Total body irradiation dose escalation decreases risk of progression and graft rejection after hematopoietic cell transplantation for myelodysplastic syndromes or myeloproliferative neoplasms

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ABSTRACT

A non-myeloablative regimen of fludarabine and 200 cGy total body irradiation combined with post-grafting immunosuppression with mycophenolate mofetil and a calcineurin inhibitor facilitates allogeneic hematopoietic cell transplantation from HLA-matched related or unrelated donors in older patients and/or those with comorbidities. However, outcomes of prior studies have been disappointing in patients with myelodysplastic syndromes or myeloproliferative neoplasms due to high incidences of progression or graft failure (together termed hematopoietic cell transplantation-failure). We hypothesized that escalating the total body irradiation dose may improve the outcomes and subsequently performed a phase II total body irradiation dose-escalation trial. Patients with median age 66 years were enrolled in two arms to receive non-myeloablative conditioning followed by hematopoietic cell transplantation with total body irradiation dose escalation for excessive hematopoietic cell transplantation-failure: Arm A: myeloproliferative neoplasm/myelodysplastic syndrome low risk (n=36); and Arm B: myelodysplastic syndrome high-risk/chronic myelomonocytic leukemia (n=41). Total body irradiation dose levels were: Level-1 (300 cGy), Level-2 (400 cGy), or Level-3 (450 cGy). Patients received intravenous fludarabine 30 mg/m² for three days. Total body irradiation was administered on day 0 followed by infusion of peripheral blood stem cells from HLA-matched related (n=30) or unrelated (n=47) donors. Post-grafting immunosuppression with mycophenolate mofetil and cyclosporine was administered. The primary end point was day 200 hematopoietic cell transplant failure, with the objective of reducing the incidence to <20%. The primary end point was reached on Arm A at dose Level-1 (300 cGy total body irradiation) with a cumulative incidence of day 200 hematopoietic cell transplant failure of 11%, and on Arm B at dose Level-3 (450 cGy) with a cumulative incidence of day 200 hematopoietic cell transplant failure of 9%. Increasing the total body irradiation dose leads to a higher success rate with non-myeloablative conditioning by reducing relapse and rejection. Further studies are necessary to decrease non-relapse mortality, especially among patients with high-risk disease. Trial registered under *clinicaltrials.gov identifier: NCT00397813*.

Introduction

Hematopoietic cell transplantation (HCT) is the only curative option for patients with myelodysplastic syndromes (MDS) or myeloproliferative neoplasms (MPN).[1] While these disorders are usually seen in older individuals, conventional HCT after

intensive cytotoxic conditioning regimen has been restricted to relatively young patients, without comorbidities. In order to address these limitations, we designed a non-myeloablative (NMA) regimen specifically for older patients and in those with comorbidities. This regimen consists of fludarabine (FLU) and 200 cGy total body irradiation (TBI) combined with post-grafting immunosuppression with mycophenolate mofetil (MMF) and a calcineurin inhibitor that facilitates allogeneic HCT with HLA-matched related (MRD) or unrelated donors (URD). Results of prior studies with this regimen have been very encouraging in most hematologic malignancies;[2-5] however, in patients with chronic myelomonocytic leukemia (CMML) or previously untreated MDS/MPN, results have not been as successful. In a previous study, the cumulative incidence of HCT failure, defined as graft rejection or disease progression before day 200 after HCT, was 46% for the entire study population, 58% for patients with CMML or MDS with excess blasts (MDS-EB), and 31% for patients with MDS [comprising MDS with single or multilineage dysplasia (MDS-SLD or MDS-MLD), MDS with ring sideroblasts (MDS-RS), MDS, unclassifiable (MDS-U) MDS with <5% blasts], or MPN. The 200-day, 1-year and 2-year probabilities of progression-free survival (PFS) were 27%, 19%, and 11%, respectively, in patients with CMML or MDS-EB, and 46%, 40%, and 37%, respectively, in patients with MDS with <5% blasts or MPN.

Furthermore, it was shown that early achievement of full donor T-cell chimerism was associated with a reduced risk of relapse (HR 0.5, *P*=0.002) in patients with hematologic malignancies receiving NMA conditioning.[6]

Taken together, these data suggest that increasing the intensity of the non-myeloablative conditioning regimen might delay disease progression until establishment of full donor chimerism and development of graft-*versus*-tumor (GvT) effects, thereby improving outcomes of patients with CMML, untreated MDS, and MPN.

Methods

Study design

A phase II TBI dose escalation trial (Figure 1) was conducted at Fred Hutchinson Cancer Research Center, Veterans Affairs Puget-Sound Health Care System, and Intermountain LDS Hospital. The Institutional Review Boards of the participating centers approved the protocol; all patients signed informed consent. An independent Data and Safety Monitoring Board oversaw the trial. The trial was registered at *ClinicalTrials.gov*, a service of the United States National Institutes of Health under number NCT00397813.

Patients

Participants were eligible if they had a World Health Organization defined diagnosis of CMML, MDS, MPN or paroxysmal nocturnal hemoglobinuria (PNH) with history of life-threatening complications.

