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ORIGINAL ARTICLE

Older recipient age is paradoxically associated with a lower incidence of chronic GVHD in Thymoglobulin recipients: a retrospective study exploring risk factors for GVHD in allogeneic transplantation with Thymoglobulin GVHD prophylaxis

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Thymoglobulin (TG) given with conditioning for allogeneic haematopoietic SCT (alloHSCT) is effective in reducing the risk of acute and chronic GVHD (cGVHD). Whether conventional risk factors for GVHD apply to TG-conditioned alloHSCT is unknown. We retrospectively studied 356 adults from three centres who received TG 4.5 mg/kg prior to alloHSCT for haematologic malignancy. Donors were unrelated in 64%. At 3 years, OS was 61% (95% confidence interval (CI) 55–67%), cumulative incidence of relapse was 28% (95% CI 23–33%) and non-relapse mortality was 19% (14–24%). The cumulative incidences of grade 2–4, and grade 3–4 acute GVHD were 23% (95% CI 19–28%) and 10% (95% CI 6–13%), respectively. The cumulative incidence of cGVHD requiring systemic immunosuppression (cGVHD-IS) at 3 years was 32% (95% CI 27–37%). On multivariate analysis, counterintuitively, recipient age over 40 was associated with a significantly decreased risk of cGVHD-IS ($P = 0.001$). We report for the first time a paradoxical association of older age with reduced cGVHD in TG recipients, and conclude that traditional risk factors for GVHD may behave differently in the context of pre-transplant TG.

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INTRODUCTION

GVHD is a common and debilitating complication of allogeneic haematopoietic SCT (alloHSCT) and is associated with an inferior quality of life.[1,2] Mortality due to acute GVHD (aGVHD) and chronic GVHD (cGVHD) is due to direct organ toxicity and opportunistic infections resulting from GVHD or its treatment-induced immune deficiency. The reported cumulative incidence of grade 2–4 aGVHD in myeloablative, T-cell replete alloHSCT is 25–45% for matched related donor transplants, and 50–60% for unrelated donor (UD) transplants.[3–7] The reported cumulative incidence of cGVHD in T-cell replete alloHSCT is 30–50% for matched sibling donor transplants, and 45–90% for UD transplants.[3,8–12]

Risk factors for aGVHD include the degree of HLA disparity, female donor/male recipient gender combination, older patient age, recipient CMV seropositivity, use of TBI, increasing donor age, and, in some studies, use of mobilised PBSCs.[13] Risk factors for cGVHD include donor and recipient age, increasing HLA disparity, female donor/male recipient combination (in addition to parity of female donors), donor relatedness (that is, HLA-matched related related donor vs HLA-matched UD) and use of mobilized PBSCs.[13] Importantly, the presence, but not the severity, of cGVHD is associated with a reduced risk of relapse of the underlying haematologic malignancy.[9]

Peri-transplant immunomodulation that preserves a graft-versus-tumour effect, but diminishes the risk of cGVHD, would substantially improve alloHSCT outcomes. T-cell depletion (TCD) of the graft potentially allows reduction of GVHD risk, while maintaining a degree of graft-versus-tumour effect.[14] TCD may be achieved by either *in vivo* or *ex vivo* graft manipulation. *In vivo* TCD is achieved by using anti-T-cell globulins (ATGs) which may be either monoclonal (for example, alemtuzumab) or polyclonal, derived from sensitised horses (ATGAM, Pfizer, New York City, NY, USA) or rabbits. The rabbit ATG formulations in current clinical use are derived from rabbits sensitised with human thymocytes (Thymoglobulin (TG), Sanofi, Cambridge, MA, USA) or cells of the Jurkat T-lymphoblastoid cell line (ATG-F, Fresenius, Bad Homburg, Germany). There are no controlled clinical data comparing the different rabbit ATG formulations. Retrospective clinical and *in vitro* data support that they should not be regarded as equivalent in specificity or efficacy.[15–18] Rabbit, but not horse-derived, ATG appears to reduce the risk of developing GVHD.[19,20]

There is extensive published experience with TG as GVHD prophylaxis, utilising different doses and schedules, both of which

