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ARTICLE

Cardiac safe hematopoietic stem cell transplantation for systemic sclerosis with poor cardiac function: a pilot safety study that decreases neutropenic interval to 5 days

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Abstract

We compared three fludarabine-based regimens for systemic sclerosis patients with a high-risk cardiac phenotype that according to EBMT criteria would be a contraindication for a high-dose cyclophosphamide (200 mg/kg) transplant regimen. All three regimens included fludarabine, ATG, and cyclophosphamide (60 mg/kg), while two regimens also included rituximab with or without IVIG. Treatment related mortality (TRM) was 2.4%. The mean number of days of neutropenia (ANC < 500) was 5.2, the mean number of platelet and red blood cell transfusions was 0.3 and 1.85, respectively. Skin score, forced vital capacity (FVC), and total lung capacity (TLC) improved with all three regimens. For patients whose regimen did not include rituximab versus those that included rituximab, 1-year overall relapse rate was higher 36% (5/14) versus 3.6% (1 of 28) ($p = 0.01$), secondary autoimmune diseases were higher 21% (3/14) versus 0% (0/28) ($p = 0.03$), and upper respiratory tract infections were higher 28% (4/14) versus 3.6% (1/28) ($p = 0.04$). In this safety study, a fludarabine-based regimen was relatively safe with a TRM of 2.4% and a neutropenic interval of only 5.2 days in systemic sclerosis patients with a high-risk cardiac phenotype. The addition of rituximab decreased 1-year relapse rate, risk of late secondary autoimmune diseases, and upper-respiratory tract infections.

Introduction

Three randomized autologous hematopoietic stem cell transplantation (HSCT) trials (ASSIST, ASTIS, SCOT) have demonstrated superiority in skin score and 5-year survival of autologous HSCT over monthly intravenous cyclophosphamide for systemic sclerosis (SSc) [1–4]. However, all three transplantation trials were complicated by a high transplant-related mortality of 6–10% [1–4]. For high dose cyclophosphamide-based regimens (200 mg/kg) (ASSIST and ASTIS trials), the main untoward toxicity has been acute cardiac failure during the transplant hospitalization [1–3, 5–7]. For the SCOT trial that decreased the dose of cyclophosphamide to 120 mg/kg but added total body irradiation, the toxicity has been predominately late radiation-induced myelodysplastic syndrome, leukemia, and solid tumors [4].

Due to the high cardiac related transplant mortality, the European Bone Marrow Transplant (EBMT) guidelines recommend extensive pretransplant cardiac evaluation including cardiac catheterization with fluid challenge and cardiac magnetic resonance imaging (MRI) [5, 8]. The guidelines recommend that patients be excluded from receiving high dose cyclophosphamide (200 mg/kg) if the resting pulmonary artery systolic pressure (PASP) or mean pulmonary artery pressure (mPAP) are >40 mmHg or 25 mmHg, respectively, or after fluid challenge (1000 cc normal saline in 10 min), the PASP or mPAP are >45 mmHg or 30 mmHg, respectively [5]. Cardiac MRI demonstration of diastolic interventricular septal flattening (D-sign) or an interventricular septal bounce at rest or during inspiration is also considered a contraindication to transplant with a high dose cyclophosphamide regimen

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because these signs are indicative of significant right ventricular overload or constrictive pericarditis [5–8].

Since the number one cause of death for systemic sclerosis, is cardiopulmonary [9], and since the number one cause of death for systemic sclerosis patients with poor cardiac function undergoing HSCT with a high dose (200 mg/kg) cyclophosphamide regimen is cardiac related [1, 6–8], we evaluated a "cardiac-safe regimen" based on high dose fludarabine (120 mg/m^2) in patients with SSc and compromised cardiac function that would otherwise be considered an exclusion for autologous HSCT using a regimen that contained high-dose (200 mg/kg) cyclophosphamide.

Methods

All patients signed consent and were initially treated compassionately off study and then on study (www.clinicaltrials.gov NCT03593902). All patients had a chest radiograph, CBC, chemistry, creatinine, liver function tests, HIV and hepatitis serology, pulmonary function test with forced vital capacity (FVC), total lung capacity (TLC), and diffusing capacity of carbon dioxide (DLCO), high resolution computed tomography of the chest, echocardiogram, cardiac magnetic resonance imaging (MRI), cardiology assessment, and a right heart catheterization measured before and after a 1000 mL intravenous normal saline bolus over 10 min. Fluid bolus was contraindicated if resting cardiac catheterization right atrial pressure was more than 12 mmHg or pulmonary capillary wedge pressure was more than 15 mmHg. Due to risk of fludarabine-related cerebellar toxicity in patients with renal insufficiency, all patients had a 24-hour urine creatinine clearance. Patients with either anemia, iron deficiency, facial telangiectasia, a history of melena, hematochezia, or history of gastric antral vascular ectasia (GAVE) underwent colonoscopy and endoscopy with argon laser coagulation [10] (even if non-bleeding) of dilated gastric antral blood vessels and repeated endoscopy and cauterization, if needed, at least 3 to 4 weeks later.

