, infusional reactions, grade 2 or greater regimen-related toxicities (RRT; through day +42, according to Bearman criteria), graft failure (primary and secondary), graft loss, acute and chronic GVHD (using National Institutes of Health consensus criteria), and unexpected serious adverse events were captured.

Immune Monitoring

Longitudinal flow cytometric analyses included the enumeration of NK cells (CD3−CD20−CD16−CD56+), and CD20+CD13+CD4+CD8− and CD20+CD13+CD4−CD8+ T cells (excluding naive [CD7+CD45RA−], central memory [Tcm; CD27+CD45RA−], effector memory [Tem; CD27−CD45RA−], and terminal effector memory subsets [Temra; CD27−/CD45RA+]). T cell subtypes were divided into CD28+ (alefacept target cells) and CD28−. The antibody clones used for the flow cytometric analysis are as follows: CD3 (UCHT1; Becton Dickinson, Franklin Lakes, NJ), CD20 (H147; Invitrogen, Grand Island, NY), CD16 (3G8; Becton Dickinson), CD56 (B159; Becton Dickinson), CD8 (RPA-T8; eBioscience, San Diego, CA), CD4 (RPA-T4; Becton Dickinson), CD2 (RPA-2.10; Becton Dickinson), CD45RA (MEM-56; Invitrogen), and CCR7 (3D12; Becton Dickinson). Testing was performed on samples from days −40, −26, −6, and 0 using a minimum of 100,000 events. TruCount beads (Becton Dickinson) were used to generate cell counts.

Retrospective Control Subjects

To provide clinical and laboratory data for comparison, we obtained data on 23 patients who did not participate in this trial and received unrelated (marrow or cord blood) HSCT at CHOA for marrow failure (acquired or inherited) after 2000. This was approved by the CHOA Institutional Review Board.

Statistical Analysis

Statistical analyses were performed using Prism version 5 for Mac OS X (GraphPad Software, La Jolla, CA). Paired t-tests were used to calculate P values comparing baseline immunologic values with those after all doses of alefacept were given and before transplant conditioning.

RESULTS

Patient Characteristics and Clinical Outcomes

Three patients were enrolled. Patient 1 was a 2-year-old girl with dyskeratosis congenita (DKC) and marrow failure requiring 17 erythrocyte and 42 platelet transfusions before undergoing 5/6 unrelated cord blood transplant with a cell dose of 11.9 × 10^7 TNCs/kg (Table 1). She tolerated her alefacept infusions without complication. Post-HSCT she developed grade 2 RRT consisting of stomatitis. Peripheral blood donor chimerism (CD3 and CD33) was 100% on day +30 post-transplant and on all subsequent analyses (Table 1; last analysis at 2 years post-transplant). On day +27 she was diagnosed with acute GVHD (aGVHD) of the skin and gastrointestinal tract (maximal grade III) that responded to systemic steroid treatment. She developed severe chronic GVHD (cGVHD) with involvement of the gastrointestinal tract and heart (recurrent pericardial effusions, possibly serositis), which was treated with steroids and drainage due to cardiac tamponade. Both of these cGVHD sequelae ultimately resolved. She had several infections post-HSCT, including pulmonary aspergillosis, recurrent cytomegalovirus viremia, and bacterial sinusitis, all of which responded to treatment. She is currently off immune suppression and without GVHD approximately 3.5 years post-transplant. She has developed chronic restrictive pulmonary disease, requiring nasal cannula oxygen, and hepatic fibrosis, both attributed to DKC.

