original reports

Naive T-Cell Depletion to Prevent Chronic Graft-Versus-Host Disease

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abstract

PURPOSE Graft-versus-host disease (GVHD) causes morbidity and mortality following allogeneic hematopoietic cell transplantation. Naive T cells (T_N) cause severe GVHD in murine models. We evaluated chronic GVHD (cGVHD) and other outcomes in three phase II clinical trials of T_N-depletion of peripheral blood stem-cell (PBSC) grafts.

METHODS One hundred thirty-eight patients with acute leukemia received T_N-depleted PBSC from HLA-matched related or unrelated donors following conditioning with high- or intermediate-dose total-body irradiation and chemotherapy. GVHD prophylaxis was with tacrolimus, with or without methotrexate or mycophenolate mofetil. Subjects received CD34-selected PBSC and a defined dose of memory T cells depleted of T_N. Median follow-up was 4 years. The primary outcome of the analysis of cumulative data from the three trials was cGVHD.

RESULTS cGVHD was very infrequent and mild (3-year cumulative incidence total, 7% [95% CI, 2 to 11]; moderate, 1% [95% CI, 0 to 2]; severe, 0%). Grade III and IV acute GVHD (aGVHD) occurred in 4% (95% CI, 1 to 8) and 0%, respectively. The cumulative incidence of grade II aGVHD, which was mostly stage 1 upper gastrointestinal GVHD, was 71% (95% CI, 64 to 79). Recipients of matched related donor and matched unrelated donor grafts had similar rates of grade III aGVHD (5% [95% CI, 0 to 9] and 4% [95% CI, 0 to 9]) and cGVHD (7% [95% CI, 2 to 13] and 6% [95% CI, 0 to 12]). Overall survival, cGVHD-free, relapse-free survival, relapse, and nonrelapse mortality were, respectively, 77% (95% CI, 71 to 85), 68% (95% CI, 61 to 76), 23% (95% CI, 16 to 30), and 8% (95% CI, 3 to 13) at 3 years.

CONCLUSION Depletion of T_N from PBSC allografts results in very low incidences of severe acute and any cGVHD, without apparent excess risks of relapse or nonrelapse mortality, distinguishing this novel graft engineering strategy from other hematopoietic cell transplantation approaches.

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ASSOCIATED CONTENT

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Data Sharing Statement

Data Supplement

Protocol

Author affiliations and support information (if applicable) appear at the end of this article.

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INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) can cure patients with advanced hematologic malignancies.[1,2] αβT cells in the graft that recognize recipient alloantigens promote engraftment by attacking host hematopoietic and immune cells and diminish relapse by killing neoplastic blood cells, thereby mediating the graft-versus-leukemia (GVL) effect. Unfortunately, alloreactive T cells can damage normal host tissues, causing graft-versus-host disease (GVHD) and necessitating pharmacologic immunosuppression or T-cell–depleted grafts. GVHD and immunosuppression both contribute to morbidity and mortality and are barriers to a broader application of HCT.[3] Chronic GVHD (cGVHD), which occurs in 30%-60% of patients receiving unmanipulated grafts, often requires prolonged immunosuppression and causes nonrelapse mortality (NRM) and a reduced quality of life.[4] cGVHD is especially problematic in recipients of peripheral blood stem-cell (PBSC) grafts; yet, PBSCs remain the most prevalent graft source.[5] Complete graft T-cell–depletion (pan-TCD) reduces GVHD but delays immune reconstitution, increases opportunistic infections, and may reduce survival.[6] In vivo partial T-cell–depletion with antithymocyte globulin (ATG) or anti–T-lymphocyte globulin (ATLG), impairment of alloreactive T cells using post-HCT cyclophosphamide (PTCy), and other strategies have also reduced GVHD, but each has limitations and cGVHD remains problematic.[6-12]

αβT cells include naive (T_N), effector (T_E), and memory (T_M) subsets, distinguishable by surface phenotype.[13] We and others demonstrated that T_N caused severe GVHD in murine models, whereas T_M caused milder or no GVHD and retained graft-versus-tumor activity.[14-20] Informed by these results and human in vitro studies demonstrating enrichment of alloreactive T cells in T_N,[21] we developed a graft engineering strategy to selectively deplete T_N from granulocyte colony-stimulating factor–mobilized PBSC

CONTEXT

Key Objective

Chronic graft-versus-host disease (cGVHD) occurs in 30%-60% of patients with leukemia who receive unmanipulated donor HLA-matched peripheral blood stem-cell (PBSC) allografts and is a major source of morbidity and mortality. In mouse models, the depletion of antigen-inexperienced naive T cells (T_N) from grafts reduced GVHD. Importantly, the remaining memory T cells retained antileukemia activity. We, therefore, conducted clinical trials to determine whether the depletion of T_N from PBSC grafts would reduce cGVHD.

Knowledge Generated

In 138 recipients of HLA-matched T_N-depleted PBSC, the incidence of cGVHD was very low (7%), resulting in a 3-year cGVHD-free, relapse-free survival of 68%. These rates were similar across subgroups, including age, related or unrelated grafts, leukemia type, and conditioning regimen intensity.

Relevance

Depletion of T_N from donor PBSC is a promising and widely applicable strategy for reducing cGVHD, which warrants comparison to other graft-versus-host disease-reduction strategies in randomized clinical trials.

using immunomagnetic beads targeting CD45RA, which is expressed on all T_N but absent on most T_M.[22] An initial clinical trial supported the hypothesis that T_N-depletion of PBSC would mitigate GVHD and protect patients from severe opportunistic infections by retaining graft pathogen-specific T_M.[23]

We now report our experience with T_N-depleted HCT in 138 patients with acute leukemia and myelodysplastic syndrome treated on three prospective phase II clinical trials. T_N-depletion resulted in low rates of severe acute GVHD (aGVHD) and exceptionally low rates of cGVHD.

METHODS

Study Design and Participants

Subjects were enrolled in three phase II trials of T_N-depleted PBSC (Data Supplement 1 [online only] and Clinical Trial Protocols NCT00914940, NCT01858740, and NCT02220985 [online only]). The preliminary results for the first 35 patients in NCT00914940 were reported previously.[23] All patients enrolled in NCT00914940 (n = 41) and NCT01858740 (n = 20), and 77 of 84 patients enrolled in NCT02220985, received T_N-depleted grafts. Donor apheresis products for seven patients on NCT02220985 did not meet the Protocol specified criteria for cell selection; by Protocol, those patients received unmanipulated PBSC (Fig 1).