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appear to influence the efficacy of TG.[21–31] Overall, TG results in an ~20–30% absolute reduction in risk of cGVHD, independent of TG total dose, disease type and status or donor relatedness. The benefit of TG is not reflected in an improvement in OS. However, it may be implied that survivors of alloHSCT incorporating TG prophylaxis have better quality of life due to less cGVHD.[32]

In studies comparing related donor and UD alloHSCT, it appears that in the presence of TG prophylaxis, donor relatedness is no longer a risk factor for developing cGVHD.[33,34] We hypothesised that other previously identified risk factors may also lose their utility for predicting aGVHD and cGVHD risk when TG is used, thus raising the need for new predictors of GVHD. We analysed the cumulative incidence of aGVHD and cGVHD in a large cohort of alloHSCT recipients treated with a uniform dose of TG.

MATERIALS AND METHODS

Patients

TG, at a dose of 4.5 mg/kg, has been routinely used since at least 2007 for GVHD prophylaxis at the University of Calgary (UC), the Royal Melbourne Hospital (RMH) and the Royal Adelaide Hospital (RAH). The only difference in TG use was a minor difference in timing: the first dose (0.5 mg/kg) was given at UC on day − 2, whereas at RMH and RAH on day − 3. The first dose was followed by two doses of 2 mg/kg on days − 1 and 0 (before graft infusion) at UC, and on days − 2 and − 1 at RMH and RAH. Additional GVHD prophylaxis was uniform across the three centres: MTX (15 mg/m^2 on day 1, and 10 mg/m^2 on days 3, 6 and 11) and cyclosporin from day − 1 until 3 months post transplant.

Inclusion criteria

Patients from UC, RAH and RMH were eligible for inclusion if they received a full dose of TG as part of a first alloHSCT from an HLA-matched sibling or UD, for haematologic malignancy, with day 0 from 1 January 2004 to 31 May 2011. This cut-off date provided a minimum of 1 year follow-up of surviving patients for analysis. Patients with related non-sibling donors and cord blood recipients were not included. All patients and donors were tissue typed using molecular methods.

Outcome measures

The outcomes of interest were the cumulative incidences of grade 2–4 and grade 3–4 aGVHD, and cGVHD requiring initiation of, or increase in, systemic immunosuppression (cGVHD-IS). aGVHD was defined according to accepted criteria,[35] and cGVHD was defined according to physician description from case record review.

Secondary outcomes were PFS and OS. PFS was defined to be the time (in months) from day 0 to any of relapse from a prior status of remission, progression from a prior status of non-remission or non-relapse death. OS was defined to be the time in months from day 0 to death from any cause. For OS and PFS, patients were censored at the date they were last known to be alive or alive and without relapse, respectively.

Collection of data

Data were extracted primarily from the transplant centre databases and secondarily from patient records of sequential eligible patients. The following information was recorded: recipient variables (sex, age at alloHSCT, day 0, disease, conditioning regimen and CMV serostatus); donor variables (sex, relationship to recipient, age at time of donation, source of stem cells (PB or BM), number of mismatched HLA alleles (out of 10) and CMV serostatus); aGVHD (grade 0–1 vs 2–4); diagnosis of cGVHD; time to initiation of systemic immunosuppression for cGVHD and other outcome variables (date of relapse, date of death, cause of death (coded as due to progressive disease or unrelated to disease)). Censoring of time-dependent outcomes was conducted on 31 May 2012.

Waiver of consent was obtained from the ethics boards of the Australian centres, whereas patients from UC had signed a Research Ethics Board-approved written consent.

Risk factors

Pairs of risk factors compared as binary variables were: UD vs related donor; < 10 HLA alleles matched vs 10 out of 10 HLA alleles matched; female donor and male recipient vs all other donor/recipient gender combinations; PB vs BM; conditioning intensity (myeloablative conditioning vs reduced-intensity conditioning); any TBI given vs not given; ablative (⩾10 Gy) TBI given vs not given; CMV seropositive vs CMV seronegative; diagnosis of CML vs all other diseases; donor age < 40 years vs 40 years and over and recipient age < 40 years vs 40 years and over. These risk factors were selected based on the large study of risk factors for GVHD by Flowers *et al.*[13]

In addition, analysis by transplant centre (UC vs RMH/RAH) was included to test for centre bias.