Patients were ≥50 and <75 years of age or were <50 years of age but at high risk for therapy-related toxicity from standard high-dose regimens; factors considered high risk included pre-existing conditions such as a chronic disease affecting kidneys, liver, lungs or heart, or previous failed HCT. Included patients were affected by MDS or MPN with less than 10% of blasts and not treated by

myelosuppressive chemotherapy (defined as chemotherapy given with the intent of inducing a complete remission; hypomethylating agents and oral cytoreductive therapy were permitted). Patients with CMML who had progressed and received myelosuppressive chemotherapy could be enrolled if they had less than 5% of marrow blasts.

Donors were either HLA-MRD or URD; unrelated donors with only a single allele disparity for HLA-A, B, or C as defined by high-resolution typing were allowed. Only granulocyte-colony stimulating factor (G-CSF) mobilized peripheral blood stem cells (PBSC) were permitted as a HCT source.

Conditioning regimens and immune suppression

The conditioning regimens (Figure 2) were FLU (30 mg/m^2/day IV) on days -4, -3, -2 and TBI of three magnitudes: 300 cGy (Level 1), 400 cGy (Level 2), or 450 cGy (Level 3) on day 0 at 6-7 cGy/min from linear accelerator, followed by HCT. For related recipients, graft-*versus*-host disease (GvHD) prophylaxis included cyclosporine (CSP) 5 mg/kg PO every 12 hours from day -3 to day +56 with subsequent taper to day +180 and mycophenolate mofetil (MMF) at 15 mg/kg PO every 12 hours from day 0 to day +27 and then discontinued. For unrelated recipients, GvHD prophylaxis included CSP 5 mg/kg PO every 12 hours from day -3 to day +100 with taper to day +180 and MMF at 15 mg/kg PO every eight hours from day 0 to day +40 and then tapering to day +96. Growth factors were not given during the first 21 days after HCT; infection prophylaxis and transfusion support were as per institutional guidelines. Bone marrow aspirates or biopsies and chimerism analyses were performed on days +28, +56, +84 and +180 after HCT.

Outcomes

The purpose of this study was to evaluate whether a more intense but still NMA conditioning regimen could reduce the combined rates of graft rejection and disease progression (together termed HCT-failure) in this group of MDS and CMML patients, while maintaining an acceptable rate of non-relapse mortality (NRM).

The primary objective was to decrease the incidence of day-200 HCT-failure to <20%. HCT-failure in this study was defined as either progression of the underlying malignancy or graft failure. Progression was defined as recurrence of disease as detected by flow cytometry, morphology, cytogenetics, or mutation analysis. For patients with myelofibrosis, persistent fibrosis was not considered evidence of progression. Graft failure was defined as <5% donor CD3 chimerism in the peripheral blood. Secondary objectives included PFS, NRM, and the kinetics of donor engraftment.

Primary cause of death was adjudicated using previously described criteria.[7] When relapse occurred, it was considered the primary cause of death regardless of other events. Additionally, acute and chronic GvHD incidences were also evaluated. Grading of acute and chronic GvHD was performed according to the established recognized standards.[8] The first day of acute GvHD of a certain grade was used to calculate cumulative incidence curves for that GvHD grade.

Statistical analysis

For purposes of this study, HCT-failure was defined as

graft rejection (defined as <5% donor T-cell chimerism) or disease progression within 200 days of transplant.

Dose escalation was carried out independently in two groups of patients:
- Arm A: patients with MPN, low-risk MDS (MDS-SLD, MDS-MLD, MDS-U, MDS-RS) or PNH;
- Arm B: patients with high-risk MDS (MDS-EB-1) or CMML.

In each arm, up to 24 patients were accrued to each TBI dose level, in groups of 6 patients, with an escalation rule triggered for excessive HCT failure. If 24 patients were successfully enrolled at a TBI dose level without triggering the escalation rule for HCT failure (or other stopping rules), then that dose level would be considered a success and accrual would be closed for that arm. ARM A was modified after completing accrual of 24 patients at a dose level, to allow accrual of up to 12 additional patients at the same dose level while accrual to Arm B continued. Stopping rules for non-relapse mortality and TBI dose escalation continued to be monitored. Cohorts of 6 patients were only defined for purposes of determining dose escalation and stopping rules; the statistical analysis was based on the operating characteristics of a sequence of cohorts of 6 patients, not on single cohorts.

All patients received FLU 30 mg/m²/day IV for three days preceding TBI.

The TBI dose levels were:
1. Dose Level 1: 300 cGy TBI
2. Dose Level 2: 400 cGy TBI
3. Dose Level 3: 450 cGy TBI

Dose escalation rules were imposed for HCT failure >20% at day +200. Stopping rules were imposed for NRM at day 200 of >25% in Arm A and >35% in Arm B. Overall survival (OS) and PFS were estimated by the Kaplan-Meier method. Cumulative incidences of relapse, NRM, and acute and chronic GvHD were estimated as previously described.[9] All analyses were performed using SAS (SAS Institute, Cary, NC, USA).

