Feasibility of conditioning with thymoglobulin and reduced intensity TBI to reduce acute GVHD in recipients of allogeneic SCT

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Murine studies using anti-T-cell antibodies for conditioning in allogeneic SCT demonstrate engraftment with low rates of GVHD. On the basis of this preclinical model, we conditioned 30 patients with advanced hematologic malignancies with rabbit antithymocyte globulin (ATG) and TBI, to reduce rates of fatal acute GVHD. Patients were enrolled in two sequential groups: cohort 1 received ATG 10 mg/kg in divided doses (days −4 to −1) + 200 cGy TBI (n = 16), and cohort 2 received ATG (days −10 to −7) + 450 cGy TBI (n = 14). Median donor blood chimerism for the combined group was 94, 93 and 93% in the first, second and third months after transplant. Only three developed grade II acute GVHD despite 43% of patients receiving unrelated donor transplants. One-year survival was 71 ± 11 and 54 ± 14%, respectively, in recipients of related and unrelated donor SCT. Donor lymphocyte infusions were needed in 12 patients for the management of relapse and for mixed donor–recipient chimerism in 4 patients. We conclude that 10 mg/kg ATG and TBI allows engraftment with a low risk of acute GVHD; however, further dose optimization of ATG is required to achieve a balance between GVHD and disease relapse.

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Introduction

Non-myeloablative conditioning regimens for allogeneic SCT have made this treatment modality available to a large number of patients not eligible for transplantation using conventional conditioning regimens. However, despite a reduction in early regimen-related toxicities and day 100 mortality with non-myeloablative regimens when compared to conventional myeloablative regimens the cumulative treatment-related mortality remains similar in the two groups.1 This has been linked to delayed onset GVHD seen following non-myeloablative allogeneic SCT, especially in recipients of transplants from unrelated donors.1–3 Additionally, even though there is a well-recognized association between GVHD and protection from relapse, 4–6 severe acute GVHD does not confer protection from relapse of leukemia4,6 and is more apt to be associated with poor survival. 7,8 Compounding the problem, those undergoing reduced intensity SCT are often elderly or have comorbidities, which puts them at significant risk for mortality in the event of developing severe GVHD. In addition, their related donor pool is limited, resulting in greater reliance on unrelated donors and increasing the risk of GVHD. Thus, severe acute and chronic GVHD remain as major barriers to the successful application of allogeneic SCT in the elderly.

A number of strategies are being studied to reduce the risk of fatal GVHD, including conventional approaches such as prolonged post-graft immunosuppression and more novel investigational strategies such as expansion and infusion of regulatory T-cells post transplant. Another potential avenue of investigation is to alter the conditioning regimen to modulate the immune function of the graft. The rationale for such an approach was established in a series of murine studies, in which unmanipulated MHC disparate BMT facilitated by low-dose thymic and whole body irradiation, combined with various regimens of CD4 and CD8 monoclonal antibodies and post-graft immunosuppression, resulted in engraftment with durable mixed lymphohematopoietic chimerism, but without the development of GVHD.9–12 This donor–recipient tolerance was largely achieved by the depletion of T cells in both the host as well as in the marrow inoculum by the monoclonal T-cell antibodies used in the preparative regimen.
In clinical SCT, antithymocyte globulin (ATG) has been used extensively in conventional myeloablative and non-
myeloablative conditioning regimens to facilitate both engraftment and GVHD prophylaxis. This effect is largely mediated by in vivo T-cell depletion produced by the ATG, similar to the effect of monoclonal T-cell antibodies in the murine model, and provides the rationale for combining it as a single agent with low-dose TBI for non-
myeloablative allogeneic SCT conditioning designed to reduce the incidence of fatal acute GVHD. We conducted sequential clinical trials evaluating the combination of rabbit ATG with reduced intensity TBI. Because of thymic involution seen in the elderly, we excluded thymic irradiation as delivered in the murine model. Likewise fludarabine, a drug commonly used in reduced intensity conditioning regimens, was excluded because of its additional myelo-
suppressive and immunosuppressive effects. We report the results of patients transplanted following conditioning with ATG given in combination with low-dose TBI.

Materials and methods

Patients and eligibility

Consecutive patients were enrolled on two Loyola University Medical Center institutional review board-approved prospective clinical trials. To be eligible, patients had to be between 18 and 70 years of age, have either a hematological malignancy or cytokine refractory renal cell carcinoma, and have adequate end organ function, status performance and either an HLA-matched related donor (MRD) or unrelated donor (URD).