Statistical analysis

Patient and transplant characteristics were compared between the transplant centres (UC vs RMH/RAH) using the χ^2-test for categorical variables such as conditioning intensity and donor relatedness, and the Mann–Whitney *U*-test for continuous variables such as donor and recipient age. Median follow-up time was calculated for the overall cohort and by transplant centre using the reverse censoring method.[36]

OS and PFS were estimated using the Kaplan–Meier product-limit method; annual survival estimates with corresponding 95% confidence intervals (95% CI) were also calculated.[37] Cumulative incidences of the competing risks of relapse and non-relapse mortality were calculated using the method of Gray.[38] Cumulative incidences of grade 2–4 aGVHD, grade 3–4 aGVHD and cGVHD-IS were calculated using the method of Gray, with relapse and non-relapse mortality acting as competing risks in each scenario.

For univariate analysis, the effect of risk factors on the cumulative incidence of grade 2–4 aGVHD, grade 3–4 aGVHD and cGVHD-IS was assessed using the method of Gray.[39] For multivariate analysis, manual backwards stepwise regression was used. Variables were only forwarded to the regression model if they met a predefined criterion of $P < 0.2$ on univariate analysis. To account for the testing of multiple hypotheses from a single data set, a Bonferroni correction was applied such that a variable was only regarded as independently statistically significant if its respective *P*-value was < 0.0167 (that is, 0.05 divided by 3) on multivariate analysis.

Analysis was performed using R (version 2.15.1, www.r-project.org).

RESULTS

Patient characteristics

A total of 356 patients were included in this analysis. Characteristics are described in Table 1. Statistically significant differences were observed between the cohorts at each centre for patient age, conditioning intensity, donor relatedness, sex mismatch and recipient CMV serostatus. For all 356 patients, 3-year OS was 61% (95% CI 55–67%) and 3-year PFS was 53% (47–59%). At 3 years, the cumulative incidence of relapse was 28% (23–33%). Non-relapse mortality was 8% (5–11%) at 100 days, and 19% (14–24%) at 3 years. Five patients experienced graft failure.

Regarding aGVHD, 355 patients were eligible for analysis of aGVHD. Eighty-three patients developed grade 2–4 aGVHD at a median of 36 days post transplant (range 10–126 days). Twenty-six patients had aGVHD of maximal grade 3, and eight patients had grade 4 aGVHD. The cumulative incidences of grade 2–4 aGVHD and grade 3–4 aGVHD were, at 100 days post transplant, 23% (95% CI 18–27%) and 9% (95% CI 6–12%), respectively, and at 180 days post transplant 23% (95% CI 19–28%) and 10% (95% CI 6–13%), respectively (Figures 1 and 2).

Regarding cGVHD, from the original cohort of 356 patients, 9 patients died before day 30, 2 patients relapsed before day 30 and 3 patients suffered graft failure. Thus 342 patients were alive, disease-free and eligible for analysis of cGVHD-IS. Hundred and nine patients developed cGVHD-IS at a median of 117 days post transplant (range 34–850 days). An additional 42 patients were diagnosed with cGVHD but did not require systemic immunosuppression. The cumulative incidences of cGVHD-IS at 1 and 3 years post transplant were 31% (26–36%) and 32% (27–37%), respectively (Figure 3).