Results

Enrollment

The study was opened on March 2006 and was closed to accrual on March 2017. Overall, 79 patients were screened, and 77 patients were enrolled. Patients' and

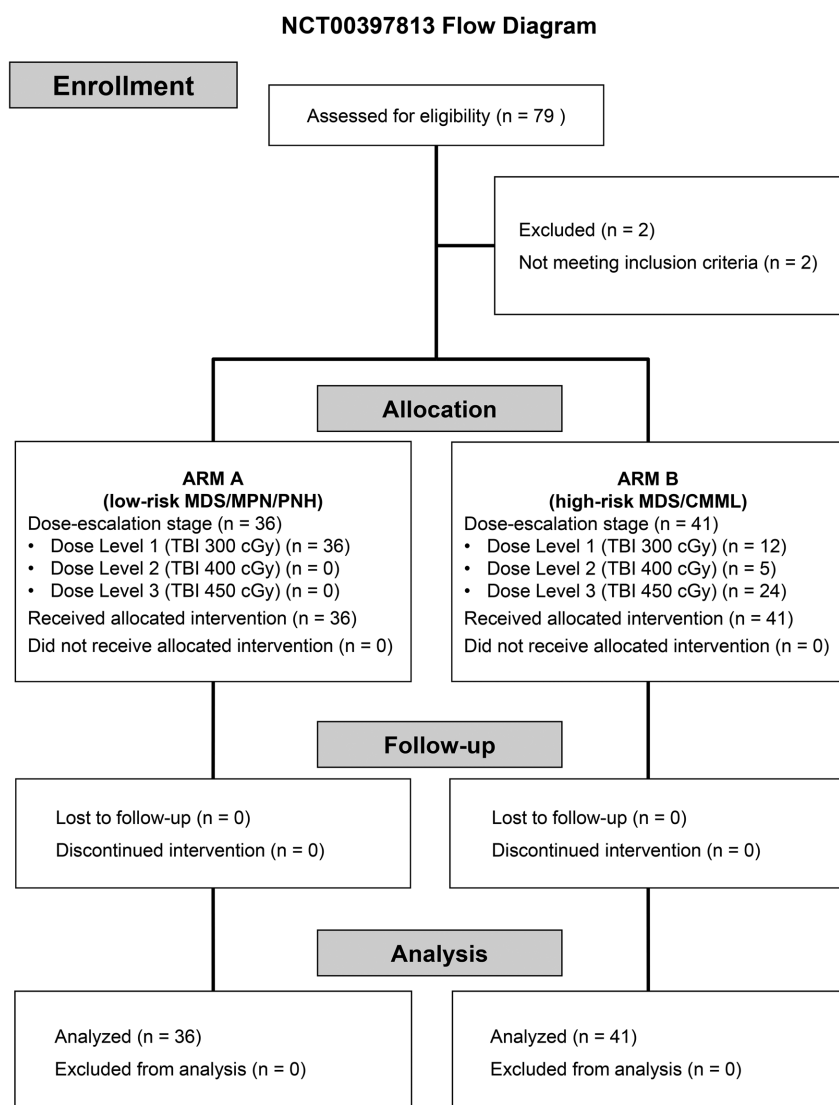**NCT00397813 Flow Diagram**

Figure 1. Flow diagram of the progress through the phases of the study. MDS: myelodysplastic syndrome; MPN: myeloproliferative neoplasm; PNH: paroxysmal nocturnal hemoglobinuria; CMML: chronic myelomonocytic leukemia; TBI: total body irradiation; cGy: centigray; n: number.

transplant characteristics are summarized in Table 1. Thirty-six patients were enrolled in Arm A and 41 in Arm B.

Engraftment

Neutrophils declined below 0.5x10⁹/L in 35 out of 36 patients in Arm A and in all patients in Arm B. The cumulative incidences of neutrophil recovery at day 28 were 100% in Arm A and 97.4% in Arm B. The median time to neutrophil >0.5x10⁹/L was 13.5 days in Arm A and 13 days in Arm B. The platelet counts did not decline below 20x10⁹/L in 9 out of 36 (25%) patients in Arm A and in 11 out of 41 (26.8%) patients in Arm B. The cumulative incidences of platelet recovery (>20x10⁹/L) at day 84 were 97.2% in Arm A and 100% in Arm B. The median times to platelet recovery were 3.5 days in Arm A and 7 days in Arm B. There were two graft failures, both in patients enrolled in Arm A.

Table 1. Patients' characteristics.