Patients provided informed consent in accordance with the Declaration of Helsinki. The study was performed after approval by the institutional review boards and the US Food and Drug Administration (Investigational Device Exemption 14160 and Investigational New Drug 15673), and in accord with an assurance approved by the Department of Health and Human Services.

Eligible patients were those referred for allogeneic HCT for a high predicted risk of relapse following chemotherapy alone. Inclusion and exclusion criteria are in Data Supplement 1. NCT00914940, NCT01858740, and NCT02220985 enrolled patients age 14-55, 0-21, and 0-60 years, respectively.

Procedures

Conditioning for NCT00914940 and NCT01858740 was high-intensity with fludarabine (25 mg/m^2 once per day for 5 days), thiotepa (5 mg/kg once per day for 2 days), and total-body irradiation (1,320 cGy).[24] In NCT02220985, younger patients mostly received the same high-intensity conditioning, whereas patients age ≥ 50 years or with comorbidities received intermediate-intensity myeloablative conditioning with fludarabine (30 mg/m^2 once per day for 5 days), cyclophosphamide (50 mg/kg once per day for 1 day), thiotepa (5 mg/kg once per day for 2 days), and total-body irradiation (400 cGy[25]; Data Supplement 1). Patients then received HLA-matched, CD34-selected PBSC, followed immediately by CD45RA-depleted cells from the CD34-negative fraction. NCT00914940 was restricted to patients with HLA-matched related donors (MRD) and used tacrolimus for GVHD prophylaxis, whereas NCT01858740 and NCT02220985 included patients with MRD or HLA-matched unrelated donors (MUD) and used tacrolimus plus methotrexate (high-intensity conditioning) or tacrolimus plus mycophenolate mofetil (intermediate-intensity conditioning).

GVHD was treated according to institutional standard practices with systemic and/or topical corticosteroids, continuation of tacrolimus, and additional therapies as deemed necessary. Duration of systemic corticosteroids (0.5-2 mg/kg prednisone per day) and subsequent taper were determined by treating physicians. Antimicrobial prophylaxis is in Data Supplement 1.

Each trial used the same graft engineering method (Data Supplement 1).[22] Donors received granulocyte colony-stimulating factor daily for 5 days, followed by PBSC collection by apheresis. Cell selection was initiated if ≥ 5×10^6 CD34$^+$ cells/kg were collected and the presence of

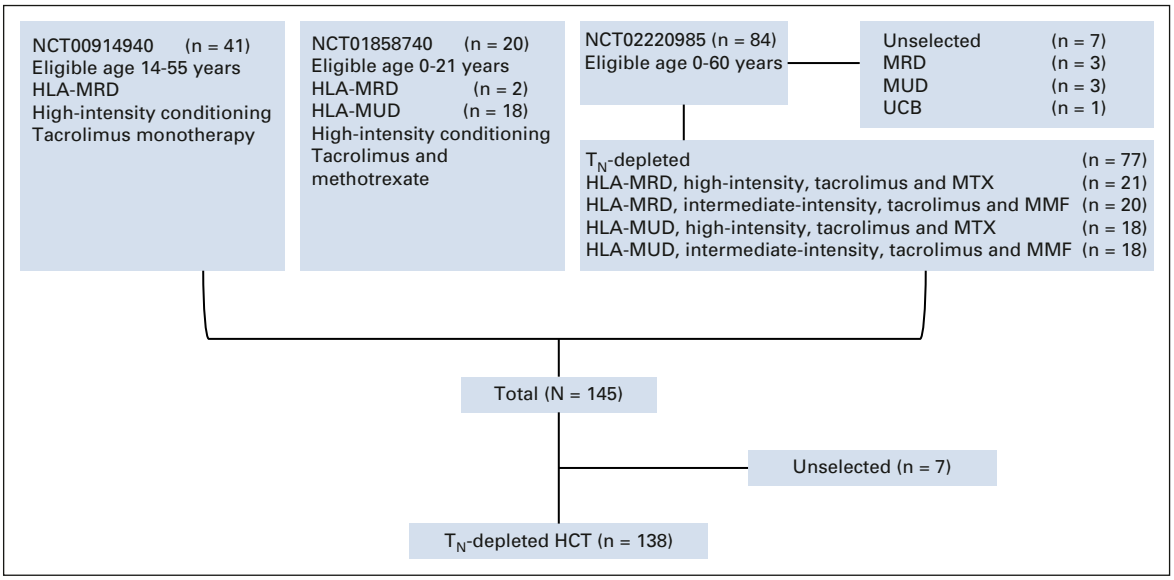

FIG 1. Study flow diagram. HCT, hematopoietic cell transplantation; MMF, mycophenolate mofetil; MRD, matched related donors; MTX, methotrexate; MUD, matched unrelated donors; T_N, naive T cells; UCB, umbilical cord blood.

$CD3^+CD45RA^-CD45RO^+$ T_M was confirmed. Positive selection of $CD34^+$ progenitor cells was followed by depletion of $CD45RA^+$ cells from the CD34-negative fraction using anti-CD45RA beads (Miltenyi Biotec; Data Supplement 1). All trials targeted $CD34^+$ cell doses of $\geq 5.0 \times 10^6$ cells/kg. In NCT00914940, $\leq 7.5 \times 10^4$ T_N/kg was targeted on the basis of estimates that more would cause GVHD. As this was consistently achieved, a goal of $\leq 5 \times 10^4$ T_N/kg was set in NCT01858740 and NCT02220985. In each trial, 10^7 $CD3^+$ T cells/kg was targeted to provide sufficient T_M to facilitate immune reconstitution.

Outcomes

The primary outcome of the analysis of cumulative data was cGVHD diagnosed by 2014 National Institutes of Health Consensus Criteria (Data Supplement 1).[26] Secondary outcomes were graft failure, grade III-IV aGVHD within 1 year of HCT (Data Supplement 1),[27] overall survival (OS), relapse, NRM, and survival free of moderate or severe cGVHD or relapse (cGVHD-free, relapse-free survival [CRFS]). The end point definition details and competing risks are in Data Supplement 1. Expert physicians graded aGVHD and reviewed cGVHD diagnosis and grading. Biopsies were performed to confirm skin and GI GVHD in most cases (Data Supplement 1). Donor chimerism was monitored by molecular techniques (Data Supplement 1).