Criteria

Inclusion criteria were diffuse systemic sclerosis defined as cutaneous involvement proximal to the elbow or knee with an modified Rodnan skin score (mRSS) of more than 14, and internal-organ involvement, which was defined as at least one of the following features: diffusing capacity of carbon monoxide (DLCO) of <80% of predicted or decline in forced vital capacity by 10% or more in the previous 12 months; pulmonary fibrosis or ground-glass appearance on high-resolution chest CT; abnormal electrocardiogram (ECG); or gastrointestinal tract involvement. Patients with limited SSc (mRSS < 14) were eligible only if they had coexistent pulmonary involvement.

Candidates were selected to meet the cardiac exclusion criteria for a transplant regimen containing high dose cyclophosphamide (200 mg/kg) as published by the EBMT [8]. Therefore, patients must have met the following cardiac inclusion criteria: either a right heart catheterization PASP > 40 mmHg at rest or >45 mmHg with fluid challenge, or mPAP > 25 mmHg at rest or >30 mmHg with fluid challenge, or diastolic interventricular septal flattening (D-sign) or septal bounce on cardiac MRI.

Exclusion criteria were cardiac tamponade, creatinine clearance <80 ml/min, malignancies, transaminases >2 × upper limit of normal, platelet count less than 100,000/ul, hepatitis B or C or HIV seropositive, or active infection. Of note, since this protocol was designed to treat patients who would be ineligible for the ASTIS or ASSIST trials, requirement for oxygen, arrhythmias, duration of disease, or pulmonary function test abnormalities, i.e. FVC, TLC, or DLCO were not a contraindication.

Mobilization regimen

Peripheral blood stem cells (PBSC) were collected 10 days after intravenous cyclophosphamide (2 g/m^2) and 5 to 10 μg/kg per day of subcutaneous filgrastim beginning 5 days after cyclophosphamide. The PBSC were cryopreserved and unmanipulated.

Transplant regimens and standard of care

Three different (14 patients each) cardiac safe transplant fludarabine-based conditioning regimens were consecutively compared (Fig. 1). All 42 patients received a fludarabine-based regimen of 120 mg/m^2 divided into 30 mg/m^2 doses on days −5 to −2, ATG 6.0 mg/kg divided as 0.5 mg/kg on days −5, 1.0 mg/kg on day −4, and 1.5 mg/kg on days −3, −2, and −1, and cyclophosphamide 60 mg/kg on day −2. Twenty-eight patients also received rituximab with 14 patients receiving rituximab 1000 mg divided as 500 mg/day on days −6 and +1 and another 14 patients received rituximab 500 mg on day-6 and IVIG (400 mg/kg) on days +1 and +8 (Fig. 1).

Inpatient transplant grade 3 and 4 toxicities were graded according to National Cancer Institute common terminology criteria for adverse events (CTCAE v2.0). Broad spectrum intravenous cefepime or piperacillin/tazobactam was started when neutropenic and oral ciprofloxacin and isavuconazonium were started on day +2 and continued until absolute neutrophil count rose above 500/ul. Fluconazole (400 mg po qd) was started upon discharge and continued for 3 months. Acyclovir (400 mg po bid) was started on admission and continued for 1 year. Bactrim (1 po double