Patient 2 was a 10-year-old boy with FA and marrow failure requiring 16 erythrocyte and 12 platelet transfusions before undergoing 5/6 unrelated cord blood transplant with cell dose of 4.6 × 10^7 TNCs/kg (Table 1). His panel reactive antibody screen was positive at class I (25%) before HSCT with specificity for A30 and A31, neither of which were donor-directed. He tolerated his alefacept infusions without complication. Post-HSCT he developed grade 2 RRT (stomatitis). Sorted (CD3 and CD33) peripheral blood chimerism studies demonstrated 100% donor chimerism on day +30 post-transplant and on all subsequent analyses (Table 1; last analysis at 13 months post-transplant). He developed aGVHD of the skin on day +20 that was maximally grade II and that responded to systemic steroids. The patient developed moderate cGVHD with involvement of...
the skin, eyes, and mouth, which responded to topical therapies. He had 2 episodes of bacteremia post-HSCT, including with Streptococcus viridans on day +1 and Staphylococcus epidermidis on day +32, both of which resolved with i.v. antibiotics. He is currently approximately 2.5 years from transplant and off immune suppression without signs of GVHD.

Patient 3 was a 9-year-old girl with idiopathic severe aplastic anemia (SAA) requiring 15 erythrocyte and 27 platelet transfusions before undergoing 8/8 matched unrelated bone marrow transplant with cell dose of $4.6 \times 10^8$ TNCs/kg (Table 1). She tolerated her alefacept infusions without complication. There was no RRT. She had 85% CD3 and 100% CD33 donor chimerism on day +1 and 60% CD3 and 100% donor at that time. The patient developed aGVHD of the skin on day +22 that was maximally grade II and that resolved with systemic steroids. She did not develop cGVHD or have any infections post-HSCT. She is currently approximately 2.5 years post-transplant and without symptoms of GVHD off immune suppression.

The retrospective control group comprised 23 patients who received unrelated marrow or cord blood (single or double) transplants for a variety of diseases, including SAA, FA, DKC, Diamond-Blackfan anemia, and Shwachman-Diamond syndrome. Four (17.4%) patients suffered rejection.

Post-Transplant Leukocyte Reconstitution

As shown in Figure 1, the leukocyte reconstitution (WBC, absolute neutrophil count [ANC], absolute monocyte count [AMC], and absolute lymphocyte count [ALC]) of the 3 patients was similar to that of the retrospective control subjects. Neutrophil engraftment (first-day ANC > 500 cells/µL for 3 consecutive days) occurred on days +13, +17, and +21 in the 3 trial patients and at a median of 21 days (±1.2 days) in the control subjects. An AMC > 200 cells/µL was achieved in the trial patients on days +12, +16, and +15 and at a median of +18.8 days (±1.0 days) in the control subjects. An ALC > 1000 cells/µL was achieved in the trial patients by days +17, +60, and +435; in historical control subjects, ALC > 1000 cells/µL was achieved by 20%, 47%, 80%, and 100% by days +60, +100, and +180 and 1 year, respectively.

Pharmacodynamic Monitoring of the Pretransplant Impact of Alefacept on T Cells and NK Cells

Flow cytometry was performed on peripheral blood samples during the alefacept treatment preconditioning phase as follows: baseline (day −40, before the first dose of alefacept), during alefacept (day −26, before the third dose of alefacept), and after the final dose of alefacept but before the start of conditioning (on day −6 or −10 depending on the conditioning regimen used) and before transplant (day 0). Hematologic and immune monitoring included the enumeration of WBC, ANC, AMC, NK cell count, total T and B lymphocyte counts, and the proportion and absolute number of CD4 and CD8 T cell subsets.

Patients 1 and 3 demonstrated no statistically significant change in the WBC, ANC, ALC, AMC, T lymphocyte, and B lymphocyte counts or their relative proportions during the alefacept treatment (Figure 1, P > .1 for all analyses when data combined). Patient 2 did demonstrate a decline in the number of WBC, granulocytes, and T and B lymphocytes with stable monocyte counts during this time period. Of note, although this patient had testing for FA at initial diagnosis that was inconclusive (and therefore received SAA-type therapy for bone marrow failure before HSCT), repeat testing before transplant confirmed a diagnosis of FA. During treatment for aplastic anemia, patient 2 had received antithymocyte globulin (horse and rabbit) and cyclosporine, which were given 6 to 12 months before receiving alefacept. In addition, he was receiving granulocyte-colony stimulating factor and low-dose prednisone (<.2 mg/kg/day) daily during alefacept preconditioning.