	ARM A (n = 36)	ARM B (n = 41)
Sex, n (%)		
Female	9 (25%)	14 (34.1%)
Male	27 (75%)	27 (65.9%)
Diagnosis, n (%)		
Myelofibrosis	18 (50%)	
Low-risk MDS (MDS-SLD, MDS-MLD, MDS-U, MDS-RS)	17 (47.2%)	
PNH	1 (2.8%)	
High-risk MDS (MDS-EB-1)		28 (68.3%)
CMML		13 (31.7%)
Treatment, n (%)		
Dose level 1 (TBI 300 cGy)	39 (100%)	12 (39.3%)
Dose level 2 (TBI 400 cGy)		5 (12.2%)
Dose level 3 (TBI 450 cGy)		24 (58.5%)
Time from diagnosis to HCT, month, median (range)	15.4 (4–336)	8.4 (1–92)
Age at transplant, years, median (range)	65 (37–71)	67 (52–74)
HCT-CI, n (%)	Median 3 (range 0–8)	Median 3 (range 0–9)
0	4 (11.1%)	7 (17.1%)
1–2	11 (30.6%)	12 (29.3%)
≥3	21 (58.3%)	22 (53.6%)
Previous stem-cell transplantation, n		
Autologous	0	2
Allogeneic	0	2
Donor, n (%)		
MRD	16 (44.4%)	14 (34.1%)
URD 10/10	18 (50%)	25 (61%)
URD 9/10	2 (5.6%)	2 (4.9%)
Neutrophils <0.5×10⁹/L, days, median (range)	13.5 (0–28)	13 (2–29)
Platelets <20×10⁹L, days, median (range)	3.5 (0–107)	7 (0–43)
HCT-failure before day +200, n		
Relapse	3	10
Graft failure	2	
Relapse, n	9	15
Death, n	24	31
Cause of death, n		
Progression/relapse	9	15
NRM non-GvHD related	6	6
	Pneumonia n = 3	Pneumonia n = 2
	Sepsis n = 1	Cardiac toxicity n = 2
	Disseminated aspergillus n = 1	Multiorgan failure n = 2
	Suicide n = 1	
NRM GvHD related	9	10
	GvHD refractory n = 4	GvHD refractory n = 3
	Pneumonia n = 2	Pneumonia n = 2
	Multiorgan failure n = 1	Sepsis n = 4
	CNS bleeding n = 1	Disseminated aspergillus n = 1
	Sepsis n = 1	
Follow up, months, median (range)	69.1 (39.1–121.8)	21 (3.4–102.6)

CMML: chronic myelomonocytic leukemia; CNS: central nervous system; GvHD: graft-*versus*-host disease; HCT-CI: hematopoietic cell transplantation comorbidity index; MDS: myelodysplastic syndrome; MDS-EB myelodysplastic syndrome with excess of blasts-1; MDS-RS: myelodysplastic syndrome with ring sideroblast; MDS-U: myelodysplastic syndrome unclassifiable; MLD: multilineage dysplasia; MRD: HLA-matched related donor; NRM: non-relapse mortality; PNH: paroxysmal nocturnal hemoglobinuria; SLD: single lineage dysplasia; TBI: total body irradiation; URD: HLA-matched unrelated donor.

Chimerism analysis

In Arm A, the day 28 median donor chimerism levels were 68% for CD3 and 100% for CD33, and the day 84 levels were 79% and 100%, respectively. In Arm B, for patients who received TBI at dose Levels-1 or 2 (300 cGy or 400 cGy), the day 28 median donor chimerism levels were 84% for CD3 and 77% for CD33, and the day 84 levels were 85% and 100%, respectively. For patients who received TBI at dose Level-3 (450 cGy) in Arm B, the day 28 median donor chimerism levels were 82% for CD3 and 100% for CD33, and the day 84 levels were 81% and 100%, respectively.

Hematopoietic cell transplantation failure and relapse

In Arm A, 4 out of 36 patients given TBI at 300 cGy had HCT-failure before day 200, three due to progression and one to graft rejection, with a cumulative incidence of 11%. The escalation rule in this arm was not reached so all patients received dose Level-1 with TBI at 300 cGy. In Arm B, 12 patients received dose Level-1 with TBI at 300 cGy and 5 had HCT-failure, all due to progression, triggering the TBI dose escalation rule. The next five patients received dose Level-2 TBI (400 cGy) and three experienced HCT-failure, all due to progression, triggering again the dose escalation rule. The cumulative incidence of HCT-failure for dose Level-1 and dose Level-2 combined was 47%. The subsequent 24 patients received dose Level-3 with TBI at 450 cGy and only two experienced HCT-failure before 200 days, with a cumulative incidence of 9%. The patients' characteristics and outcomes for the high-risk patients enrolled on Arm B (separated by TBI dose) are shown in *Online Supplementary Table S1* (Table 2 and Figure 3A and E).

The cumulative incidences of progression in Arm A at one year, two years, and five years was 17%, 19%, and 22%, respectively. Patients who received TBI at dose Levels-1 or 2 (300 cGy or 400 cGy) had cumulative incidences of progression at one year, two years, and five years of 53%, 53% and 53%, respectively. Patients who received TBI at dose Level-3 (450 cGy) in Arm B had cumulative incidences of progression at one year, two years, and five years of 24%, 24% and 32%, respectively.

Graft-*versus*-host disease

The cumulative incidences of grades II-IV and grade III-IV acute GvHD at day 100 were 58% and 13%, respectively. The cumulative incidences of chronic GvHD at one year and two years were 38% and 45%, respectively: one, 21, and eight patients developed mild, moderate, and severe chronic GvHD, respectively. No statistically significant differences were seen between each arm with regards to incidence of acute or chronic GvHD (cumulative incidences in each arm are shown in Table 2) (Figure 4).