Statistical Analysis

Data were analyzed as of December 2020. Probabilities of OS, relapse-free survival (RFS), CRFS, and GVHD-free, relapse-free survival (GRFS) were estimated with the Kaplan-Meier method. Probabilities of engraftment, death not preceded by relapse, recurrent malignancy, aGVHD and cGVHD, permanent discontinuation of systemic corticosteroids, and discontinuation of all systemic immune suppression were summarized by cumulative incidence estimates with competing risks (Data Supplement 1). The relationship between aGVHD and relapse was explored using a Cox proportional hazards model with aGVHD as a time-dependent covariate, adjusting for relapse-risk group and conditioning intensity. Clinical outcome analyses were conducted using R 3.6.0.

RESULTS

Subjects and Graft Engineering

From December 2009 to March 2020, 145 patients age 1-60 years with acute leukemia or advanced myelodysplastic syndrome were enrolled at one of three centers on one of three phase II trials of T_N-depleted PBSC grafts (Data Supplement 1). All patients received Protocol-specified conditioning and post-HCT pharmacologic immunosuppression. Donor PBSC aphereses meeting requirements for cell selection were available for 138 of 145 patients; these patients received T_N-depleted grafts meeting release criteria (Data Supplement 1). The characteristics and outcomes of the recipients of unmanipulated PBSC (n = 6) or umbilical cord blood (n = 1) and an intent-to-treat analysis are in Data Supplement 1.

Cell selection targets were achieved for all 138 T_N-depleted PBSC recipients. Grafts contained a median of 8.4×10^6 $CD34^+$ cells/kg ($3.8\text{-}20 \times 10^6$) and 1×10^7 $CD3^+$ T cells/kg ($1.6\text{-}10.2 \times 10^6$), including a median of 2,500 T_N/kg (300-74,600; interquartile range [IQR], 1,500-4,100; 95%

TABLE 1. Demographic, Clinical, and Transplantation Characteristics of T_N-Depleted PBSC Recipients at Baseline[a]

Characteristic	T_N-Depleted PBSC Recipients (n = 138)
Median age, years (range)	37 (1-60)
Sex, No. (%)	
Male	56 (41)
Female	82 (59)
Performance status score, No. (%)[b]	
≥ 90	103 (75)
< 90	35 (25)
Diagnosis, No. (%)	
Myeloid	72 (52)
AML	59 (43)
MDS with excess blasts	6 (4.4)
Blastic plasmacytoid dendritic cell neoplasm	1 (0.7)
CML with a history of myeloid blast crisis	2 (1.5)
Mixed phenotype acute leukemia	4 (2.9)
Lymphoid	66 (48)
ALL	64 (46)
CML with a history of lymphoid blast crisis	2 (1.5)
Disease risk, No. (%)	
Standard-risk (CR1, no residual disease)	85 (62)
High-risk (beyond CR1 and/or residual disease)[c]	53 (38)
Donor, No. (%)	
HLA-MRD	84 (61)
HLA-MUD	54 (39)
Conditioning regimen, No. (%)	
High-intensity	100 (72)
Intermediate-intensity	38 (28)
GVHD pharmacologic prophylaxis, No. (%)	
Tacrolimus monotherapy	41 (29.7)
Tacrolimus and methotrexate	59 (43)
Tacrolimus and MMF	38 (27.5)
Graft characteristics, No. of cells, median (range)	
$CD34^+ \times 10^6$/kg	8.4 (3.8-19.9)
$CD3^+ \times 10^6$/kg	10 (1.6-10.2)
$CD3^+CD45RA^+ CD45RO^-$ $(T_N) \times 10^4$/kg	0.25 (0.03-7.46)
CMV serostatus, No. (%)	
CMV-seropositive	93 (67.3)
Recipient-positive, donor-positive	55 (40)
Recipient-positive, donor-negative	38 (27.5)
CMV-seronegative	42 (30.4)
Recipient-negative, donor-positive	13 (9.4)
Recipient-negative, donor-negative	29 (21)
CMV serostatus equivocal (recipient)	3 (2.2)
EBV serostatus, No. (%)	
Recipient-seropositive	127 (92)
Recipient-seronegative	11 (8)
Protocol, No. (%)	
NCT00914940	41 (29.7)
NCT01858740	20 (14.5)
NCT02220985	77 (55.8)

Abbreviations: ALL, acute lymphoid leukemia; AML, acute myeloid leukemia; CML, chronic myelogenous leukemia; CMV, cytomegalovirus; CR1, first complete remission; CR2, second complete remission; EBV, Epstein-Barr virus; GVHD, graft-versus-host disease; MDS, myelodysplastic syndrome; MMF, mycophenolate mofetil; MRD, matched related donors; MUD, matched unrelated donors; PBSC, peripheral blood stem cell; T_N, naive T cells.

[a]Percentages may not total 100 because of rounding.

[b]Performance status was measured by the Karnofsky (age ≥ 16 years) or Lansky (age < 16 years) performance status scales. A lower score represents more disability.

[c]Among the 53 high-risk patients, residual disease was present at the time of transplantation in 31 (58%), four with refractory disease (≥ 5% blasts) and 27 with measurable residual disease and < 5% blasts. Among those high-risk patients without residual disease, 9 (17%) had ALL in CR2, 5 (9%) had AML or ALL in CR3, 4 (7.5%) had AML with marrow and extramedullary disease in CR2, 2 (4%) had CML in blast crisis, and 2 (4%) had AML in CR2 with intermediate-risk or normal cytogenetics.

< 10,000 T_N/kg; Table 1). Products administered across trials, institutions, and from MRD and MUD sources did not significantly differ (Data Supplement 1).

Donor Cell Engraftment, Chimerism, and Immune Reconstitution

Neutrophil engraftment occurred at a median of 15 days (9-29) and platelet counts exceeded 20,000/mm^3 without transfusion at a median of 14 days (8-111; Data Supplement 1). Six patients died during the first 100 days, before neutrophil (n = 1) or platelet engraftment (n = 6). Myeloid ($CD33^+$) cells were ≥ 95% donor-derived in all recipients at most or all time points. $CD3^+$ T cells were ≥ 95% donor-derived in most

recipients at all time points (Data Supplement 1). There were no graft rejections. Two patients developed secondary graft failure at nine and 16 months, one with 100% donor myeloid and T-cell chimerism and one with 100% donor myeloid and stable mixed T-cell chimerism (50%-70%).

