Treatment of Fanconi anemia patients using fludarabine and low-dose total body irradiation followed by unrelated donor hematopoietic cell transplantation

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Abstract

A non-myeloablative conditioning regimen consisting of fludarabine (FLU) and 2 Gy total body irradiation (TBI) has been used with great experience and engraftment success without promoting excessive non-relapse mortality (NRM) in medically infirm patients requiring hematopoietic cell transplantation (HCT). Here, we studied this same low-toxicity regimen as a means to promote engraftment of unrelated donor peripheral blood stem cells (PBSC) in patients with Fanconi Anemia (FA). All patients tolerated the regimen well with no mucositis or other severe toxicity. Of six patients transplanted, five achieved stable mixed or full donor chimerism. Acute and chronic graft-versus-host disease (GVHD) occurred in four and three patients, respectively. Three patients are alive and well a median of 45.9 (range, 20.9–68.1) months after transplant. In summary, this FLU-based regimen facilitates stable engraftment of unrelated PBSC but is associated with significant chronic GVHD.

Keywords

Fanconi anemia; HLA-matched unrelated donor transplant; fludarabine

CONFLICT OF INTEREST

The authors have no conflicts of interest.
INTRODUCTION

FA is a rare, heterogeneous disorder characterized by progressive marrow failure and susceptibility to malignancies. Hematopoietic cell transplantation (HCT), while the only known cure for the hematological manifestations of this disease, is associated with considerable toxicity in FA patients due to their underlying DNA repair defects and inability to tolerate conventional doses of cytotoxic therapy. Studies incorporating reduced doses of chemotherapy have allowed high rates of engraftment and excellent survival with minimal toxicity when using human leukocyte antigen (HLA)-matched sibling donors. However, similar results have been more difficult to achieve when using HLA-matched unrelated donors due to higher rates of both graft rejection and GVHD. A retrospective analysis of unrelated donor transplants conducted by the CIBMTR registry showed a benefit when using FLU, rather than non-FLU, based regimens. However, to promote engraftment in the unrelated donor setting, FLU historically has been combined with other agents, such as TBI at moderate doses, which has contributed to additional toxicity. In particular, irradiation at doses exceeding 4–5 Gy has been associated with additional acute toxicity and secondary malignancies in FA patients, especially squamous cell carcinomas of the head and neck.

There has also been some precedence in eliminating TBI altogether when using unrelated donors to reduce these late effects. Accordingly, we reasoned that a well-established non-myeloablative conditioning regimen typically reserved for high-risk, medically fragile patients that uses FLU and low-dose TBI, in combination with cyclosporine (CSP) and mycophenolate mofetil (MMF), could also be attempted in FA patients to facilitate engraftment without excessive toxicity. Even when used to salvage a high-risk, non-FA population who rejected prior allogeneic transplants, this regimen was able to promote sustained engraftment in 87% of patients, with four of five patients who failed to engraft having myelofibrosis.

This study was also designed to reduce and possibly eliminate the already low-dose of TBI for future cohorts based on high rates of engraftment. Finally, since we have shown that FA patients undergoing HLA-matched sibling transplants can tolerate a dose of cyclophosphamide (CY) 60 mg/kg for conditioning, we hypothesized that a regimen that incorporated no alkylating agents and only a low-dose of TBI in the HLA-matched, unrelated donor setting would also be similarly tolerable. Thus, the goal of this trial was to evaluate engraftment and toxicity associated with FLU and low dose TBI in patients with FA.

METHODS

Patient characteristics

Between August 2001 and September 2006, six patients confirmed as having FA by chromosomal breakage analysis and requiring allogeneic transplantation for aplastic anemia but having no HLA-matched sibling donors were enrolled on FHCRC Protocol 1444 at four different centers. Participating centers included Fred Hutchinson Cancer Research Center, Seattle, WA; Children’s Memorial Hospital, Chicago, IL; Monroe Carell Jr. Children’s Hospital at Vanderbilt, Nashville, TN; and Primary Children’s Medical Center, Salt Lake City, UT. All patients and/or legal guardians provided informed consent using Institutional
Review Board approved documents. Patients were between the ages of 9.9–12.1 (median, 10.6) years at the time of transplant. Additional patient characteristics are detailed in Table 1.

**Donor characteristics**

Patients and donors were typed at HLA-A, B, C, DRB1, and DQB1 using high-resolution DNA-based methods. Donors were matched with the patient for HLA-DRB1 and DQB1, and either matched (n=4) or mismatched for a single allele at HLA-A, B, or C (n=2). PBSC were mobilized with granulocyte-colony stimulating factor (G-CSF) at a dose of 10 μg/kg for 5 days. All donors provided informed consent per National Marrow Donor Program (NMDP) guidelines. Additional donor characteristics can be found in Table 1.