Non-relapse mortality

The cumulative incidences of NRM in Arm A at one year, two years, and five years were 17%, 22%, and 43%, respectively. Patients who received TBI at dose Levels-1 or 2 (300 cGy or 400 cGy) had cumulative incidences of NRM at one year, two years, and five years of 29%, 35%, and 35%, respectively. Patients in Arm B who received TBI at dose Level-3 (450 cGy) had cumulative incidences of NRM at one year, two years, and five years of 31%, 43%, and 51%, respectively (Table 2 and Figure 3D).
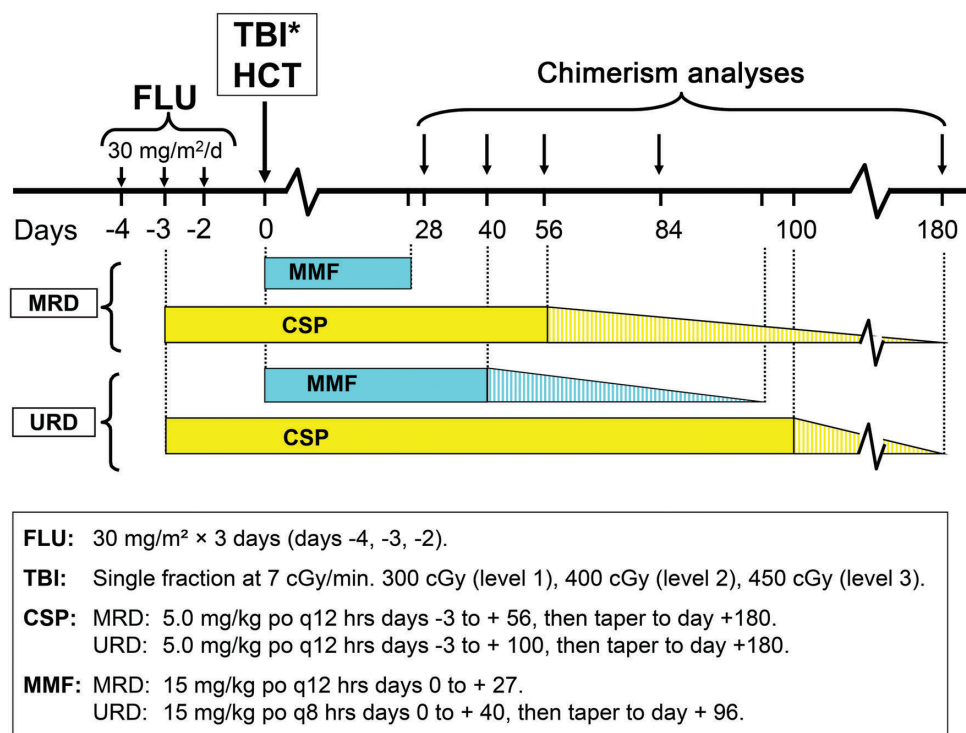

Figure 2. Outline of treatment plan involving conditioning regimens and graft-*versus*-host disease (GvHD) prophylaxis. FLU: fludarabine; TBI: total body irradiation; HCT: hematopoietic cell transplantation; MRD: HLA-matched related donor; URD: HLA-matched unrelated donor; MMF: mycophenolate mofetil; CSP: cyclosporine; cGy: centigray; hrs: hours.

Causes of death

Twenty-four of 36 patients in Arm A died; GvHD and disease progression were the primary causes of death for nine patients each. In Arm B, 31 out of 41 patients died; progression was the primary cause of death for 15 patients (Table 1).

Overall survival and progression-free survival

In Arm A, OS at one year, two years, and five years were 72%, 61%, and 38%, respectively, and PFS were 68%, 58%, and 35%, respectively. Patients who received TBI at dose Levels 1 or 2 (300 cGy or 400 cGy) in Arm B had OS at one year, two years, and five years of 24%, 12%, and 12%, respectively, and PFS of 18%, 12%, and 12%, respectively. Patients in Arm B who received TBI at dose Level-3 (450 cGy) had OS at one year, two years, and five years of 60%, 37%, and 22%, respectively, and PFS of 45%, 33%, and 17%, respectively (Table 2 and Figure 3B and C).

Discussion

In the early 2000s, at Fred Hutchinson Cancer Research Center, a regimen for NMA conditioning using the combination of fludarabine and 200 cGy TBI was developed. This regimen allowed for donor cell engraftment but was characterized by a high incidence of early disease progression or graft rejection,[2-5] especially for high-risk patients. In 2006, Scott *et al.*[10] published results on 38 patients affected by MDS or acute myeloid leukemia (AML) who received

an NMA regimen with 3-year rates of OS, PFS, and NRM of 27%, 28%, and 41%, respectively. These results were retrospectively compared with patients who underwent myeloablative conditioning, and no statistically significant differences were seen. Disease progression was the main issue with a cumulative incidence of 31% at three years. Laport *et al.*[11] reported data from our retrospective study on 148 patients diagnosed with MDS or MPN who underwent allogeneic HCT after NMA conditioning. PFS and OS at three years were both 27% for all patients, with a progression incidence of 41%. When stratifying data, PFS rates at three years for patients with *de novo* MDS (n=40), treatment-related MDS (n=25), MPN (n=27), and CMML (n=7) were 22%, 29%, 37%, and 43%, respectively, and OS rates were 20%, 27%, 43% and 43%, respectively. For all patients, NRM at three years was 32%. In both analyses, disease progression was the major cause of treatment failure and the leading cause of death; it appears that this regimen may not have conferred enough cytoreduction for adequate disease control. The data from these retrospective reviews formed the basis for the current study.