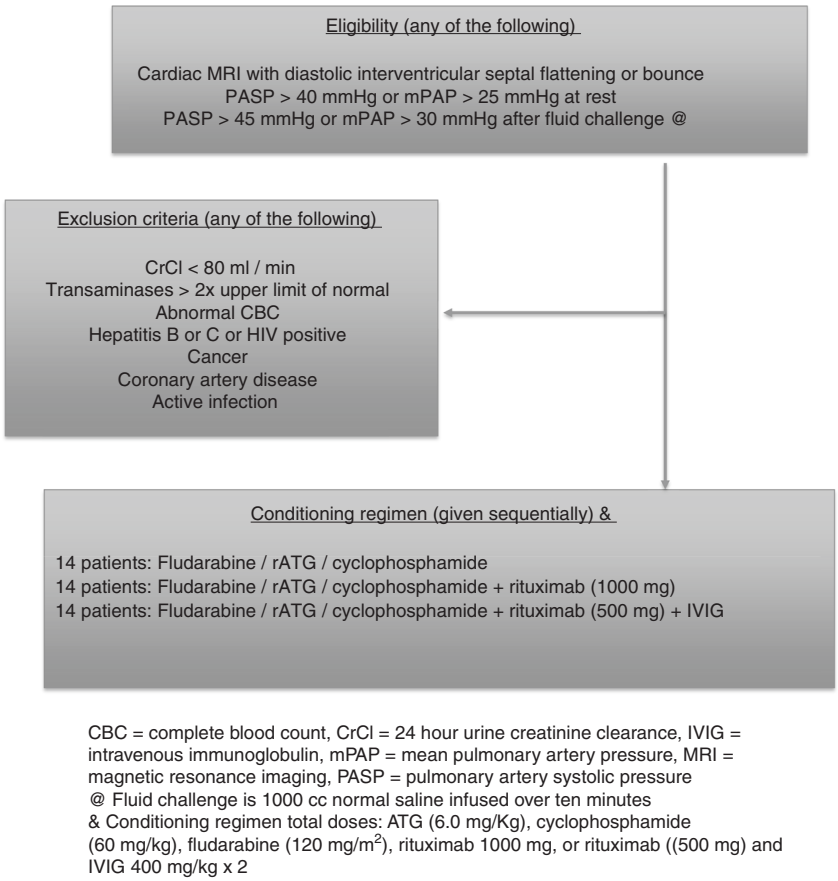

Fig. 1 Treatment design.

CBC = complete blood count, CrCl = 24 hour urine creatinine clearance, IVIG = intravenous immunoglobulin, mPAP = mean pulmonary artery pressure, MRI = magnetic resonance imaging, PASP = pulmonary artery systolic pressure
@ Fluid challenge is 1000 cc normal saline infused over ten minutes
& Conditioning regimen total doses: ATG (6.0 mg/Kg), cyclophosphamide (60 mg/kg), fludarabine (120 mg/m^2), rituximab 1000 mg, or rituximab ((500 mg) and IVIG 400 mg/kg x 2

strength TIW) was started when the platelet count rose above 100,000/ul and continued for 3 months. Patients were contacted to return at 6 months and 1 year.

Outcome

The primary objective was survival. Secondary objectives included modified Rodnan skin score (mRSS) and pulmonary function tests (FVC, TLC, DLCO). Relapse is defined as any of the following: an increase of mRSS by 25%, decline in FVC by 10%, renal crises, or restarting immune modulating or immune suppressive medications.

Statistical methods

Continuous variables (as age, FVC, TLC, DLCO, PASP, and mPAP) were summarized as means and standard deviations and their differences between groups were assessed via the Wilcoxon rank-sum test. Categorical variables (as sex and relapse) were summarized as counts and proportions, and differences in categorical variables between groups were assessed via Fisher's exact test. A two-sided p value was considered statistically significant.

Results

Demographics

Forty-five patients were evaluated of which three were excluded one each for a diagnosis of generalized morphea, scleroderma sine scleroderma, and CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia). Ten patients had limited systemic sclerosis and 32 had diffuse systemic sclerosis. The mean age was 46.3 (range 20–65) years old, 15 were male, 27 were female, and mean duration of disease before HSCT was 70.5 (SD 59, range 11–204) months. Before transplantation, 13 patients required chronic supplemental oxygen. All patients had interstitial lung disease. In terms of gastrointestinal involvement, all had gastroesophageal reflux disease (GERD), 38 patients had a patulous esophagus, 9 patients had GAVE, while none had documented small bowel involvement. Only one patient had a history of scleroderma renal crisis. Forty-one patients were antinuclear antibody (ANA) positive, 19 were Scl-70 positive, and 11 were anti-RNA polymerase positive. Pretransplant immune medications taken by patients are shown in Table 1.

Table 1 Pretransplant patient demographics.