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Table 1. Cohort characteristics

	Total	*UC*	*RMH/RAH*	P-*value*
Number of patients	356	272	84	—
Patient age, years Median (range)	48 (17–66)	50 (19–66)	45.5 (17–62)	0.005
Disease [a]				
Acute leukaemia	206 (58%)	151 (56%)	55 (65%)	0.32
CML	21 (6%)	16 (6%)	5 (6%)	—
Other myeloid malignancy	53 (15%)	45 (17%)	8 (10%)	—
Other lymphoid malignancy	76 (21%)	60 (22%)	16 (19%)	—
Conditioning intensity [b]				
MAC	334 (94%)	270 (99%)	64 (76%)	< 0.001
RIC	22 (6%)	2 (1%)	20 (24%)	—
TBI				
Yes	226 (63%)	178 (65%)	48 (57%)	0.19
No	130 (37%)	94 (35%)	36 (43%)	—
Donor relatedness				
Sibling	128 (36%)	128 (47%)	0	< 0.001
Unrelated	228 (64%)	144 (53%)	84 (100%)	—
Donor age, years				
Median (range)	36 (12–69)	37 (12–69)	35 (20–59)	0.39
HLA matching				
10/10	312 (88%)	237 (87%)	75 (89%)	0.71
8–9/10	44 (12%)	35 (13%)	9 (11%)	—
Graft source				
PB	344 (97%)	265 (97%)	79 (94%)	0.16
BM	12 (3%)	7 (3%)	5 (6%)	—
Sex mismatch				
Female donor to male recipient (RD/UD)	66 (19%) (32/38)	59 (22%) (29/30)	7 (8%) (0/7)	0.02
All other	290 (81%)	213 (78%)	77 (92%)	—
Recipient CMV serostatus				
D+R+	106 (30%)	79 (29%)	27 (32%)	0.02
D − R+	89 (25%)	60 (22%)	29 (35%)	—
D+R −	33 (9%)	24 (9%)	9 (11%)	—
D − R −	119 (33%)	100 (37%)	19 (23%)	—
Indeterminate	9 (3%)	9 (3%)	0	—
Median follow-up, months				
All (reverse censoring method)	27.2	27.1	28.0	0.25
Survivors	26.3 (2.2–62.2)	26.2 (2.2–49.9)	26.2 (3.6–62.2)	—

Abbreviations: D = donor; MAC = myeloablative conditioning; PK = pharmacokinetics; R = recipient; RD = related donor; RAH = Royal Adelaide Hospital; RMH = Royal Melbourne Hospital; RIC = reduced-intensity conditioning; UC = University of Calgary; UD = unrelated donor. [a]Acute leukaemia: AML, UC 109, RMH/RAH 39; acute promyelocytic leukaemia, UC 5, RMH/RAH 0; ALL, UC 48, RMH/RAH 14; acute biphenotypic leukaemia, UC 5, RMH/RAH 2. Other myeloid malignancy: myelodysplastic syndrome, UC 31, RMH/RAH 5; myeloproliferative neoplasms, UC 13, RMH/RAH 2; MDS/MPN, UC 7, RMH/RAH 1. Other lymphoid malignancy: CLL, UC 17, RMH/RAH 2; Hodgkin lymphoma, UC 4, RMH/RAH 1; non-Hodgkin lymphoma, UC 38, RMH/RAH 11; plasma cell myeloma, UC 1, RMH/RAH 2. [b]For Calgary patients, most patients received fludarabine 250 mg/m^2, BU 12.8 mg/kg, PK adjusted and TBI 4 Gy. A minority of patients received fludarabine 250 mg/m^2 and BU 12.8 mg/kg, PK adjusted. Very few patients received a different regimen. For RMH/RAH patients, myeloablative conditioning regimens were CY 120 mg/kg+TBI 12 Gy ($n = 37$), etoposide 60 mg/kg+TBI 13.2 Gy ($n = 11$), BU 12.8 mg/kg PK adjusted+CY 120 mg/kg ($n = 8$) and fludarabine 250 mg/m^2+BU 12.8 mg/kg, PK adjusted ($n = 8$) and RIC regimens were fludarabine 125 mg/m^2+melphalan 140 mg/m^2 ($n = 16$) and fludarabine 125 mg/m^2+CY or TBI 2 Gy ($n = 4$).