Lymphocyte recovery was similar to that observed in the unmanipulated PBSC graft recipients (Data Supplement 1), and the numbers of $CD4^+$ and $CD8^+$ T cells exceeded the values reported for pan-TCD in the early post-HCT period.[23,28] $CD8^+$ and $CD4^+$ T_N were generally not observed during the first 6 months post-HCT, as expected.

Graft-Versus-Host Disease

The cumulative incidences of grades III and IV aGVHD were 4% (95% CI, 1 to 8) and 0%, respectively (Fig 2A). The cumulative incidence of grade II aGVHD, which was mostly stage 1 (upper) gastrointestinal GVHD, was 71% (95% CI, 64 to 79; Fig 2B, Data Supplement 1). The incidences of aGVHD are summarized by subgroups in Data Supplement 1. The incidence of grade III aGVHD was very low, regardless of graft source (MRD, 5% [95% CI, 0 to 9]; MUD, 4% [95% CI, 0 to 9]), or conditioning intensity (high-intensity, 5% [95% CI, 1 to 9]; intermediate-intensity, 3% [95% CI, 0 to 8]). The clinical pattern of aGVHD, stage, and response to corticosteroids are in Data Supplement 1. Systemic treatment with agents other than corticosteroids was required in only two subjects. The cumulative incidence of stage 1, 2, 3, and 4 gastrointestinal aGVHD was 69% (95% CI, 61 to 77), 2.2 (95% CI, 0 to 4.7), 1.5% (95% CI, 0 to 3.5), and 0.7% (95% CI, 0 to 2.2), respectively. Although stage 1 gastrointestinal aGVHD, manifested by anorexia and nausea, occurred frequently, it responded to prednisone within seven days in most patients (Data Supplement 1). Thirteen subjects with gastrointestinal aGVHD required only topical beclomethasone with or without budesonide. Of note, grade II-III aGVHD was associated with decreased risks of relapse and death (Fig 3, Data Supplement 1).

Three-year cumulative incidences of mild, moderate, and severe cGVHD were 6% (95% CI, 2 to 10), 1% (95% CI, 0 to 2) and 0%, respectively (Fig 2C). Six of nine patients with cGVHD completed prednisone at a median of 667 days (259-1,366) post-HCT; the others required only tacrolimus (n = 2) or topical corticosteroids (n = 1). Two of the six who received prednisone required additional therapies (Data

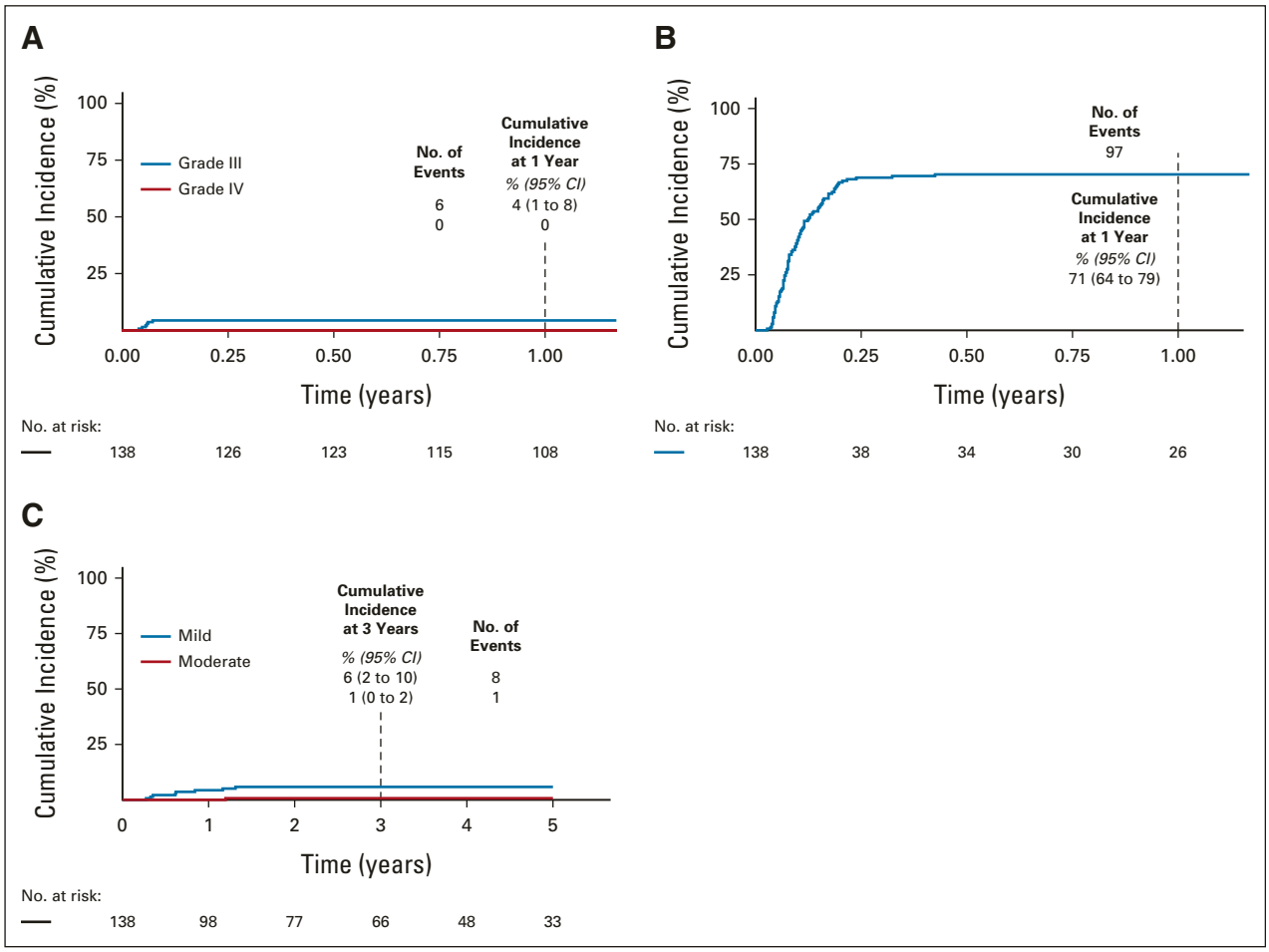

FIG 2. Cumulative incidence of (A) grade III-IV aGVHD, (B) grade II aGVHD, and (C) cGVHD. aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease.