ATG + TBI conditioning regimens

The patients enrolled in the first cohort (cohort 1) received rabbit-ATG (Thymoglobulin; Genzyme, Cambridge, MA, USA) at a dose of 2.5 mg/kg/day given intravenously on days –4 through –1, followed by 200 cGy TBI on day 0. PBSCs or unmanipulated BM were infused on day 0. GVHD prophylaxis was with tacrolimus (0.03 mg/kg twice daily) given orally starting on day –4 maintaining levels of approximately 10–15 μg/l, with taper beginning approximately 8 weeks after transplant based on whole blood chimerism results. MTX was given i.v. at a dose of 5 mg/m² on days 1, 3 and 6. Owing to secondary graft loss observed in 3 out of 16 patients in cohort 1, this regimen was modified, with patients in the second cohort (cohort 2) receiving the same dose of rabbit ATG, and administered earlier than in the previous cohort, from days –10 through –7, with a higher TBI dose, 450 cGy given in three fractions of 150 cGy each, on days –1 and 0. Tacrolimus was given orally from days –2 to 90 with taper commencing on day 90 based on whole blood chimerism results. Mycophenolate mofetil (MMF) was given orally at a dose of 15 mg/kg twice daily from days 0 to 28. G-CSF was given at a dose of 5 μg/kg/day from day 5 until myeloid engraftment. PBSCs were collected from all MRD using G-CSF 10 μg/kg/day given s.c. on days 1 through 5, and BM was collected from URD whenever possible.

Chimerism and lymphocyte reconstitution

Donor engraftment was measured using chimerism anal-

yses performed at approximately 4, 8 and 12, weeks following transplant on whole blood, granulocytes and T

cells. Subsequent chimerism measurements were performed at the physician’s discretion. For lineage-specific chimer-

ism, peripheral blood cell separations were accomplished using immunomagnetic beads (Dynal Inc., Carlsbad, CA, USA) enriching for CD15 and CD3 expressing cells. DNA was isolated using Qiagen columns (Qiagen Inc., Valencia, CA, USA); the ABI Profiler and the ABI Identifiler kits (Applied Biosystems, Foster City, CA, USA) were used to determine the short tandem repeat alleles of the donor and patient. Results were expressed as the proportion of donor derived DNA. Immunophenotypic analysis of the blood for lymphocyte subset reconstitution was performed using a single-platform technique on a Coulter Epics XL flow cytometer (Beckman Coulter Inc., Miami, FL, USA). A three-color approach was employed using antibodies to CD3, CD16/56 and CD45 (Beckman Coulter Inc., Miami, FL, USA). CD16/56 expression on peripheral blood cells at the transplant physician’s discretion. Overall survival was measured from the day following transplant. Acute GVHD was scored according to the Glucksberg criteria. Chronic GVHD with isolated skin involvement or liver enzyme elevation not requiring therapy was classified as limited and other forms as extensive. Patients receiving DLI were censored for chronic GVHD observations. Disease-specific criteria were used for diagnosing relapse or progression.

The initial study (cohort 1) was stopped early because of secondary graft loss observed in 3 out of 16 patients (19%). We hypothesized that this was attributable to excessive T-cell depletion of the graft by the ATG and inadequate host immunosuppression by this regimen. To address these concerns, the conditioning regimen was modified for cohort 2. The reported estimate of the elimination half-life of active ATG (capable of binding lymphocytes) is approximately 7–10 days, and therefore the ATG infusion was completed 1 week before stem cell infusion to attenuate the degree of donor T-cell depletion achieved by this regimen. TBI dose was also increased to 4.5 Gy, based on canine transplant studies, in which 4.5 Gy TBI followed by CsA resulted in allo-engraftment and 4.0 Gy TBI followed by canine-G-CSF allowed hematopoietic recovery.
without stem cell rescue. MMF replaced MTX for post transplant immunosuppression because of marginally superior engraftment reported in the preclinical canine model when MMF was given with CsA instead of MTX (one out of five rejecting with CsA + MMF as compared with three out of six with CsA + MTX). Engraftment failure rate of ≥10% and GVHD rate of ≥30% were defined as early stopping criteria for maintaining patient safety in this cohort. Backup autologous stem cell collection was mandated for the first 10 patients. This phase of the study has been completed and we report the experience with this clinical trial.