**Transplant characteristics**

The conditioning regimen consisted of FLU 30 mg/m$^2$ from days −4 to −2 and 2 Gy TBI day 0.9,10 PBSC were T-cell replete and infused on day 0. Infused cell doses can be found in Table 1. Post-grafting immunosuppression consisted of CSP from days −3 to +100 then tapered to day +177, and MMF from days 0 to +40 then tapered to day +96.

**Outcome measures and statistical methods**

Graft rejection was defined as <5%, mixed chimerism between 5–95%, and full donor chimerism as >95% donor CD3 chimerism. CD3 chimerism was evaluated on days +28, +84, and +365 (or more frequently) either by fluorescent in situ hybridization for sex-mismatched transplants or analysis of genomic DNA for variable number of tandem repeats for sex-matched transplants. GVHD was graded per established methods.$^{12}$ Late-onset acute GVHD was defined as any acute GVHD occurring beyond day +100. An infectious episode was defined only if a pathogen was identified and treatment prescribed. Due to the small sample size of this cohort, variables that may have affected outcomes were not incorporated into a multi-variate analysis. Stopping rules for grade IV regimen-related toxicity prior to day +28 were not met. Protocol oversight was maintained by a dedicated Data Safety Monitoring Board which met at a minimum of every 6 months.

**RESULTS**

**Engraftment and hematopoietic recovery**

Engraftment was achieved in five of six patients, in whom three developed mixed and two developed full donor chimerism. Median time to achieve an absolute neutrophil count of 500/mm$^3$ was day +16 (range, +13 to +17) and platelet count >50,000/mm$^3$ without transfusion was day +15 (range, +14 to +18). All five patients had improvement from baseline hematological blood counts no matter the extent of donor chimerism. Graft failure occurred in one patient and was not rectified after a second PBSC transplant on day +34 from the same donor with no additional conditioning. However, engraftment subsequently was achieved when the patient was conditioned with FLU and antithymocyte globulin prior to receiving a third PBSC transplant from the same donor. He is currently alive and doing well with ameliorated blood counts and mixed donor chimerism (Figure 1).
Regimen-related toxicity

One patient experienced nausea and vomiting related to the preparative regimen. Otherwise, all other patients tolerated the conditioning regimen and PBSC infusion well with no complications. No patients developed mucositis. Other than transient mild hyperbilirubinemia in two patients (3.1 and 3.6 mg/dL) within the first 30 days after transplant, both liver and kidney functions remained within normal limits. No other adverse regimen-related toxicities were noted within the first 100 days after transplant.

Graft-versus-host disease

Four patients developed acute GVHD with stage II skin (n=3), stage III gut (n=1) and stage III liver (n=1) resulting in overall grades of acute GVHD of I (n=1), II (n=2), and III (n=1). In all cases, systemic steroids were given and the MMF taper was held. No patient developed late-onset acute GVHD. Of the five evaluable patients, three developed refractory chronic GVHD at 83, 84, and 102 days after HCT that required prolonged treatment with systemic steroids as well as tertiary immunosuppressive therapy.

Infections

All three patients with chronic GVHD developed multiple episodes of late systemic infections. Patient 1 had fifteen (bacterial, 10; viral, 4; fungal, 1) infections at a median of 293 (range, 23–690) days, Patient 5 had fourteen (bacterial, 12; viral, 2) infections at a median of 415 (range, 139–741) days, and Patient 6 had twelve (bacterial, 9; viral, 1; fungal, 2) infections at a median of 432 (range, 65–487) days after HCT. Patient 4 was treated for one bacterial infection before being taken off study for graft rejection, while Patients 2 and 3, neither of whom developed chronic GVHD, had any documented infections.

Overall survival

With a median follow-up of 26 (16.3–68.1) months after HCT, three of six patients are alive and well. As of last follow-up, all three remain transfusion independent and have not developed any secondary malignancies to date, and two have discontinued all immunosuppressive drugs.