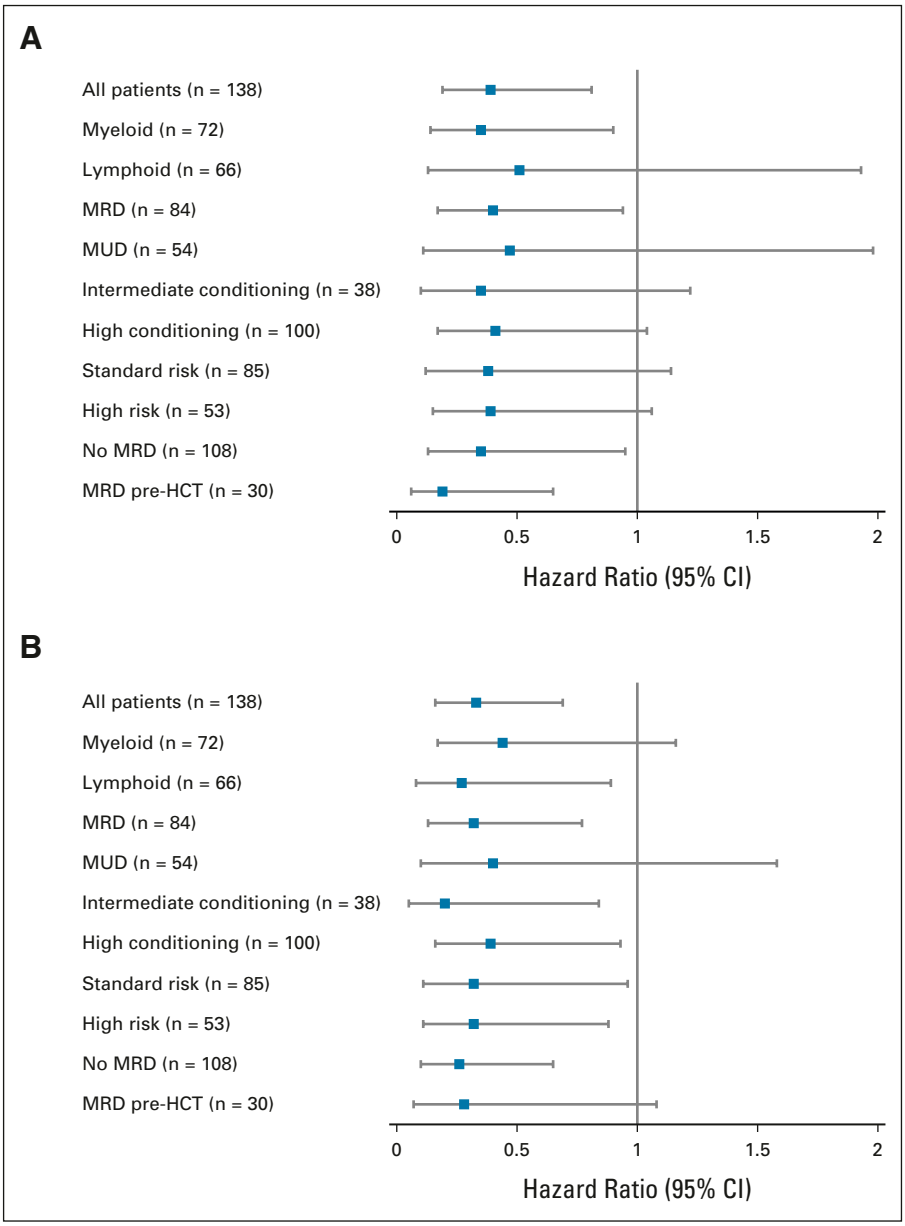

FIG 3. Relative risks of (A) relapse and (B) death in naive T cells-depleted peripheral blood stem cell recipients who developed acute graft-versus-host disease (grade II-III) compared with those who did not. HCT, hematopoietic cell transplantation; MRD, matched related donors; MUD, matched unrelated donors.

Supplement 1). The incidences of cGVHD are summarized by subgroup in Data Supplement 1. The incidence of cGVHD was similar in patients who received T_N-depleted MRD (7% [95% CI, 2 to 13]) or MUD (6% [95% CI, 0 to 12]) PBSC. Of the 94 patients who were alive at 2 years post-HCT, three were receiving systemic immunosuppression for cGVHD management.

Relapse and Survival

Median follow-up of surviving patients was 1,485 days (262-1,826 days). The 3-year cumulative incidence of relapse was 23% (95% CI, 16 to 30; Fig 4A). Relapse was 27% (95% CI, 17 to 37) and 17% (95% CI, 7 to 27) in recipients of MRD or MUD grafts, 19% (95% CI, 11 to 27) and 35% (95% CI, 18 to 52) after high-intensity or intermediate-intensity conditioning, 27% (95% CI, 17 to 38) and 19% (95% CI, 9 to 28) in those with myeloid malignancies or lymphoid leukemia, and 18% (95% CI, 7 to 29) and 26% (95% CI, 17 to 36) in those age $<$ 30 and $\geq$ 30 years, respectively (Data Supplement 1). The median time to relapse was 206 days (56-1,091; IQR, 111-343 days). Among the 31 patients who relapsed, 90% were not on prednisone at the time and most had discontinued (10 of 31) or were rapidly tapered off immunosuppression without developing GVHD (16 of 18 tapered successfully, two flared GVHD, and three had rapid leukemia progression precluding a taper). Of the 31 patients