Repeated measures analysis of variance was used to evaluate within-group and between-group differences in engraftment between cohorts. All tests were based on determining statistical significance at an α-level of 0.05. Survival analysis was performed using the Kaplan–Meier method. All statistical analyses were performed using SPSS for Windows (SPSS 12.0.2, SPSS Inc., Chicago, IL, USA).

Results

Patients

Sixteen patients were enrolled in cohort 1 (recipients of ATG on days −4 to −1 and 2 Gy TBI), and 14 patients have been enrolled in cohort 2 (ATG on days −10 to −7 and 4.5 Gy TBI). Patient characteristics are given in Tables 1 and 2. This was a poor risk group of patients, including 12 patients with active disease at the time of transplant; these included chemotherapy-related myelodysplastic syndrome (MDS) (three) (two with complex karyotype), post-autologous transplant persistent multiple myeloma (two), relapsed CLL (two), refractory follicular lymphoma (one) (Jehovah’s Witness), myelodysplastic syndrome evolving into AML (one), AML with persistent monosomy 7 following reinduction chemotherapy (one), cytokine refractory renal cell carcinoma (one) and refractory Ph-negative chronic myeloproliferative disorder (one) (Table 2).

PBSCs from MRD were used in 17 patients, and 13 received allografts from URD (PBSC n=4, BM n=9). Donors for MRD transplants were significantly older than those for the URD transplants with a median donor age of 58 years (range 27–77) as compared with 35 years (24–57) (unpaired Student’s t-test P = 0.0001). URD transplant recipients received a significantly lower mononuclear cell (MNC) dose when compared with MRD (median 2.53 × 10^6 MNC per kg vs 7.3 × 10^6 MNC per kg; unpaired Student’s t-test P = 0.0001). Three patients received stem cells from HLA-mismatched URD; two were mismatched at HLA-C and one at HLA-DRB1.

Engraftment and chimerism

In the patients receiving 450 cGy TBI (cohort 2), the median time to neutrophil engraftment (absolute neutrophil count 0.5 × 10^9/L) was 11 days (range 0–18 days), with two patients not developing neutropenia following conditioning. Predominantly donor-derived hematopoiesis was promptly established in both cohorts following transplant-ation. The median whole blood hematopoietic chimerism in the two cohorts was 94, 93 and 93% donor-derived on weeks 4, 8 and 12, respectively. Despite receiving a lower MNC dose and marrow in most cases, recipients of URD allografts were 96% donor chimeric at 4 weeks, 95% at 8 weeks and 94% at 12 weeks, displaying durable engraftment when compared with recipients of MRD, especially in cohort 1 where persistent mixed chimerism was often seen in the third month following transplant as immunosuppression was tapered (Figure 1). Additionally, in the combined cohorts, whole blood donor chimerism at 8-week post transplant appears to be predictive of treatment failure at 1-year post transplant (cumulative incidence of relapse, graft loss or death) with 8 out of 10 evaluable patients with <90% donor chimerism developing treatment failure vs 7 out of 18 evaluable patients with ≥90% donor chimerism (Fisher’s exact test P = 0.043).

Lineage-specific chimerism was measured at 1, 2 and 3 months following transplant in the two cohorts. Significant differences were observed in T-cell engraftment between the two cohorts: 78 ± 22, 77 ± 22, 67 ± 26% mean donor-derived T cells in fourteen 2 Gy recipients vs 94 ± 7, 94 ± 6, 96 ± 5% in ten 4.5 Gy TBI recipients; RMANOVA P = 0.004 (Figure 2a). Lymphoid recovery was more robust in 4.5 Gy recipients (mean absolute lymphocyte

Table 1 Patient characteristics

|                | 2 Gy TBI | 4.5 Gy TBI |
|----------------|----------|------------|
| n              | 16       | 14         |
| Age (years)    | 57 (43–70) | 61 (50–69) |
| Gender         |          |            |
| Female         | 7        | 5          |
| Diagnosis      |          |            |
| Multiple myeloma | 5 | 4         |
| NHL            | 5        | 4          |
| CLL            | 3        | 2          |
| MDS            | 1        | 3          |
| AML            | 1        | 1          |
| Others         | 1        |            |
| Prior regimens |          |            |
| MRD            | 2 (1–5)  | 3 (1–4)    |
| URD            | 7        | 6          |
| Donor          |          |            |
| Male           | 9        | 6          |
| Female         | 7        | 8          |
| Marrow         | 3        | 6          |
| Disease status |          |            |
| CR             | 2        | 6          |
| PR stable      | 6        | 4          |
| Untreated relapse | 8 | 4        |
| CMV+ donor–recipient pairs | 8 | 11 |
| ABO mismatch   |          |            |
| Major          | 6 | 4 |
| Minor          | 8 | 4 |
| Donor–recipient gender mismatch | 4 | 7 |

Abbreviations: MDS = myelodysplastic syndrome; MRD = matched-related donor; NHL = Non-Hodgkin’s lymphoma; URD = unmatched-related donor.