Cliff *et al.*[12] had previously shown that an escalation in TBI dose to 15.75 Gy from 12 Gy reduced relapse/progression (13% *vs.* 35%). This result demonstrated that a decrease in tumor burden before transplantation led to a decreased risk of disease progression; however, most patients with MDS or MPN are not candidates for myeloablative TBI conditioning regimens either due to advanced age or comorbidities.

Taking into consideration these data, along with results from pre-clinical studies,[13,14] we hypothesized that raising

Table 2. Results.

	% at 100 days	% at 1 year	% at 2 years	% at 5 years
Overall survival				
Arm A TBI dose Level-1(300 cGy)		72	61	38
Arm B TBI dose Level-1 or 2 (300–400 cGy)		24	12	12
Arm B TBI dose Level-3 (450 cGy)		60	37	22
Progression-free survival				
Arm A TBI dose Level-1(300 cGy)		68	58	35
Arm B TBI dose Level-1 or 2 (300–400 cGy)		18	12	12
Arm B TBI dose Level-3 (450 cGy)		45	33	17
Relapse incidence				
Arm A TBI dose Level-1(300 cGy)		17	19	22
Arm B TBI dose Level-1 or 2 (300–400 cGy)		53	53	53
Arm B TBI dose Level-3 (450cGy)		24	24	32
Non-relapse mortality				
Arm A TBI dose Level-1(300 cGy)		17	22	43
Arm B TBI dose Level-1 or 2 (300–400 cGy)		29	35	35
Arm B TBI dose Level-3 (450 cGy)		31	43	51
Chronic GvHD		38	45	
Arm A TBI dose Level-1(300 cGy)			53	
Arm B TBI dose Level-1 or 2 (300–400 cGy)			35	
Arm B TBI dose Level-3 (450 cGy)			40	
Acute GvHD (grades 2-4)	58			
Arm A TBI dose Level-1(300 cGy)	56			
Arm B TBI dose Level-1 or 2 (300–400 cGy)	65			
Arm B TBI dose Level-3 (450 cGy)	57			
Acute GvHD (grades 3-4)	13			
Arm A TBI dose Level-1(300 cGy)	17			
Arm B TBI dose Level-1 or 2 (300–400 cGy)	12			
Arm B TBI dose Level-3 (450 cGy)	9			

TBI: total body irradiation; GvHD: graft-*versus*-host disease.

the TBI dose from the originally used 200 cGy could improve the outcome of HCT in the NMA setting, thereby reducing the risk of disease progression in patients who are not considered candidates for myeloablative conditioning.

In agreement with this hypothesis, we showed improvement in outcome by raising the TBI dose from 200 cGy to 300 cGy in our cohort of low-risk patients (MDS-SLD, MDS-MLD, MDS-RS, MDS-U and MPN). Similarly, the day 200 failure rate for high-risk patients (MDS-EB-1/CMML) was reduced to 9% when TBI was raised to 450 cGy while the lower TBI doses of 300 cGy