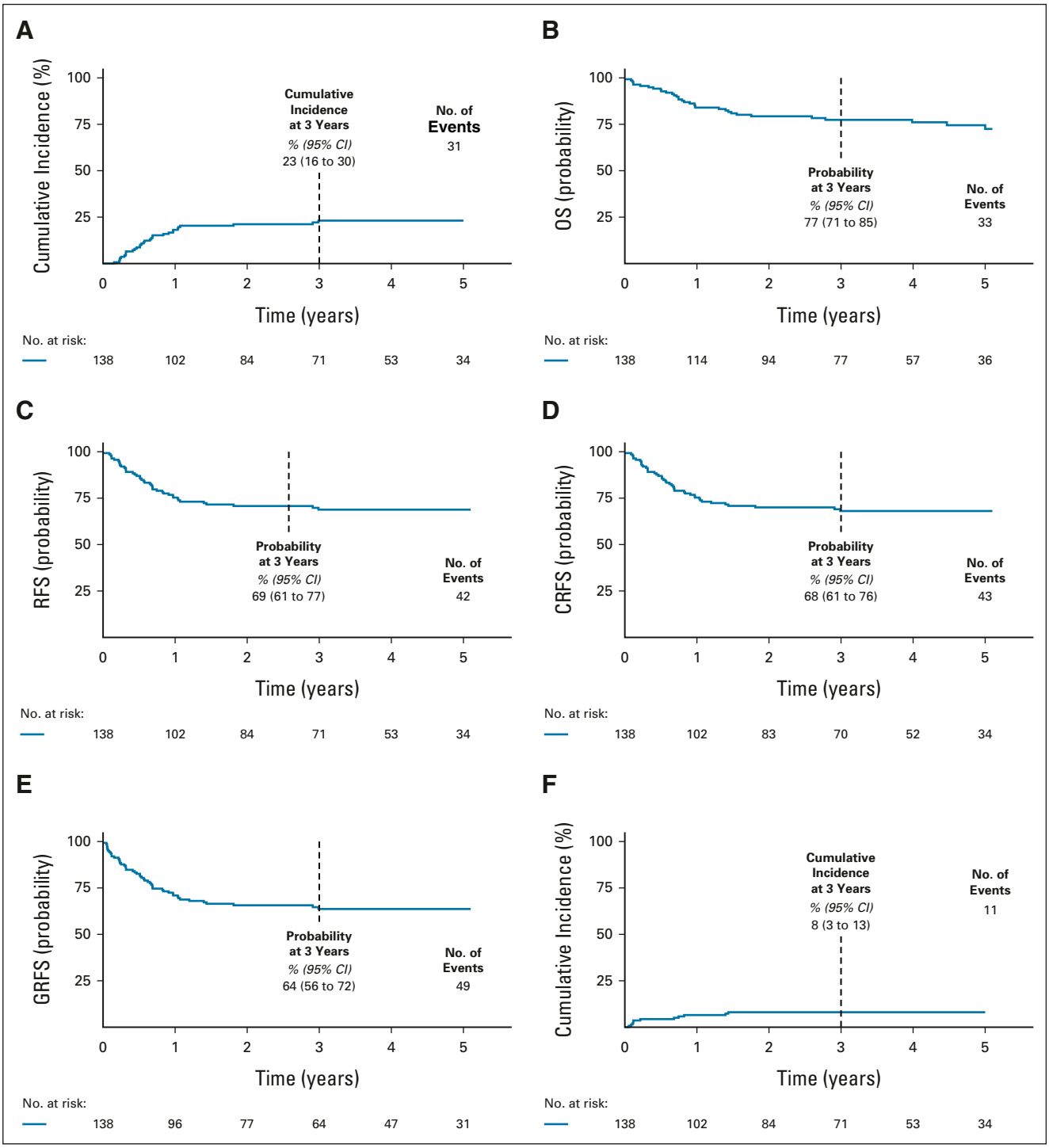

FIG 4. Relapse and survival. (A) Cumulative incidence of relapse, (B) probability of OS, (C) probability of RFS, (D) probability of CRFS, (E) probability of GRFS, and (F) cumulative incidence of NRM. CRFS, cGVHD-free, relapse-free survival; GRFS, GVHD-free, relapse-free survival; NRM, nonrelapse mortality; OS, overall survival; RFS, relapse-free survival.

who relapsed, the median survival from relapse was 273 days (4-1,615 days to death or last follow-up; IQR, 85-590 days). Twenty-one relapsed patients were treated with donor lymphocyte infusions or other immunotherapies; 11 were subsequently in complete remission for at least 6 months (Data Supplement 1).

OS, RFS, CRFS, and GRFS at 3 years were 77% (95% CI, 71 to 85), 69% (95% CI, 61 to 77), 68% (95% CI, 61 to 76), and 64% (95% CI, 56 to 72), respectively (Figs 4B-4E). These probabilities are summarized by subgroup in the Data Supplement (OS, RFS, CRFS, and GRFS) and Figure 5 (CRFS). In recipients of intermediate-intensity