Others, Ph-chronic myeloproliferative disorder and renal cell carcinoma.
### Table 2: Patient outcomes

| UPN | Diagnosis (Donor) | Diagnosis of Tx (number of prior regimen*) | AGVHD Grade | Week 8 chimerism, % | Post transplant disease status | DLI (number of infusions) | CGVHD Status at last contact (chimerism, %) | Status at last contact (months) |
|-----|------------------|-------------------------------------------|-------------|----------------------|-------------------------------|--------------------------|---------------------------------------------|--------------------------------|
| 1   | NHL-transformed (MRD) | PR5 (6°) | No | 100 | CR | Yes (1°) | Ext* | CR (100) | Alive (75) |
| 2   | CLL (MRD) | PR2 (3) | No | 96 | Persistent disease | Yes (3) | No | CR (91) | Alive (72) |
| 3   | MM (URD) | Persistent disease (4°) | II | 97 | Stable disease | No | No | Progression free (100) | Alive (69) |
| 4   | CLL (MRD) | R1 | No | 90 | CR | Yes (1°) | No | CR (100) | Alive (69) |
| 5   | MM (URD) | CR1 (2°) | No | 96 | CR | No | No | CR (100) | Alive (64) |
| 6   | NHL-DLCL (MRD) | CR3 (4°) | No | 100 | CR | No | No | CR (95) | Alive (64) |
| 7   | NHL-follicular (MRD) | Persistent disease (4°) | No | 95 | Persistent disease | Yes (1°) | Ext* | CR (100) | Alive (67) |
| 8   | NHL-composite (MRD) | PR2 (2) | No | 81 | Relapse | Yes (2°) | No | Progressive disease (7) | Dead (16) |
| 9   | MDS→AML (MRD) | Persistent disease (1) | No | ND | Relapse | Yes (1°) | NE | Progressive disease (1) | Dead (3) |
| 10  | Rx MDS (WM) (MRD) | Persistent disease (5) | No | 87 | CR | Yes (1°) | Ext* | CR (96) | Alive (59) |
| 11  | MM (URD) | Persistent disease (4°) | No | ND | Relapse | No | NE | NE (100) | Alive (71) |
| 12  | CMPD (Ph-) (MRD) | Persistent disease (1) | No | 86 | Relapse | Yes (1°) | No | Refractory disease (12) | Dead (13) |
| 13  | CLL (MRD) | R1 (1) | No | 95 | CR | No | Lim | CR (100) | Alive (54) |
| 14  | NHL-follicular (URD) | PR2 (2) | No | 97 | CR | No | Lim | CR (100) | Alive (48) |
| 15  | MM (URD) | PR1 (2°) | II | 100 | CR | No | Lim | CR (100) | Alive (44) |
| 16  | MM (URD) | PR2 (4°) | No | 93 | CR | No | Ext | CR (100) | Alive (37) |