To determine the impact of alefacept preconditioning on CD4+ and CD8+ cells, the CD4+ and CD8+ T naive, Tem, Tcm, and Temra were divided into CD2hi (putative alefacept target

Figure 1. Impact of alefacept preconditioning on post-transplant hematologic reconstitution. Hematologic reconstitution was monitored in all 3 patients receiving alefacept treatment and compared with a cohort of 23 historical control subjects. Black circles indicate patient 1; blue squares, patient 2; red triangles, patient 3; black diamonds (with broken line), historical control subjects (mean ± SEM). (This figure is available in color online at www.bbmt.org).
Figure 2. Impact of alefacept preconditioning on CD4⁺ and CD8⁺ naive and memory T cell subsets and on NK cells. Longitudinal immune monitoring was performed on peripheral blood samples using flow cytometry. Samples were drawn at baseline (day -40, before the first dose of alefacept), during alefacept (day -26, before the third dose of alefacept), after the final dose of alefacept but before the start of conditioning (on day 0 or 10 depending on the conditioning regimen used), and before transplant (day 0). CD20⁻CD3⁺CD4⁺ and CD20⁻CD3⁺CD4⁻CD8⁻ T cells were divided into naive (CCR7⁺CD45RA⁺), Tcm (CCR7⁺CD45RA⁻), Tem (CCR7⁻CD45RA⁻), and Temra (CCR7⁻CD45RA⁺). T cell subsets were further divided into CD2hi (alefacept target cells) and CD2lo. NK cells were classified as CD3⁻CD20⁻CD16⁻CD56hi/lo. Black circles indicate patient 1; blue squares, patient 2; red triangles, patient 3. Solid lines denote CD2hi cells and dashed lines denote CD2lo. *P < 0.05 comparing baseline with post-alefacept values on combined data. (A) Top panels show comparison of the percent of CD4⁺ T naive, Tcm, Tem, and Temra that were CD2hi for each patient at baseline and after all alefacept doses were completed. Bottom panels show comparison of absolute numbers (cells/μL) of CD4⁺ T naive, Tcm, Tem, and Temra that were CD2hi for each patient at baseline and after all alefacept doses were completed. (B) Top panels show comparison of the percent of CD8⁺ T naive, Tcm, Tem, and Temra that were CD2hi for each patient at baseline and after all alefacept doses were completed. Bottom panels show comparison of absolute numbers (cells/μL) of CD8⁺ T naive, Tcm, Tem, and Temra that were CD2hi for each patient at baseline and after all alefacept doses were completed. (C) Top panels show comparison of the percentage of total lymphocytes attributed to NK cells or NK cell subpopulations (cells/μL) for each patient at baseline and after all alefacept doses were completed. Bottom panels show comparison of the absolute number of NK cells or NK cell subpopulations (cells/μL) for each patient at baseline and after all alefacept doses were completed. (This figure is available in color online at www.bbmt.org).
T cell subsets, predominantly in CD8
[15-17,19-23,38]. As shown in Figure 2, all 3 patients had a decrease in the frequency of CD2\hi CD4\- Tem and Temra and of CD2\hi CD8\- Tcm and Tem (which, even for this small number of patients, reached statistical significance, P < .05 for all combined analyses). The frequency of the more rare CD2\hi CD4\- Tem and CD8\- Temra populations declined in all 3 patients, although this was not statistically significant (P = .09 and .08, respectively, on combined data). Although the absolute number of the CD2\hi cells also declined during alefacept treatment, this did not reach statistical significance.