DISCUSSION

FA is characterized by an intrinsic sensitivity to DNA-damaging agents, and thereby presents a challenging therapeutic balance between providing enough conditioning to allow durable engraftment without promoting excessive acute toxicity. Here we studied the use of FLU in combination with TBI in the conditioning of FA patients prior to unrelated donor grafts. The regimen which included 2 Gy TBI was well-tolerated and engraftment was attained in most cases. Similar to what has been reported for non-FA patients transplanted for malignant diseases, these findings demonstrated that low-intensity conditioning with FLU allowed for stable engraftment. However, chronic refractory GVHD remained a substantial problem in our population, and novel strategies to reduce GVHD will be required in future protocols.
Several patients developed late infections complicating their clinical course. Because most infections were seen years following HCT and only in those patients having chronic GVHD, it is likely that functional defects from chronic GVHD itself, as well as effects of chronic immunosuppression, are the likely culprits rather than acute regimen-related toxicity.

The major problem identified with this regimen was the high rate of chronic GVHD, which contributed to the deaths of three patients. The most likely etiology is the use of G-CSF-mobilized PBSC grafts, which are associated with higher rates of chronic GVHD compared to marrow grafts.\textsuperscript{13, 14} Despite the presence of chronic GVHD and the immunological drive of donor cells to promote GVHD in two of three patients with mixed chimerism, conversion to full donor chimerism was not observed. The etiology of this is unclear, but similar observations have been noted when transplanting other non-malignant diseases treated with a conditioning regimen similar to ours.\textsuperscript{15} Of note, the long-term significance of prolonged mixed chimerism is uncertain in FA. Theoretically, while the persistence of residual FA cells may not necessarily affect long-term engraftment, there is concern that these cells may transform into leukemia or myelodysplastic syndrome over time. However, when evaluating results of a joint study between our group and Curitiba, Brazil,\textsuperscript{2} of the 43 patients who underwent HLA-matched sibling transplantation and conditioned with CY 60 mg/kg, over half developed mixed donor-host chimerism, and to date none have developed leukemia or MDS with a median follow-up of 6.7 (range 3.4–10.6) years (personal communication, R. Pasquini and C. Bonfim). As the follow-up of most patients on this study is still relatively early, such observations are interesting but the benefit of mixed chimerism cannot be confirmed without the ongoing longitudinal follow-up. This is in contrast to what has been reported in the natural history of FA, where without transplant, one study indicated an actuarial risk is as high as 67% by the age of 40.\textsuperscript{16}

Finally, development of chronic GVHD on our study could be attributed to the inadequacy of early post-grafting immunosuppression. Although prolonged immunosuppression with CSP and MMF helped establish stable engraftment in our protocol, the prevention of GVHD will require a more aggressive elimination of actively proliferating alloreactive donor T cell clones that emerge early after HCT. One approach worth considering would be using only marrow grafts to reduce the number of T cells, or adding other agents to target more specific \textit{in vivo} T cell depletion, such as either adding antithymocyte globulin during conditioning,\textsuperscript{17, 18} or as shown in non-FA patients, adding a single dose of CY after HCT.\textsuperscript{19} Another approach would be to deplete donor T cells \textit{ex vivo} and infusing CD34-selected stem cells.\textsuperscript{20} It is thus clear from our results and published studies that successor transplant trials for FA need to incorporate more efficient T cell suppression/depletion strategies while attempting dose de-escalation or elimination of TBI to decrease toxicity and late-effects.

Other groups have published FLU-based regimens for unrelated donors using marrow and/or T-cell depletion methods with promising results. Wagner et al. evaluated CIBMTR registry data demonstrating the survival advantage of FLU-based regimens.\textsuperscript{4} Yabe et al.\textsuperscript{17} reported on 27 FA patients receiving alternative donor transplants, of which 24 received 5–6/6 unrelated donor marrow. Using a regimen containing 3–4.5 Gy thoracoabdominal irradiation (TAI) or TBI, FLU, CY, and ATG, engraftment was seen in 26/27, acute GVHD grades II-III in 3/26, and chronic GVHD in 8/26 patients, resulting in an overall survival of 96.3%.

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Chaudhury et al. reported on alternative donor transplants, of which ten of 18 received CD34+, T-cell-depleted PBSC or marrow grafts from HLA-matched or mismatched unrelated donors preceded by 450 cGy TBI, FLU 150 mg/m², and CY 40 mg/kg. Outcomes for the entire group showed that all engrafted, one patient developed Grades II-III GVHD which progressed to chronic GVHD, and the 5-year disease-free survival was 66.6%. Some groups have also incorporated alemtuzumab, a monoclonal antibody against CD52, as a means to deplete T cells in vivo with promising results. In contrast, MacMillan et al. reported on a TBI dose-escalation study on which 21 of 29 patients received unrelated donor T-cell depleted marrow grafts. Conditioning included CY 40 mg/kg, TBI 450 or 600 cGy, and ATG. Results showed that for the entire cohort, engraftment was 63%, grades II-IV acute GVHD was 32%, chronic GVHD 0%, and 1-year overall survival was low at 34%. Thus, ongoing prospective trials incorporating minimally toxic yet potently immunosuppressive strategies are necessary for successfully transplanting patients with FA.