### Abbreviations:
- Composite = follicular and large cell lymphoma; DLI = donor lymphocyte infusions; DLCL = diffuse large cell lymphoma; Ext = extensive chronic GVHD; MRD = matched-related donor; MCL= mantle cell; MDS/Rx MDS = de novo/therapy-induced myelodysplastic syndrome; MM = multiple myeloma; Lim = limited chronic GVHD; N = no; NE = not evaluable; NHL = non-Hodgkin’s lymphoma; RCC = renal cell carcinoma; URD = unrelated donor; Transform = follicular transformed to large cell; WM = Waldenstrom's macroglobulinemia; Y = yes.
- *Previous autologous transplant.
- °GVHD developed following DLI.
- †Complications requiring intervention in the first 6 months following transplantation:
  1. Pericarditis and pneumonia.
  2. Fever and pancytopenia.
  3. Monoartricular arthritis (knee) + Staphylococcus aureus bacteremia.
  4. Fever/hypotension; CMV viremia.
  5. Pneumonitis Parainfluenza virus type iila + pancytopenia.
  6. One patient each with minor and major ABO incompatibilities developed early and delayed alloimmune hemolysis, respectively.
  7. CNS toxoplasmosis.
  8. Two patients developed immune thrombocytopenia.
  9. Limbic encephalitis → death.
  10. Staphylococcus aureus bacteremia/pneumonia + endocarditis; Escherichia coli sepsis with ARDS; pulmonary emboli → death.
  11. Steroid psychosis; Staphylococcus epidermidis sepsis; pulmonary aspergillosis.
  12. CNS toxoplasmosis.
  13. Clostridium difficile colitis with septic shock → death.
  14. Jehovah’s Witness.
  15. Chemotherapy given before DLI.
Figure 1  Whole blood chimerism following antithymocyte globulin-TBI and allogeneic hematopoietic SCT plotted over the first 3 months. Box-whisker plot depicting whole blood chimerism values in patients undergoing matched-related donor (MRD) and unmatched-related donor (URD) SCT.

Figure 2  Lineage-specific chimerism and lymphocyte reconstitution following antithymocyte globulin-TBI and allogeneic hematopoietic SCT in the first 3 months following transplant (a); mean T-cell chimerism, (b) absolute lymphocyte counts, (c) neutrophil chimerism, plotted over time for the two cohorts; (time in months following transplant), and (d) neutrophil (solid lines) and T-cell (dashed lines) chimerism over time in three patients (depicted in different colors) demonstrating declining T-cell chimerism with preserved neutrophil chimerism. Asterisks indicate donor lymphocyte infusion.
count 0.2 ± 0.1 × 10^9/l, 0.4 ± 0.4, 0.5 ± 0.3 in thirteen 2 Gy recipients vs 0.4 ± 0.4, 0.9 ± 0.6, 1.2 ± 1.1 in ten 4.5 Gy TBI recipients; RMANOVA \( P = 0.023 \) (Figure 2b). No significant difference was identified in neutrophil engraftment between the two groups (RMANOVA \( P = 0.97 \)) (Figure 2c). Five patients (all MRD, four in cohort 1) demonstrated stable donor-derived hematopoiesis (neutrophil chimerism \( \geq 90\% \) over the first 3 months and day 100 BM chimerism \( \geq 80\% \)) while sustaining a sharp decline in T-cell chimerism (<50%) in the same time period, suggesting the emergence of myeloid tolerance (Figure 2d).

When lymphocyte subsets were analyzed, rapid normalization of NK cell counts (CD3–, CD16/56+) was seen as opposed to T cells (CD3+) (Figure 3a). On further analysis, the majority of NK cells were found to be CD16/56dim for both URD and MRD recipients (Figure 3b). Interestingly, URD recipients missing \( \geq 1 \) killer immunoglobulin-like receptor (KIR) ligand (either HLA-Bw4, HLA-C1 or HLA-C2 sero-group) were less likely to relapse compared to those who had no missing KIR ligands (one out of seven vs five out of six patients relapsing; Fisher’s exact test \( P = 0.029 \)), but a similar effect was not seen in recipients of MRD transplants.

**GVHD**

Acute GVHD in the first 100 days was seen in only 3 out of 29 evaluable patients (10%) in this study. Acute GVHD was grade II in all instances and responded promptly to initial therapy with corticosteroids. Two recipients of URD SCT developed steroid-responsive \textit{de novo} extensive chronic GVHD; one of these had received BM from a DRB1-mismatched URD. Limited chronic GVHD was seen in three other recipients of URD marrow.

**Survival and DLI**

At a median follow-up of 64 months in the surviving patients in cohort 1 and 26 months in cohort 2, (Table 2) 14 out of 30 patients are alive. The cause of death was disease progression in 13 patients. Three patients died in remission, one each of limbic encephalitis, Clostridium difficile colitis with sepsis and pulmonary emboli. Overall 1-year survivals (± s.e.) are 71 ± 11 and 54 ± 14%, respectively, in recipients of MRD and URD SCT by the Kaplan–Meier method.