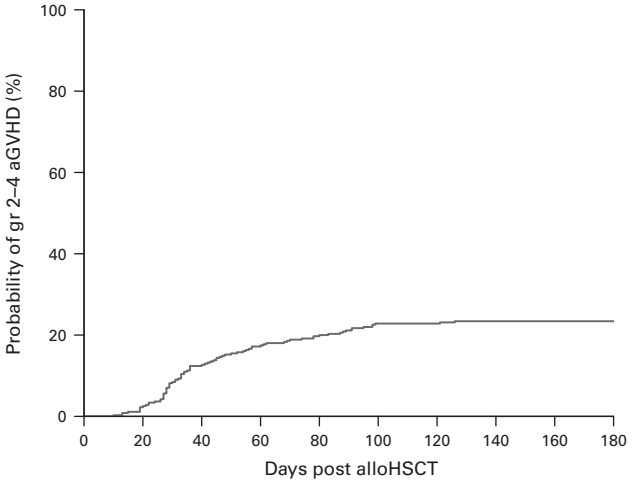

Figure 1. Cumulative incidence of grade 2–4 acute GVHD.

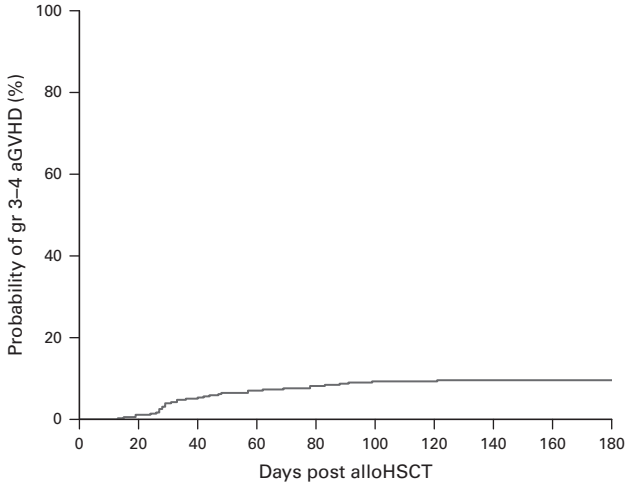

Figure 2. Cumulative incidence of grade 3–4 acute GVHD.

Univariate analysis

Table 2 shows the results of univariate analysis for the effect of risk factors on the cumulative incidence of acute and chronic GVHD. Transplantation from a donor with one or two HLA mismatches was associated with an increased risk of both grade 2–4 aGVHD ($P = 0.021$) and grade 3–4 aGVHD ($P = 0.034$), and transplantation for a disease other than CML was associated with a decreased risk of both grade 2–4 aGVHD ($P = 0.021$) and grade 3–4 aGVHD ($P = 0.003$). Regarding cGVHD-IS, recipient age >40 was associated with a decreased risk ($P = 0.018$, Figure 4), as was conditioning incorporating any TBI ($P = 0.002$, Figure 5). However, in a subgroup analysis of patients conditioned with ablative doses of TBI ($n = 48$) versus no TBI ($n = 130$), there was no association with risk of cGVHD-IS ($P = 0.370$).

Multivariate analysis

Results of regression modelling are shown in Table 3. No variables were independently associated with an increased risk for grade 2–4 aGVHD. Transplantation for a disease other than CML remained significantly associated with a decreased risk

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of grade 3–4 aGVHD ($P = 0.003$, hazard ratio (HR) 0.3, 95% CI 0.1–0.6). Factors independently associated with a significantly decreased risk of cGVHD-IS were recipient age >40 ($P = 0.001$, HR 0.5, 95% CI 0.3–0.8) and use of TBI in conditioning ($P < 0.001$, HR 0.5, 95% CI 0.3–0.7). For all three multivariate models, each independently associated variable remained significantly associated ($P < 0.0167$) after the addition of transplant centre as a variable.

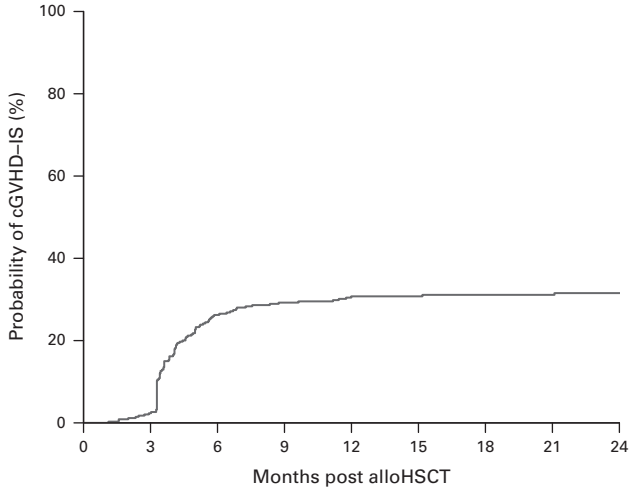

Figure 3. Cumulative incidence of chronic GVHD requiring immunosuppression (cGVHD-IS).