We conclude that in patients with FA, our regimen with FLU and low dose TBI was able to achieve stable engraftment with minimal early toxicity, but it was associated with unacceptably high rates of chronic GVHD for this patient population.

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References

1. Flowers MED, Doney KC, Storb R, Deeg HJ, Sanders JE, Sullivan KM, et al. Marrow transplantation for Fanconi anemia with or without leukemic transformation: an update of the Seattle experience. Bone Marrow Transplant. 1992; 9:167–173. [PubMed: 1511254]
2. Bonfim CM, de Medeiros CR, Bitencourt MA, Zanis-Neto J, Funke VA, Setubal DC, et al. HLA-matched related donor hematopoietic cell transplantation in 43 patients with fanconi anemia conditioned with 60 mg/kg of cyclophosphamide. Biol Blood Marrow Transplant. 2007; 13:1455–1460. [PubMed: 18022575]
3. Guardiola P, Pasquini R, Dokal I, Ortega JJ, van Weel-Sipman M, Marsh JC, et al. Outcome of 69 allogeneic stem cell transplantations for Fanconi anemia using HLA-matched unrelated donors: a study on behalf of the European Group for Blood and Marrow Transplantation. Blood. 2000; 95:422–429. [PubMed: 10627445]
4. Wagner JE, Eapen M, MacMillan ML, Harris RE, Pasquini R, Boulad F, et al. Unrelated donor bone marrow transplantation for the treatment of Fanconi anemia. Blood. 2007; 109:2256–2262. [PubMed: 17038525]
5. MacMillan ML, Auerbach AD, Davies SM, Defor TE, Gillio A, Giller R, et al. Haematopoietic cell transplantation in patients with Fanconia anaemia using alternate donors: results of a total body irradiation dose escalation trial. Br J Haematol. 2000; 109:121–129. [PubMed: 10848791]
6. Deeg HJ, Socié G, Schoch G, Henry-Amar M, Witherspoon RP, Devergie A, et al. Malignancies after marrow transplantation for aplastic anemia and Fanconi anemia: a joint Seattle and Paris analysis of results in 700 patients. Blood. 1996; 87:386–392. [PubMed: 8547667]
7. Masserot C, Peffault de Latour R, Rocha V, Leblanc T, Rigolet A, Pascal F, et al. Head and neck squamous cell carcinoma in 13 patients with Fanconi anemia after hematopoietic stem cell transplantation. Cancer. 2008; 113:3315–3322. [PubMed: 18831513]
8. Motwani J, Lawson SE, Darbyshire PJ. Successful HSCT using nonradiotherapy-based conditioning regimens and alternative donors in patients with Fanconi anaemia--experience in a single UK centre. Bone Marrow Transplant. 2005; 36:405–410. [PubMed: 15995715]

9. McSweeney PA, Niederwieser D, Shizuru JA, Sandmaier BM, Molina AJ, Maloney DG, et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. Blood. 2001; 97:3390–3400. [PubMed: 11369628]

10. Maris MB, Niederwieser D, Sandmaier BM, Storer B, Stuart M, Maloney D, et al. HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies. Blood. 2003; 102:2021–2030. [PubMed: 12791654]

11. Gyurkocza B, Cao TM, Storb RF, Lange T, Leisenring W, Franke GN, et al. Salvage allogeneic hematopoietic cell transplantation with fludarabine and low-dose total body irradiation after rejection of first allografts. Biol Blood Marrow Transplant. 2009; 15:1314–1322. [PubMed: 19747640]

12. Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation. 1974; 18:295–304. [PubMed: 4153799]

13. Champlin RE, Schmitz N, Horowitz MM, Chapuis B, Chopra R, Cornelissen JJ, et al. Blood stem cells compared with bone marrow as a source of hematopoietic cells for allogeneic transplantation. Blood. 2000; 95:3702–3709. [PubMed: 10845900]

14. Blaise D, Kuentz M, Fortanier C, Bourhis JH, Milpied N, Sutton L, et al. Randomized trial of bone marrow versus lenograstim-primed blood cell allogeneic transplantation in patients with early-stage leukemia: a report from the Société Française de Greffe de Moelle. J Clin Oncol. 2000; 18:537–571. [PubMed: 10653869]