Sixteen out of 29 (55%) evaluable patients were given donor cell infusions because of either persistent/relapsing disease (\( n = 12 \)) or declining chimerism (\( n = 4 \)), at a median of 113 days post transplant (days 63–425). Median CD3+ cell dose infused was 3.9 × 10^9/kg (0.7–6 × 10^9) in the seven patients receiving G-CSF mobilized cells, and 0.4 × 10^9/kg in the nine patients who received unstimulated DLI (0.1–0.6 × 10^9). Two patients received high-dose therapy (effectively a second transplant, one each conditioned with BU + CY and melphalan), and two other patients received single-agent fludarabine before cell infusion. (Table 2)

One patient in cohort 2 died of pneumonitis of presumed infectious origin one month after DLI without disease or chimerism reassessment. Increased donor chimerism was seen in 12 of the remaining 15 evaluable patients. Following DLI, 7 out of 15 patients developed GVHD requiring corticosteroid therapy, and four of these patients were refractory to corticosteroid therapy. Eight patients had disease progression despite DLI; in three patients, this occurred despite the development of acute GVHD, and all three died as a consequence of steroid refractory GVHD complicating the relapse therapy. Only 4 out of 15 patients receiving DLI remain alive in remission, of which one is alive with disease progression (Table 2). Three recipients of MRD (all in cohort 1) developed graft loss despite receiving chemotherapy before DLI. Only one other patient who received chemotherapy prior to DLI developed full donor chimerism. This led to modification of the conditioning regimen to intensify the host immunosuppression in cohort 2.

**Toxicity**

The ATG infusions were well tolerated even in elderly patients. Six patients developed grade III infusional toxicity; atrial fibrillation (three); fever with dyspnea or rash (three). GI toxicities were grade II or less in all patients; nausea and diarrhea were the most frequent complaints. Oral mucositis was absent. No instances of
veno-occlusive disease or pulmonary toxicities were seen. One-year non-relapse mortality is 10% (3 out of 30 patients). Despite the high dose of ATG used, opportunistic infections were not seen at a higher frequency than expected in these heavily pretreated patients (Table 2).

Discussion

We report results from a well-tolerated and simple ATG-based conditioning regimen for allografting following which there were no cases of grade III–IV acute GVHD before day 100 even in patients undergoing URD SCT. As is often seen when T-cell depletion methodology of any kind is applied, initial mixed T-cell chimerism was seen in patients receiving a lower dose of TBI and administration of ATG proximal to the actual transplant. T-cell engraftment was more robust after modification of the conditioning regimen to complete the ATG administration 1 week ahead of stem cell infusion and with a higher dose of TBI. We hypothesize that this is related to a decline in active ATG levels by the time of allograft infusion and more complete immunosuppression of the recipient with the higher TBI dose, allowing reliable lymphoid engraftment with minimal acute GVHD, providing a platform for later adoptive immunotherapy with DLI. However, despite the improvement in lymphoid engraftment, relapse and late opportunistic infections limited successful outcomes in patients treated in the second cohort.

Grades III and IV acute GVHD remain major risk factors for treatment-related mortality in allogeneic SCT recipients conditioned with reduced intensity regimens such as 2Gy TBI and fludarabine (hazard ratio 9.5; 95% confidence interval 4.7, 19). Patients receiving URD transplants fare poorly, with a 15 and 26% cumulative incidence of grades III and IV acute GVHD at 1 year and a corresponding mortality rate of 18 and 29% reported with two different GVHD prophylaxis regimens consisting of extended course MMF and CsA. To address this problem, the group at Massachusetts General Hospital has adapted their murine model to the clinical setting, using CY, thymic irradiation and either ATG or MEDI-507 (z-CD2) to facilitate allogeneic SCT with matched and mismatched related donors, reporting a low incidence of grades II–IV acute GVHD (29%). However, nearly 50% of patients required DLI for mixed donor-recipient chimerism. A more recent update of this trial reported a 27% incidence of graft loss among 82 patients, with graft loss occurring over the first few months with declining T-cell chimerism despite DLI. Similar to the findings reported in this trial, we observed a low incidence of acute and de novo chronic GVHD in our patients; however, a number of patients developed GVHD after DLI given for disease persistence or progression, negating the beneficial effect of acute GVHD risk reduction seen early on following transplant.