and 400 cGy failed to improve results. We achieved the primary objective of reducing transplant failure to less than 20% by day 200, which resulted in PFS at two years of 58% in the low-risk group and 33% in the high-risk group. NRM was not significantly increased after escalating TBI to 300 cGy, but 450 cGy TBI led to toxicity with a resultant NRM of 43% at two years after HCT. Limitations of the current study include the slow accrual over ten years. While donor selection and GvHD prophylaxis remained unchanged during this interval, and there were no major changes in antimicrobial treatments, radiological techniques and ancillary therapies, incremental
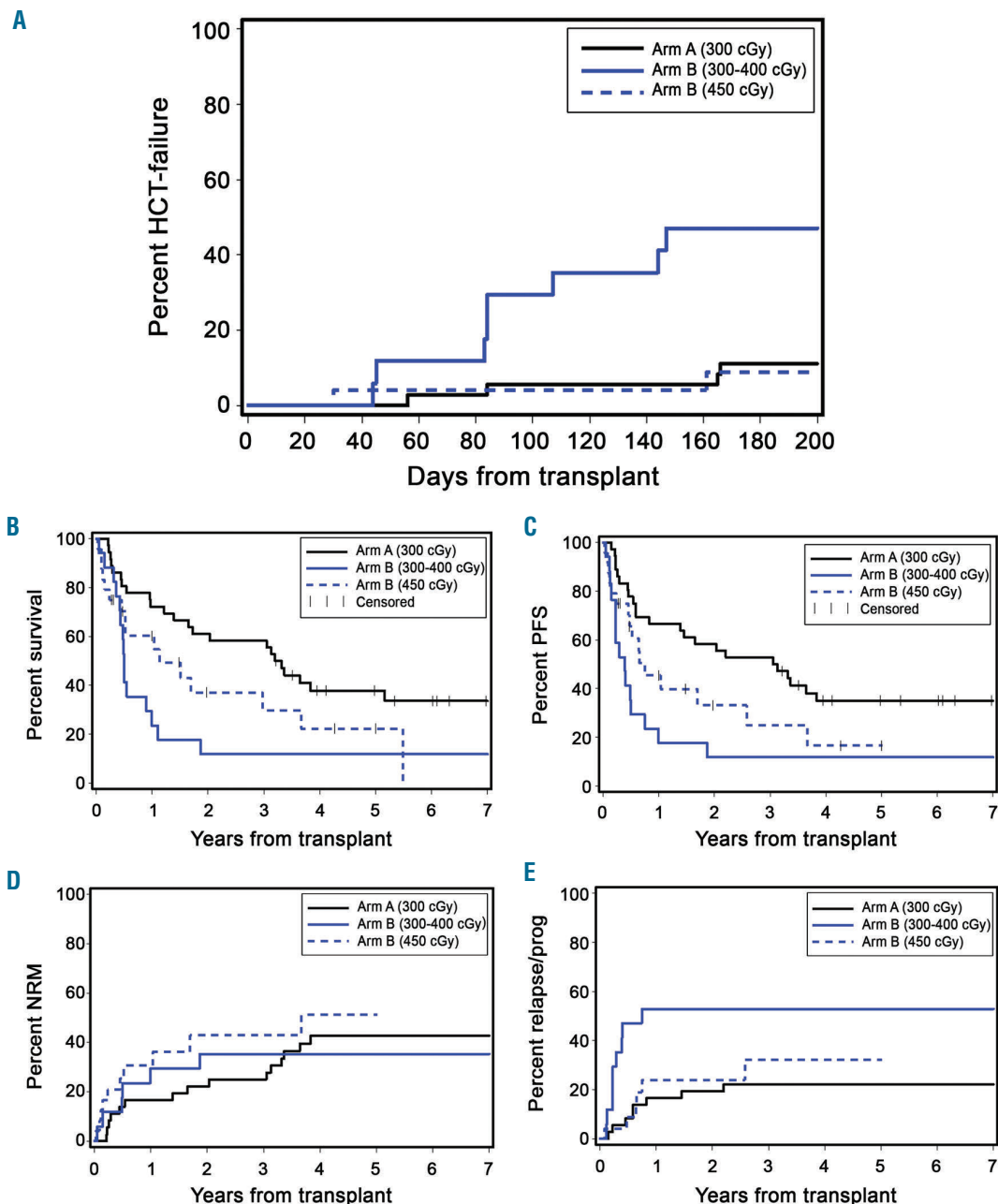

Figure 3. Patients enrolled in Arm A receiving 300 cGy total body irradiation (TBI) and patients enrolled in Arm B receiving 450 cGy TBI had reduced day 200 hematopoietic cell transplantation (HCT) failure. Transplant outcomes by Arm and TBI dose: (A) HCT-failure, (B) overall survival, (C) progression-free survival (PFS), (D) non-relapse mortality (NRM), and (E) relapse incidence.

improvements could be a bias. The slow accrual was due to competing protocols at our Centers. Another limitation regards high-risk patients who received dose level-2 TBI (400 cGy). Only five patients were enrolled but, as per protocol design, we met dose escalation rules because of transplant failure. It is possible that, with greater numbers, better results could be achieved.

The incidence of non-relapse mortality remains an issue, being high especially for high-risk patients (cumulative incidence of 43% at 2 years). There was no statistically significant difference regarding TBI-dose and GvHD incidence. Different strategies are needed to reduce the toxicity of 450 cGy TBI. Fractionation of the TBI dose was investigated in two preclinical canine experiments, which showed that fractionation of 450cGy TBI was significantly less immunosuppressive with a higher rate of graft rejection than when given as a single dose.[15,16] Stelljes *et al.* demonstrated that a conditioning regimen of 8Gy fractionated TBI and FLU was feasible with low NRM in patients with AML (8% at 2 years for patients in CR, 37% at 2 years for patients with active disease).[17] A novel approach to replace or augment TBI while reducing toxicity, consists of using targeted radioimmunotherapy.[18] Monoclonal antibodies to CD45 and CD20 coupled to beta (β)-emitting radionuclides (iodine-131 or yttrium-90) have been studied[19,20] showing good results but evident disadvantages (e.g. off-target effects and the need to use isolation rooms) in clinical trials. However, the recent substitution with alpha (α)-emitting radionuclides (bismuth-213 and astatine-211) has given encouraging results,[21,22] especially for astatine-211.[23] Clinical trials are currently in progress using astatine-211 (*clinicaltrials.gov identifier: NCT03128034*).