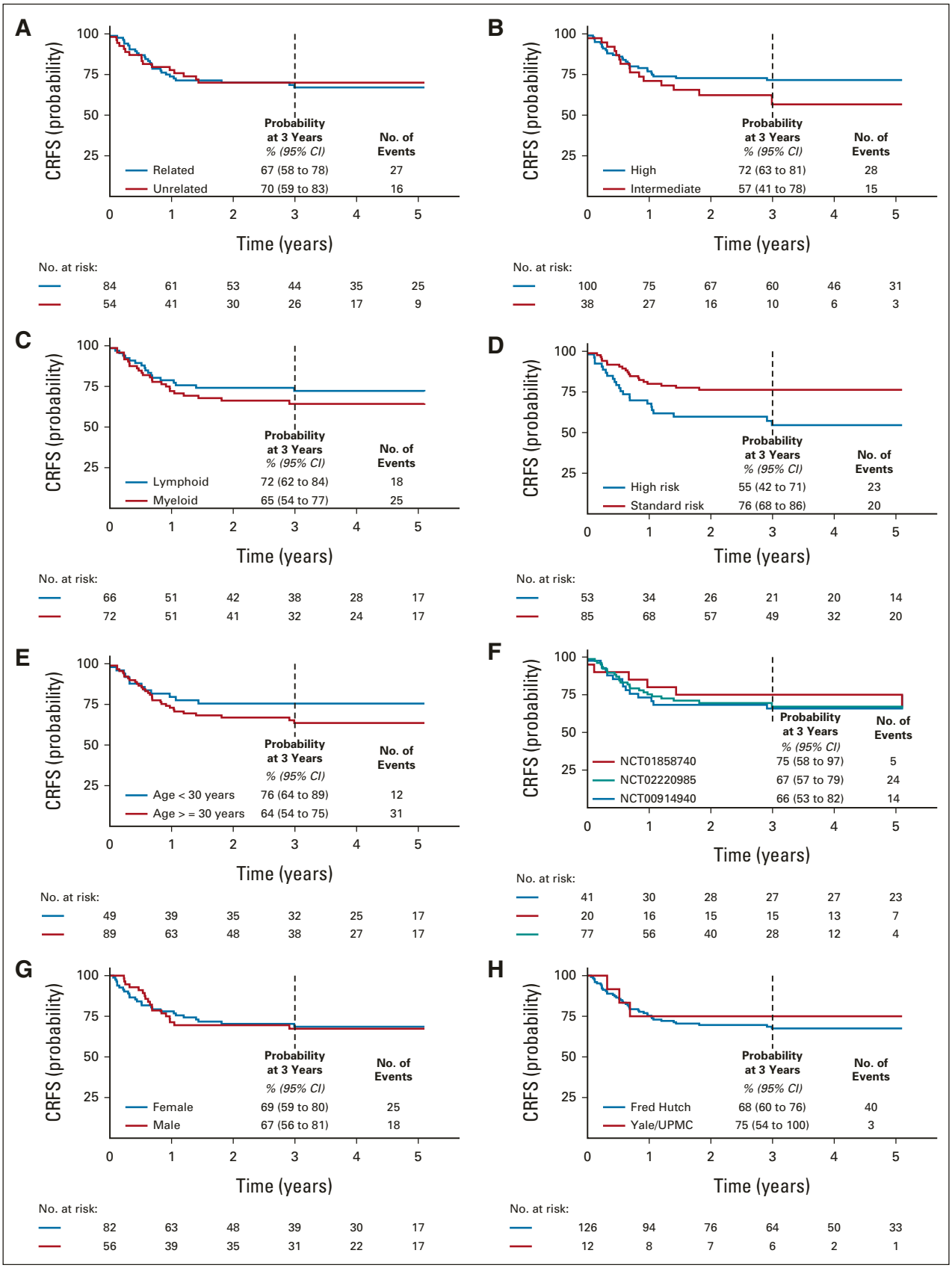

FIG 5. CRFS in subject subgroups. Probability of CRFS by (A) donor source, (B) conditioning intensity, (C) lymphoid or myeloid malignancy, (D) disease risk, (E) age, (F) clinical trial, (G) sex, and (H) institution. CRFS, cGVHD-free, relapse-free survival; UPMC, University of Pittsburgh Medical Center.

conditioning (n = 38; median age, 54 years; 15-60, IQR, 50-57), the OS, RFS, CRFS, and GRFS at 3 years were 78% (95% CI, 66 to 93), 60% (95% CI, 45 to 80), 57% (95% CI, 41 to 78), and 54% (95% CI, 38 to 75), respectively (Data Supplement 1, Fig 5). An intent-to-treat analysis is in Data Supplement 1.

NRM, Causes of Death, Adverse Events, and Infectious Complications

Among recipients of T_N-depleted grafts, the cumulative incidences of NRM at 100 days and 3 years were 4% (95% CI, 1 to 8) and 8% (95% CI, 3 to 13), respectively (Fig 4F). The 3-year NRM estimates were 6% (95% CI, 1 to 11) and 11% (95% CI, 3 to 20) for recipients of MRD and MUD grafts, 9% (95% CI, 3 to 15) and 5% (95% CI, 0 to 13) for recipients of high-intensity and intermediate-intensity conditioning, and 6% (95% CI, 0 to 13) and 9% (95% CI, 3 to 15) in those age < 30 and ≥ 30 years, respectively (Data Supplement 1). No serious reactions occurred with cell infusions (Data Supplement 1). Types and frequencies of grades 3-5 nonhematologic adverse effects were typical of myeloablative HCT recipients (Data Supplement 2, online only). NRM causes are in Data Supplement 1.

Infections are detailed in Data Supplement 1. Epstein-Barr virus (EBV) reactivation was uncommon (EBV $\geq$ 1,000 copies/mL plasma in three [2.1%] subjects); only one of 138 subjects required rituximab to manage EBV reactivation. One and two subjects had human herpesvirus 6 ($>$ 1,000 copies/mL) or adenovirus ($>$ 300 copies/mL) reactivations. The cumulative incidence of cytomegalovirus reactivation ($\geq$ 500 IU/mL) at day 180 (12%) was similar to that reported after pan-TCD (11.6%) and T-cell–replete (9.6%) HCT.[6] Possible, probable, or proven cytomegalovirus disease occurred in 4.3% of subjects.

DISCUSSION

Here, we report on 138 recipients of T_N-depleted HLA-matched allografts. Cell processing was reliable using widely available commercial technology. aGVHD was mild and corticosteroid-responsive; use of second-line agents to treat aGVHD was rarely required. Strikingly, only 7% of patients developed cGVHD, which was also mostly mild and steroid-responsive. The decrement in cGVHD was not associated with an apparent increase in relapse or fatal infections, yielding favorable OS, RFS, and CRFS rates regardless of graft source, conditioning intensity, or patient age.

Prior studies of pharmacologic approaches for GVHD reduction with unmanipulated grafts have reported low rates of severe aGVHD but have not reduced cGVHD to the levels observed with T_N-depletion (Data Supplement 1). In the recent multiarm Blood and Marrow Transplant Clinical Trials Network (BMT CTN) 1301 randomized controlled trial (RCT) of GVHD prophylaxis methods, the arm using bone marrow transplantation with PTCy for GVHD prophylaxis reported a 2-year incidence of moderate-to-severe cGVHD of 27%, similar to the tacrolimus and methotrexate arm (33.7%).[6] Other studies of PTCy in HLA-matched HCT reported cGVHD and moderate-to-severe cGVHD rates of 13%-42% and up to 30%, respectively (Data Supplement 1). In RCTs, ATG or ATLG reduced aGVHD, cGVHD, or both; however, cGVHD and moderate-severe cGVHD rates were 16%-34% and 8%-16%, respectively (Data Supplement 1). In our trials, severe aGVHD rates were comparable to those observed with the addition of sitagliptin[29] abatacept,[30] ATG or ATLG to standard immunosuppression with unmanipulated HCT, and to bone marrow transplantation with PTCy.[6] However, overall (7%) and moderate-to-severe (1%) cGVHD were less frequent with T_N-depletion.

Ex vivo pan-TCD reduces both aGVHD and cGVHD (Data Supplement 1).[28,31] In BMT CTN 1301, the pan-TCD PBSC arm had a low 2-year incidence of cGVHD (22.5% total and 8.9% moderate-to-severe)[6] but high NRM (21.5%), partially because of opportunistic infections. By contrast, with T_N-depletion, cGVHD was very infrequent and NRM was only 8% at 3 years, reflecting the paucity of fatal opportunistic infections. In particular, high-level EBV reactivation, which can lead to fatal lymphoproliferative disorders and is more common with pan-TCD,[6,28] was rare in T_N-depleted graft recipients, consistent with the transfer of donor EBV-specific T_M. The higher NRM with pan-TCD could be partially because of the administration of ATG combined with ex-vivo graft lymphocyte depletion. By contrast, T_N-depleted grafts were used without ATG, thereby preserving graft T_M. The 8% incidence of NRM in T_N-depleted HCT is similar or lower than NRM reported in other forms of HCT (Data Supplement 1).