We also observed poor outcomes in most patients who failed to achieve near complete donor chimerism (≥90% donor-derived hematopoiesis) early on following transplantation. This was most likely attributable to the high dose of ATG used in our study, with resultant significant delay in lymphoid recovery secondary to T-cell depletion contributing to both graft loss as well as relapse seen in these patients. Although delaying stem cell infusion following ATG and intensifying host immunosuppression by increasing the TBI dose in the second cohort has resulted in more robust lymphoid engraftment, the disease relapse rate remained high in this later cohort of patients. This may largely be due to the poor prognosis of the patients we treated, that is, patients with persistent or untreated high-risk MDS, AML and post-autograft recurrence of multiple myeloma, but a role for in vivo T-cell depletion in producing higher relapse rate cannot be excluded. It remains to be determined whether this strategy would be more successful in patients traditionally treated with non-myeloablative transplants, that is, heavily pre-treated chemotheraphy-sensitive CLL, follicular and mantel cell Non-Hodgkin’s lymphoma, and first-remission multiple myeloma. Other patients may require a greater degree of pretransplant cytoreduction before undergoing ATG-based non-myeloablative conditioning.

Despite the negative outcome of this trial, there are several lessons to be learned from this study. Even though moderate levels of residual recipient chimerism (≥10%) early on appear to confer a greater risk of treatment failure in our small heterogeneous group of patients, several elderly patients with MRD SCT maintained disease remission even though they had mixed recipient-donor hematopoietic chimerism (5–10% recipient DNA), suggesting a beneficial impact of residual host hematopoiesis. In a murine allograft model, superior DLI-mediated antitumor effect was observed in mice which were mixed chimeric as opposed to full donor chimeric, with the host MHC class I expression being critical for these antitumor responses. Greater effectiveness of adoptive immunotherapy in the setting of mixed chimerism is also illustrated by canine studies in which DLI given to recipients of BMT from DLA-identical donors, following 10Gy TBI and ATG, resulted in conversion to full donor chimerism without GVHD, when given around day 60, whereas earlier infusion resulted in lethal GVHD. Another explanation for this observation may be through invoking the immune homeostatic effects of lympho-depletion, which is recognized for its immunostimulatory effect in patients undergoing therapy for cancer and may indeed have a similar role in the setting of T-cell-depleted non-myeloablative allografts incorporating strategies to perform adoptive immunotherapy with DLI. All these lines of reasoning suggest a potential beneficial effect of adoptive immunotherapy with DLI on the platform of early mixed chimerism achieved by T-cell-depleting regimens. Such conditioning regimens may in the future also allow an assessment of tumor-specific vaccines in the context of an allogeneic SCT.

Establishment of early donor-derived hematopoiesis without significant GVHD in the setting of T-cell depletion suggests a role for the KIR-dependent activity of donor-derived NK cells in establishing donor hematopoiesis. In our patients a rapid normalization of NK cell counts following transplantation was observed. This early NK cell recovery may be particularly useful in recipients of URD transplants, where a trend toward fewer relapses in patients with missing KIR Ligands, belonging to HLA-C1, C2 or...
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becomes an attractive option as a means of maximizing the conditioning for transplanting high-risk elderly patients. The use of URD with such ATG-based universal disparity in KIR genotypes in unrelated donor-recipient pairs, the use of URD with such ATG-based regimen may be attributed to ATG affecting NK cell reconstitution by altering the T-cell content of the graft. The rapid reconstitution of CD16/56dim NK cells that we observe may also be related to the positive outcomes seen in URD recipients with missing KIR ligands, as these cells are known to mediate cytotoxicity against tumor targets.

Given the nearly universal disparity in KIR genotypes in unrelated donor-recipient pairs, the use of URD with such ATG-based conditioning for transplanting high-risk elderly patients becomes an attractive option as a means of maximizing the potential for GVL effects while reducing GVHD risk.

Donor-recipient tolerance achieved through SCT is being intensively investigated as a means for achieving solid organ allograft tolerance. On the basis of our observation of persistent myeloid engraftment despite declining donor-derived T-cell chimerism, we hypothesize that ATG-based regimens such as ours can be successfully used in promoting solid organ transplant tolerance.

Thymoglobulin and reduced intensity TBI appears to facilitate multilineage engraftment with minimal acute GVHD and provides a platform for later DLI-mediated adoptive immunotherapy. However, an unacceptable relapse rate was observed in these pilot studies, admittedly performed in high-risk patients. A randomized clinical trial of thymoglobulin and 450 cGy TBI is being developed that compares lower doses of thymoglobulin, which have been shown to retain effectiveness in preventing acute GVHD without resulting in increased opportunistic infections.

Optimization of ATG dosing may allow us to finally reign in acute GVHD, whereas preserving the anti-malignancy effect of an allograft in elderly patients.

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