Given their ubiquitous expression of CD2, NK cells, including both CD56\hi and CD56\lo subsets, were examined during preconditioning with alefacept. The frequency and number of all NK cells decreased during alefacept treatment in all 3 patients (Figure 2C), with the largest decline observed in patient 1. The absolute number of the CD56\hi NK subset likewise decreased in all 3 patients, and the frequency of these cells decreased in patients 1 and 3.

DISCUSSION

This is the first study to report the use of alefacept, a recombinant LFA-3/IgG1 fusion protein targeting CD2, as a component of the conditioning regimen in patients undergoing HSCT. We report the clinical outcomes and immune effects of alefacept preconditioning in our first 3 patients before the drug was removed from production by the drug company in 2011. Alefacept was well tolerated in this study, with no infusional reactions or drug-related toxicity. Although all 3 patients were at heightened risk for graft rejection by virtue of their NMDs, their extensive transfusion history, the use of mismatched donors (2 being 5/6 cord blood units), and the use of a reduced-intensity conditioning regimen, all 3 achieved sustained, full-donor engraftment [15-17,19-23,38].

In this series we found that alefacept decreased memory T cell subsets, predominantly in CD8\- T cells, had no effect on naive T cells, and led to a decrease in NK cells. All 3 patients had a notable decline in the percentage of CD2\hi CD8\- memory cell subsets, which was statistically significant for Tcm and Tem. This corresponded to an individual decline in the number of CD2\hi CD8\- Tem and Temra cells in all 3 patients. Consistent with the expression of CD2 on NK cells, we also observed a decrease in both the absolute numbers and proportions of these cells in all 3 patients.

Animal models indicate that in the setting of MHC-matched transplantation, rejection induced by transfusion-related alloimmunization is mediated primarily by T cells [39]. Given the challenges to demonstrating donor-directed cellular immunity clinically, we did not attempt to assess it. Therefore, we cannot ascertain the extent to which pretransplant transfusions may have posed a barrier to engraftment in our 3 patients. Although a much larger study would be needed to accurately gauge alefacept’s efficacy, that all 3 patients achieved full donor engraftment nevertheless raises the possibility that it might promote engraftment, especially because the incidence of rejection was 17.4% in our control group. Also, even higher incidences have been observed in registry studies of unrelated HSCT for FA, DKC, and other forms of marrow failure [10,17,40].

The immune effects of alefacept have been described primarily in psoriasis and after solid organ transplant [30,36,41-43], although its use has recently been reported in pediatric patients with newly diagnosed type 1 diabetes mellitus [44]. In these populations, alefacept was found to predominantly decrease CD4\+ and CD8\+ memory T cells with only a modest depletion of NK cells [41,42]. Although NK cells are uniformly CD2 high [45-47] and therefore should be targeted by alefacept, previous studies have not demonstrated any significant effect [41,42]. However, unlike in psoriasis, solid organ transplant, and type 1 diabetes, our study used a higher dose of alefacept and shorter total course. Although our data do not specifically address this, it is possible that this higher alefacept dose was able to more efficiently target NK cells than the lower doses used in previous studies. This represents an important area for future investigation.

Of note, the degree of memory T cell depletion was also lower in this study compared with previous results in psoriasis patients, where alefacept led to a steep decline in CD4\+ and CD8\+ memory T cell counts (CD45RO\+ [30,36]. The decrease in memory T cells continued through the 12 weeks of therapy in the psoriasis patients; however, most of the decline occurred in the first 6 weeks of therapy with counts dropping by greater than 50% [30,36]. Given that depletion of memory T cells by alefacept has been shown to rely on ADCC, which is prominently mediated by NK cells [46], it is possible that the current regimen favored depletion of NK cells. Also, with fewer of these cells available, ADCC of memory T cell populations was less effective. Although depletion of NK cells may have beneficial effects on engraftment, it is important to note that NK cells have also been shown to be integral to tumor and viral immunity post-HSCT [48] and that there have been selected reports of malignancy in patients receiving long-term alefacept for autoimmune indications [43,49].