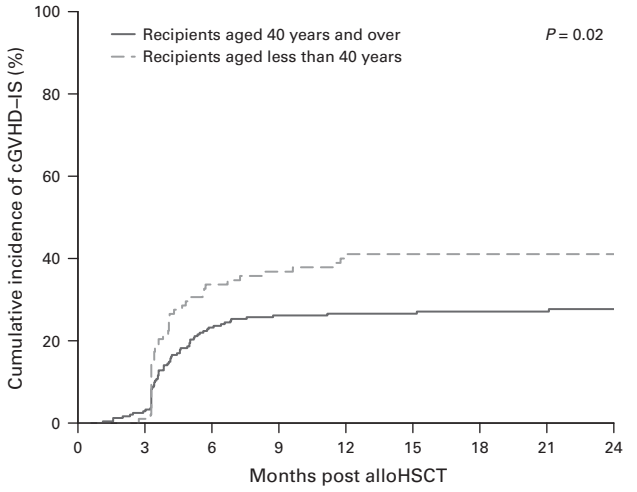

Figure 4. Cumulative incidence of chronic GVHD requiring immunosuppression (cGVHD-IS) according to recipient age.

Table 2. Univariate analysis of aGVHD and cGVHD-IS

Variable type	*Reference variable*	*Experimental variable*	*Grade 2–4 aGVHD*	*Grade 3–4 aGVHD*	*cGVHD-IS*
Recipient age	<40 years	$\geqslant 40$ years	$P = 0.076$ HR 0.7 95%CI 0.4–1.0	$P = 0.090$ HR 0.6 95%CI 0.3–1.1	$P = 0.018$ HR 0.6 95%CI 0.4–0.9
Donor age	<40 years	$\geqslant 40$ years	$P = 0.370$ HR 1.2 95%CI 0.8–1.9	$P = 0.468$ HR 1.3 95%CI 0.7–2.5	$P = 0.461$ HR 0.9 95%CI 0.6–1.3
Donor relatedness	Sibling	Unrelated	$P = 0.215$ HR 1.4 95%CI 0.8–2.1	$P = 0.238$ HR 1.6 95%CI 0.7–3.4	$P = 0.707$ HR 1.1 95%CI 0.7–1.6
Conditioning intensity	Reduced intensity	Myeloablative	$P = 0.540$ HR 1.4 95%CI 0.5–3.7	$P = 0.424$ HR 2.2 95%CI 0.3–16.3	$P = 0.134$ HR 2.4 95%CI 0.8–7.6
TBI in conditioning	No	Yes	$P = 0.489$ HR 0.9 95%CI 0.6–1.3	$P = 0.821$ HR 0.9 95%CI 0.5–1.8	$P = 0.002$ HR 0.5 95%CI 0.4–0.8
Ablative TBI in conditioning	No	Yes	$P = 0.692$ HR 1.1 95%CI 0.6–2.1	$P = 0.861$ HR 0.9 95%CI 0.3–2.6	$P = 0.370$ HR 0.8 95%CI 0.5–1.3
Recipient CMV serostatus	CMV negative	CMV positive	$P = 0.703$ HR 0.9 95%CI 0.6–1.4	$P = 0.475$ HR = 1.3 95%CI 0.6–2.7	$P = 0.934$ HR 1.0 95%CI 0.7–1.5
Female donor to male recipient	No	Yes	$P = 0.438$ HR 1.2 95%CI 0.7–2.0	$P = 0.915$ HR 1.0 95%CI 0.4–2.3	$P = 0.557$ HR 1.2 95%CI 0.7–1.9
HLA mismatch	No	Yes, 1–2 alleles	$P = 0.021$ HR 1.9 95%CI 1.1–3.3	$P = 0.034$ HR 2.4 95%CI 1.1–5.4	$P = 0.006$ HR 1.6 95%CI 1.0–2.7
Graft source	BM	PB	$P = 0.839$ HR 0.9 95%CI 0.3–2.9	$P = 0.888$ HR 1.2 95%CI 0.2–8.5	$P = 0.733$ HR 0.8 95%CI 0.3–2.3
Diagnosis of CML	Yes	No	$P = 0.021$ HR 0.4 95%CI 0.2–0.9	$P = 0.003$ HR 0.3 95%CI 0.1–0.6	$P = 0.454$ HR 0.8 95%CI 0.4–1.6
Transplant centre	RMH/RAH	UC	$P = 0.945$ HR 1.0 95%CI 0.6–1.6	$P = 0.667$ HR 1.2 95%CI 0.5–2.7	$P = 0.234$ HR 1.3 95%CI 0.8–2.1