15. Burroughs LM, Storb R, Leisenring WM, Pulsipher MA, Loken MR, Torgerson TR, et al. Intensive postgrafting immune suppression combined with nonmyeloablative conditioning for transplantation of HLA-identical hematopoietic cell grafts: results of a pilot study for treatment of primary immunodeficiency disorders. Bone Marrow Transplant. 2007; 40:633–642. [PubMed: 17660844]

16. Rosenberg PS, Greene MH, Alter BP. Cancer incidence in persons with Fanconi anemia [erratum appears in Blood. 2003 Mar 15;101(6):2136]. Blood. 2003; 101:822–826. [PubMed: 12393424]

17. Yabe H, Inoue H, Matsumoto M, Hamanoue S, Koike T, Ishiguro H, et al. Allogeneic hematopoietic cell transplantation from alternative donors with a conditioning regimen of low-dose irradiation, fludarabine and cyclophosphamide in Fanconi anaemia. Br J Haematol. 2006; 134:208–212. [PubMed: 16846479]

18. Tan PL, Wagner JE, Auerbach AD, Defor TE, Slungaard A, MacMillan ML. Successful engraftment without radiation after fludarabine-based regimen in Fanconi anemia patients undergoing genotypically identical donor hematopoietic cell transplantation. Pediatric Blood and Cancer. 2006; 46:630–636. [PubMed: 16078221]

19. Luznik L, O’Donnell PV, Symons HJ, Chen AR, Leffell MS, Zahurak M, et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, post-transplantation cyclophosphamide. Biol Blood Marrow Transplant. 2008; 14:641–650. [PubMed: 18489989]

20. Chaudhury S, Auerbach AD, Kernan NA, Small TN, Prockop SE, Scaradavou A, et al. Fludarabine-based cytoreductive regimen and T-cell-depleted grafts from alternative donors for the treatment of high-risk patients with Fanconi anemia (Review). Br J Haematol. 2008; 140:644–655. [PubMed: 18302713]

21. Bitan M, Or R, Shapira MY, Aker M, Resnick IB, Ackerstein A, et al. Fludarabine-based reduced intensity conditioning for stem cell transplantation of Fanconi anemia patients from fully matched and unrelated donors. Biol Blood Marrow Transplant. 2006; 12:712–718. [PubMed: 16785060]

22. de La Fuente J, Reiss S, McCoy M, Vulliamy T, Roberts IA, Rahemtulla A, et al. Non-TBI stem cell transplantation protocol for Fanconi anaemia using HLA-compatible sibling and unrelated donors. Bone Marrow Transplant. 2003; 32:653–656. [PubMed: 13130311]
Figure 1.
(a) Absolute neutrophil counts (ANC), (b) absolute lymphocyte counts (ALC), and (c) platelet counts following hematopoietic cell transplantation show improvement of baseline hematological counts whether converting to full or maintaining mixed donor CD3 chimerism (d).
Table 1

| Pt | Age (yrs) | Gender | Time from dx to HCT (yrs) | ABO | Cyto-genetics | CD34 cell dose/kg (10^6) | CD3 cell dose/kg (10^8) | Rejection (days after HCT) | Acute GVHD | Chronic GVHD (days after HCT) | Chimerism | Outcome |
|----|-----------|--------|---------------------------|-----|--------------|-------------------------|-----------------------|--------------------------|------------|-------------------------------|-----------|---------|
| 1 | 11.5      | M/F    | 2.6                       | A+  | −/−          | 1.10                    | 3.5                    | N                        | 2          | Y (+84)                       | Partial   | Died due to MOF/chronic GVHD |
| 2 | 10.3      | M/F    | 1.0                       | O−/A+ | −/−         | 2.8                     | 4.3                    | N                        | 2          | N                             | Mixed     | Died day +839 due to MOF/BOOP/chronic GVHD |
| 3 | 10.0      | M/F    | 0.7                       | B−/A− | −/−         | 9.5                     | 0.7                    | Y (+30)                  | 0          | N                             | NE        | Alive and well 68.1 months after HCT |
| 4 | 12.2      | H/L    | 8.3                       | A−/A+ | −/−         | 6.1                     | 3.7                    | N                        | 3          | Y (+83)                       | Full      | Died day +839 due to MOF/BOOP/chronic GVHD |
| 5 | 10.2      | M/F    | 2.8                       | A+/O+ | −/−         | 8.0                     | 2.2                    | N                        | 2          | Y (+84)                       | Mixed     | Died day +839 due to MOF/chronic GVHD |

MOF: multi-organ failure; BOOP: bronchiolitis obliterans organizing pneumonia