Although cGVHD and severe aGVHD rates were low with T_N-depletion, a syndrome of corticosteroid-responsive stage 1 upper GI aGVHD, characterized by nausea and anorexia and associated with a mild increase in apoptotic epithelial cells, was frequently observed. This syndrome was associated with protection from relapse and death, suggesting a connection to GVL and an alloimmune mechanism. Given that aGVHD is the greatest risk factor for cGVHD,[32] the disconnect between aGVHD and cGVHD in T_N-depleted HCT implies fundamental functional differences between the progeny of alloreactive T_M and T_N, such that T_M cause only limited aGVHD and rare cGVHD, yet provide some protection from relapse.

T cell receptor (TCR)–repertoire–dependent and –independent differences between T_N and T_M have been studied in mouse GVHD models.[18-20,33-36] T_N have a more diverse TCR repertoire and a greater frequency of minor histocompatibility (H) antigen-reactive T cells.[21] The remaining T_M in T_N-depleted grafts may target fewer minor H antigens with fewer unique TCRs targeting each. This might generate a sufficient alloresponse to induce limited aGVHD and some GVL but be generally insufficient to initiate or sustain cGVHD.[37] Other cell-intrinsic, repertoire-independent differences between T_N and T_M, such as where they traffic and their potential for clonal expansion and differentiation, may also contribute to their

disparate capacities to induce severe or cGVHD.[18-20,34-36] Testing these hypotheses in humans will require the ability to broadly characterize minor H antigen-specific T cells in tissues of HCT recipients, which is not practical with current technologies.

Relative to published cohorts that received myeloablative conditioning and unmanipulated MUD or MRD grafts, an increase in leukemia relapse was not observed in T_N-depleted graft recipients (Data Supplement 1), suggesting GVL activity was at least partially retained. Although (62%) of our subjects were in CR1 without measurable residual disease, such patients remain at risk of relapse, with 3-year relapse rates of 22% reported in 235 patients transplanted with AML in remission with no measurable disease by multiparametric flow cytometry,[38] and relapse of 20% reported even in patients with AML transplanted in a measurable residual disease-negative remission by highly sensitive next-generation sequencing.[39] Thus, the 23% cumulative incidence of relapse in our cohort, and 17% among our CR1 measurable residual disease-negative subjects, is consistent with previous experience. Randomized trials comparing T_N-depletion to other strategies would better clarify any impact of T_N-depletion on relapse.

Drugs used to prevent and treat GVHD may limit the use and efficacy of promising engineered T-cell immunotherapies for post-HCT relapse.[40-42] Because of the shorter duration of pharmacologic immunosuppression, T_N-depleted HCT may facilitate such post-HCT immunotherapies. Notably, immunosuppression was discontinued in most of our patients with early relapse without subsequent GVHD, suggesting that routine immunosuppression tapering after T_N-depleted HCT could be accelerated.

The strengths of our research include the low rate of cGVHD and high CRFS in a sizable cohort of 138 patients across three clinical trials, observed across major subgroups including recipients of MRD and MUD grafts, high- and intermediate-intensity conditioning regimens, and immunosuppression with tacrolimus monotherapy or tacrolimus with methotrexate or MMF. Our results support the use of graft T_N-depletion as a broadly applicable approach for cGVHD reduction (Data Supplement 1, Fig 5).

Study limitations include the possibility that the lower total T-cell dose administered with T_N-depleted grafts might explain the cGVHD reduction. Although the T-cell dose in our trials (10^7 $CD3^+$ cells/kg) is less than in unselected PBSC grafts (around 20-30 $\times$ 10^7/kg), it is in the same range as the $CD3^+$ content of bone marrow grafts (2-3 $\times$ 10^7/kg). In the randomized trial of PBSC versus BMT[5] for which the T-cell doses were subsequently published,[43] the incidence of cGVHD at 2 years in the PBSC group was 53% with a median T-cell dose of 24.6 $\times$ 10^7/kg, compared with 41% with a median T-cell dose of 2.3 $\times$ 10^7/kg in the BMT group. If a 90% difference in the T-cell content of grafts (24.6 $\times$ 10^7 to 2.3 $\times$ 10^7/kg) is associated with a decrease in cGVHD of 53% to 41%, it seems highly unlikely that a 95% reduction in total T cells (24.6 $\times$ 10^7 to 1.0 $\times$ 10^7/kg) would result in a cGVHD rate of $<$ 10%, and much more likely that the multilog reduction of the T_N content of the graft was responsible.

We acknowledge the limitations of single-arm phase II studies, including the risk of type 1 errors. Given the size and composition of our cohorts and the magnitude of the reduction in cGVHD observed across all trials and subgroups, it is unlikely that a randomized trial would disprove that T_N-depletion results in a much lower incidence of cGVHD (7% [95% CI, 2 to 11]) than unselected PBSC transplantation with tacrolimus and methotrexate GVHD prophylaxis. Historical cGVHD rates at Fred Hutch, the center that enrolled $>$ 90% of the patients, were 45% for MUD and 42% for MRD recipients.[44] Rates of cGVHD reported in the literature have remained stable with incidences of all cGVHD and moderate-severe cGVHD of 37%-45% and 34%-36%, respectively, reported in the control arms of phase III RCTs between 2019 and 2021 (Data Supplement 1). However, randomized trials will be important to confirm beyond doubt the cGVHD reduction and to definitively determine how relapse, survival, and composite end points such as GRFS in recipients of T_N-depleted HCT compare to patients treated with standard HCT and other GVHD-reduction strategies. To address this, we have initiated two phase II RCTs. NCT03970096 compares T_N-depleted PBSCT to T-cell–replete PBSCT with tacrolimus and methotrexate or with PTCy, with the goal of determining whether T_N-depleted HSC is sufficiently promising to justify comparing it to other approaches in a pivotal phase III RCT. NCT03779854 is a Pediatric Transplantation and Cellular Therapy Consortium trial that compares T_N-depleted PBSCT to T-cell–replete BMT with tacrolimus and methotrexate.

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DISCLAIMER

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Naive T-Cell Depletion to Prevent Chronic Graft-Versus-Host Disease

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