Abbreviations: aGVHD = acute GVHD; cGVHD-IS = chronic cGVHD requiring systemic immunosuppression; CI = confidence interval; HR = hazard ratio.

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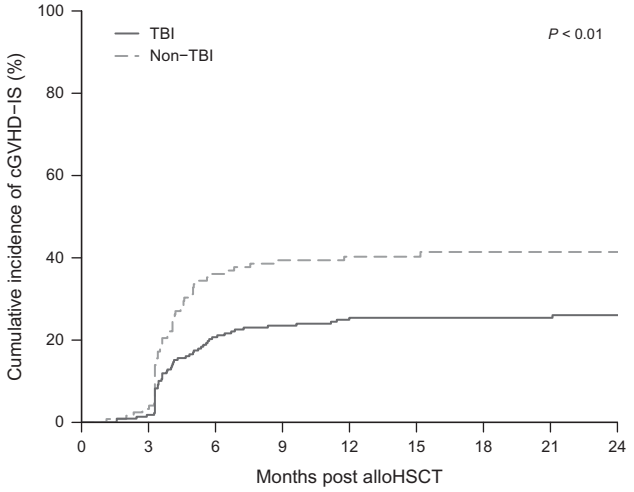

Figure 5. Cumulative incidence of chronic GVHD requiring immunosuppression (cGVHD-IS) according to conditioning with or without TBI.

Table 3. Multivariate analysis

Variable type[a]	*Reference variable*	*Experimental variable*	*HR*	*95% CI*	P-*value*
Grade 2–4 aGVHD					
No variables were significant on multivariate analysis					
Grade 3–4 aGVHD					
CML diagnosis	Yes	No	0.3	0.1–0.6	0.003
cGVHD-IS					
Recipient age	< 40 years	40+ years	0.5	0.3–0.8	0.001
Any TBI in conditioning	No	Yes	0.5	0.3–0.7	< 0.001

Abbreviations: aGVHD = acute GVHD; cGVHD-IS = chronic cGVHD requiring systemic immunosuppression; CI = confidence interval; HR = hazard ratio. [a]Variables tested in the multivariate models for grade 2–4 and grade 3–4 aGVHD and cGVHD-IS were HLA mismatch, CML diagnosis, recipient age, donor age, TBI, conditioning intensity, female donor to male recipient, graft source and transplant centre.

DISCUSSION

The most remarkable finding of this retrospective study of a large cohort of alloHSCT recipients receiving a uniform dose of TG is that older age may be a protective factor for cGVHD-IS in the context of TG administration. In the absence of TG, increasing recipient age has consistently been associated with an increased risk of cGVHD following allogeneic transplantation.[13,40–42] The mechanisms underlying the interaction of age, *in vivo* TCD and development of cGVHD-IS in our patients are unclear. We hypothesised that there may be age-related variation in TG pharmacokinetics, and were able to investigate this using data from the UC group. Serum TG concentrations at day 7 and day 28 post alloHSCT were available for 220 and 247 patients, respectively. The TG concentration was measured by flow cytometry as described.[43] Using Spearman rank correlation test, there was no significant correlation between recipient age and the day 7 level ($r = -0.07$, $P = 0.32$) and no significant correlation between recipient age and the day 28 level ($r = +0.04$, $P = 0.50$) (Storek, personal communication, August 2013). Thus, the increased incidence of cGVHD with lower recipient age cannot be attributed to age-related variation in TG pharmacokinetics. Other possible explanations include age-related variation in T-cell subsets and/or TG pharmacodynamics.