The high incidence of GvHD irrespective of TBI dose remains a problem, especially for grades III-IV acute GvHD and chronic GvHD that reached cumulative incidences of 13% at 200 days and 45% at two years. These complications impacted OS and NRM, accounting for one-third of all deaths. Novel approaches are currently being investigated that are aimed at reducing GvHD incidence: the triple-drug combination using tacrolimus, mycophenolate mofetil, and sirolimus has been studied with encouraging results,[24] and a phase III trial evaluated the addition of sirolimus to cyclosporine and MMF in order to reduce GvHD without impairing graft-*versus*-tumor effect with significantly less acute GvHD and less NRM with superior overall and progression-free survival (*clinicaltrials.gov identifier: NCT04251575*).[25]

Lim *et al.*[26] recently described results from a large retrospective study by the European Group for Blood and Marrow Transplantation (EBMT) registry which included 1,333 patients with MDS, who were older than 50 years, of whom 833 (62%) underwent reduced intensity conditioning (RIC). The RIC regimens included FLU plus intermediate doses of one or two alkylating agents including busulfan, melphalan, cyclophosphamide, or thiotepa, or low-dose TBI (200-400 cGy) with or without anti-thymocyte globulin or alemtuzumab. OS, NRM and relapse incidence (RI) rates at four years were 32%, 32% and 41%, respectively. The median age of patients undergoing RIC was 59 years (range 50-74.7), which was younger than current patients, and no comorbidity data was reported. Similarly, McClune *et al.*[27] performed another large retrospective analysis of data of the Center for International Blood and Marrow Transplant Research (CIBMTR) involving 1,080 patients of whom 535 had MDS and received an RIC or NMA conditioning regimen before transplantation. Of this cohort, 181 patients were over 60 years of age but, again, no comorbidity scores were reported. At two years after HCT, results for patients 60-64 years of age or over 65 years old included OS 45% and 38%, RFS 35% and 36%, NRM 35% and 39%, and relapse rate of 29% and 21%, respectively. Recently Scott *et al.*[28] concluded a randomized prospective trial showing that more intensive conditioning might be better than RIC for patients with AML or MDS. A reduction in NRM for RIC (4.4% *vs.* 15.8% at 18 months) was offset by increased disease progression (37% *vs.* 3.7% at 18 months), although there were no statistically significant differences in OS and relapse-free survival between the two groups of patients.

Storb and Sandmaier[29] analyzed results of eleven different retrospective studies using RIC or NMA regimens for various hematologic diseases reported by registries or individual transplant centers on nearly 9,000 patients. With a median follow up of three years (range 1.75-5), the incidence of disease progression was 43% (range 22-65%), NRM 34% (range 6-38%), and OS 38% (22-65%).

Non-myeloablative conditioning has extended the use of allogeneic HCT for patients with myeloid malignancies who were previously not considered candidates for HCT because of advanced age or comorbidities. Initial results with a regimen based on 200 cGy TBI was associated with a relatively
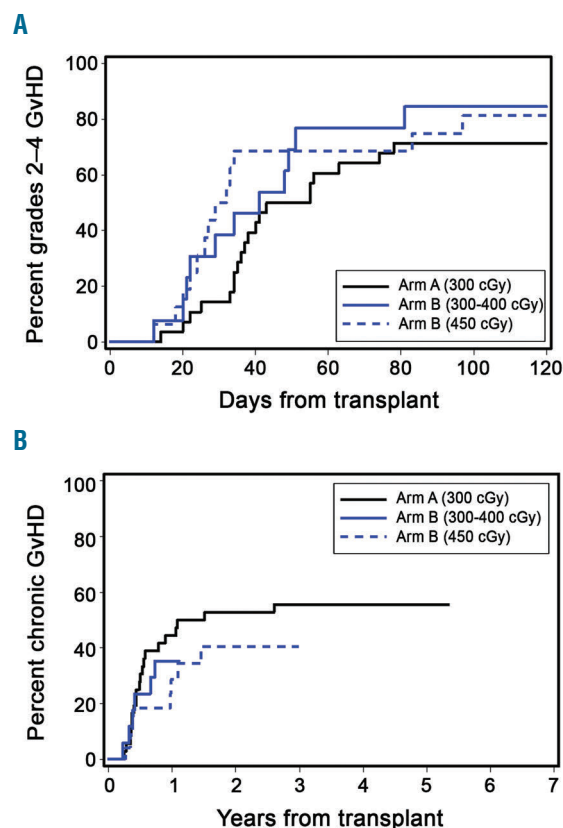
Figure 4. Incidences of graft-*versus*-host disease (GvHD) were comparable between different Arms and TBI doses. (A) Acute GvHD, (B) chronic GvHD.

high rate of disease progression or relapse. We showed that an incremental increase in TBI resulted in a lower risk of disease progression/relapse among patients with MDS, MPN, and CMML. However, the escalation of the TBI dose to 450 cGy was associated with increased NRM, offsetting the benefit achieved from decreased disease recurrence. Future studies will, therefore, employ targeted radioimmunotherapy in order to reduce relapse/progression without added toxicity and without increasing NRM.

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