Two limitations of our study beyond its small size are that we did not perform pharmacokinetic testing and did not rigorously assess immune reconstitution. Together, these limitations make it difficult to determine to what extent if any that alefacept may have hindered immune reconstitution. Further studies with this drug, or other agents targeting CD2, would benefit from a detailed analysis of pharmacokinetics, a determination of the impact of pretransplant treatment on post-transplant reconstitution of NK and T cells subsets, and functional immune studies (including response to vaccines) in treated patients.

In addition to alefacept, other agents, such as antibodies directed against LFA-1 (efalizumab [50,51]) or CD2 (siplizumab) [51-54], have been studied with the goal of depleting memory T cells. However, unlike alefacept, these drugs do not selectively deplete memory T cells and may have more serious off-target effects on protective immunity to new pathogens [55]. Unfortunately, in 2011 production of alefacept was halted based on fiscal, not clinical, factors. This decision has had a significant negative impact on multiple fields in addition to HSCT, including systemic autoimmune disease, solid organ transplant, and type 1 diabetes [42-44,56]. The results of the current case series add to the strong rationale for reviving alefacept or similar agents, which deserve expanded analysis for their utility in multiple immune-mediated diseases. This case series also suggests that further studies using higher dosing schedules of alefacept may impact NK cells in addition to memory T cells and that an escalating dose of alefacept may alter the cell types targeted to favor either memory T cells or NK cells. This may make a significant clinical impact on patients undergoing HSCT for NMDs who are at high risk for rejection by both innate and adaptive immune mechanisms.
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REFERENCES

Sensitive Replicate Real-Time Quantitative PCR of BCR-ABL Shows Deep Molecular Responses in Long-Term Post–Allogeneic Stem Cell Transplantation Chronic Myeloid Leukemia Patients

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ABSTRACT

Real-time quantitative PCR (RT-qPCR) is commonly used for follow-up of chronic myeloid leukemia (CML) patients treated with tyrosine kinase inhibitors, but its current sensitivity does not allow detection of very low BCR-ABL levels. Therefore RT-qPCR negativity is not synonymous with complete molecular response. Replicate RT-qPCR had shown increased sensitivity in tyrosine kinase inhibitor–treated patients and was, therefore, used here to evaluate whether RT-qPCR–negative post–allogeneic stem cell transplantation (SCT) patients harbor detectable disease. Samples from 12 patients were tested at 2 time points using 82 replicates of BCR-ABL RT-qPCR. One patient (38 months after SCT) had detectable transcripts at baseline and none at the follow-up test, done at a median of 107 months after SCT. This suggests cure from CML in the majority of allogeneic SCT patients who have no transcripts detectable by replicate RT-qPCR for BCR-ABL.

INTRODUCTION

Treatment with ABL tyrosine kinase inhibitors (TKI) has become the cornerstone of chronic myeloid leukemia (CML) therapy in recent years. Chronic phase CML patients achieving deep molecular responses (MR) on long-term TKI therapy may be thought of as having a “functional” cure and withholding TKI treatment in patients with continuous deep responses is currently under intensive investigation [1,2].

Nevertheless, allogeneic (Allo) stem cell transplantation (SCT) is still the only modality with proven efficacy in advanced phase cases and is a valid option in TKI-resistant cases. Absence of BCR-ABL transcripts after SCT with sensitive methods (ie, achieving PCR negativity) correlates with long-term disease-free survival, whereas re-emergence of BCR-ABL transcripts has been shown to predict disease relapse and is, therefore, used to trigger therapeutic interventions, such as the addition of a TKI or donor lymphocyte infusion [3–6]. With the widespread use of PCR to detect BCR-ABL transcripts, it has become clear that achieving PCR negativity is not synonymous with molecular complete response but rather depends on the sensitivity of the method used and technical variables, such as the RNA quality in each specific sample [7]. Real-time quantitative PCR (RT-qPCR) is the most commonly used method for follow-up of CML