In this TG-conditioned cohort, TBI was also independently associated with a reduction in risk of cGVHD-IS (HR 0.5). However, confoundingly, ablative doses of TBI showed no influence on any type of GVHD. Ablative TBI has been associated with reduced risk of cGVHD when compared against ablative BU doses in a prospective study of matched sibling transplantation.[44] However, other studies have shown an association of TBI with increased rates of aGVHD and cGVHD.[13,45] However, these studies would have generally reported on recipients of ablative doses of TBI. We acknowledge that in our cohort, TBI doses were variable—the majority of patients received 4 Gy TBI, and most of these were from the UC cohort—and thus cannot be certain of the generalisability of this association. Radiation diminishes regulatory T-cell numbers, promotes aberrant T-cell trafficking to target organs and lowers the threshold for generation of autoreactive T-cell clones.[46–48] However, little is known about the impact of the combination of anti-thymocyte globulin and TBI on T-cell biology, even though the combination has been used and refined, for many years.[49–52] Perhaps co-administration of TBI and TG reduces pro-inflammatory cytokines in the patient, leading to less T-cell activation post donor T-cell infusion. Another possibility is that TBI in the presence of TG may kill or inhibit recipient DCs that are known to present allo-Ags to donor T cells.[53] Our group is currently exploring factors associated with developing infusion-related reactions to TG; this data may provide some clues to the mechanism.

The influence of CML on the risk of aGVHD and cGVHD is conflicting in the literature.[5,13,54] Ours is the first report of CML being associated with increased risk of aGVHD *in the context of TG use*, and the large effect size (HR 0.2 for diseases other than CML, which equates to HR 4.1 for CML) is striking. As most of these patients were transplanted in the era of widespread use of tyrosine kinase inhibitors, patients with CML in need of alloHSCT likely had advanced disease, which may have influenced aGVHD risk. However, the small numbers of CML patients in our study suggests that caution be applied to this finding.

We acknowledge a number of limitations of our study. Most obvious is the retrospective nature of the study and the potential for bias in data collection that inevitably exists with such an exercise. The use of a surrogate endpoint of immunosuppression initiation for cGVHD raises concerns about non-standard grading, may introduce bias from variable practices in managing cGVHD, and limits comparability with larger studies. However, the earliest transplanted patients in our study predated the development of the National Institutes of Health guidelines for diagnosis,[55] and the cGVHD-IS endpoint has previously been used in large prospective randomised studies.[11] We also acknowledge that an uncontrolled variable is total dose of corticosteroid that may have been used for indications other than GVHD—for example, engraftment syndrome or reactions to TG—which may influence the risk of GVHD. On a similar note we would have liked to evaluate the impact of aGVHD as a risk factor for cGVHD. However, we had insufficient data regarding timing of onset and offset of aGVHD to appropriately deal with this as time-dependent variable.

In spite of these limitations, we feel the size of the effect and the multivariate analysis strengthens support for the idea that older recipient age may indeed be protective for cGVHD in TG recipients. This novel finding warrants confirmation in larger studies, and perhaps registry data offer the ideal platform for this. If verified, centres using TG may view patient selection and conditioning differently, particularly with respect to older patients. The other implication is that traditional risk factors for GVHD need to be regarded differently in the context of *in vivo* TCD, as this study highlights, and clearly there is scope to explore

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identification of novel biomarkers of GVHD risk that apply in the setting of *in vivo* TCD.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DISCLAIMER

This company was not involved in the design, collection, analysis or interpretation of this study, but they were given the opportunity to review this abstract prior to submission. The decision to submit for publication was made by the authors independently.

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