Parameter: # patients and where indicated includes mean/standard deviation (range)	All patients	Flu/Cy/ATG	Flu/Cy/ATG with rituximab ± IVIG	*P* value between regimens with versus without rituximab
Patients	42	14	28	ND
Diffuse/limited SSc	32/10	11/3	21/7	0.99
Age (years)	46.3/11.9 (20–65)	40/11 (20–56)	49/11 (25–65)	0.02
Sex	M = 15, F = 27	M = 4, F = 10	M = 11, F = 17	0.73
Duration of disease in months	70.5/59 (11–204)	69/47 (13–171)	71/64 (11–204)	0.59
mRSS	18.1/12 (3–48)	18.4/9.4 (5–36)	18/13 (3–48)	0.66
FVC % predicted	62.5/16 (32–95)	56.3/16 (32–94)	65.8/15.6 (41–95)	0.07
TLC % predicted	70/16.5 (34–107)	62.3/18.5 (34–102)	73.8/14.4 (51–107)	0.04
DLCO (Hgb corrected) % predicted	49.4/16.4 (24–89.6)	38.7/12.6 (24–66)	53.8/15.8 (30–89.6)	0.005
Oxygen dependent	13/42	5/14	8/28	0.73
PASP > 40 mmHg	2/42	1/14	1/28	0.99
PASP > 45 mmHg with fluid (mmHg)	9/42	3/14	6/28	0.99
mPAP > 25 mmHg	3/28	0/14	3/28	0.54
mPAP > 30 mmHg with fluid challenge (mmHg)	14/42	8/14	6/28	0.04
Holter with cardiac SVE arrhythmias	14/14	3/3	11/11	NA
Holter with cardiac VE arrhythmias	14/14	3/3	11/11	NA
Holter with conduction blocks[a]	5/14	0/3	5/11	0.26
Cardiac MRI interventricular septal flattening (D-sign) or septal bounce	36/42	13/14	23/28	0.99
Abnormal LVEF, range <55% or >72%	0/42	0/14	0/28	NA
BNP elevated	9/42	3/14	6/28	0.99
EKG: abnormal[b]	27/42	10/14	17/28	0.73
Cardiac MRI myocardial late gadolinium enhancement	10/42	2/14	8/28	0.45
Cardiac MRI high ECV (>27%)	29/42	9/14	20/28	0.73
Diastolic dysfunction (grade 1 and 2)	10/42	2/14	8/28	0.45
Non-ischemic diffuse wall hypokinesis	4/42	0/14	4/28	0.28
Pericardial effusions	15/42	5/14	10/28	0.99
Patulous esophagus	38/42	13/14	25/28	0.99
Prior renal crises	1/42	0/14	1/28	0.99
GAVE	8/42	1/14	7/28	0.23
ANA positive	41/42	14/14	27/28	0.99
Scl-70 positive	19/42	8/14	11/28	0.33
anti-RNA polymerase positive	11/42	2/14	9/28	0.28

Table 1 (continued)

Parameter: # patients and where indicated includes mean/standard deviation (range)	All patients	Flu/Cy/ATG	Flu/Cy/ATG with rituximab ± IVIG	*P* value between regimens with versus without rituximab
Pre-HSCT immune medications				
Mycophenolate mofitel	35/42	9/14	26/28	0.03
Corticosteroids	16 /42	7/14	9/28	0.32
Cyclophosphamide	12/42	6/14	6/28	0.17
Rituximab	11/42	2 /14	9/28	0.28
Methotrexate	14/42	6 /14	8/28	0.49
Hydroxychloroquine	7/42	1/14	6/28	0.39
Intravenous immunoglobulin	2 /42	1/14	1/28	0.99
TNF-inhibitor (etanercept, adalimumab, infliximab)	4/42	2/14	2/28	0.59
Tocilizumab	1/42	0/14	1/28	0.99
Azathioprine	3/42	2/14	1/28	0.25

ANA antinuclear antibody, *ATG* anti-thymocyte globulin, *BNP* brain natriuretic peptide, *Cy* cyclophosphamide, *DLCO* diffusing capacity of the lung for carbon dioxide, *ECV* extracellular volume, *EKG* electrocardiogram, *F* female, *Flu* fludarabine, *FVC* forced vital capacity, *GAVE* gastric antral vascular ectasia, *HSCT* hematopoietic stem cell transplantation, *LVEF* left ventricular ejection fraction. *M* male, *mPAP* mean pulmonary artery pressure, *mRSS* modified Rodnan skin score, *MRI* magnetic resonance imaging, *PASP* pulmonary artery systolic pressure, *SSc* systemic sclerosis, *SVE* supraventricular ectopy (includes supraventricular tachycardia, or premature atrial contractions), *TLC* total lung capacity, *TNF* tumor necrosis factor, *VE* ventricular ectopy (includes premature ventricular contractions, bigeminy, trigeminy, or non-sustained ventricular tachycardia)

[a]Conduction blocks include 1st degree atrioventricular blocks, bundle branch blocks, or interventricular conduction delay.

[b]EKG abnormalities include: sinus tachycardia, atrial or ventricular premature contractions, supraventricular tachycardia, sinus bradycardia, flipped T waves, flipped P waves, low QRS amplitude, prolonged QT, 1st degree atrioventricular block, right bundle branch block, left anterior fascicular block, non-ischemic septal, anterior, or inferior infarcts.

In this sequential, non-randomized open-label study, patients who did not receive rituximab in their regimen compared with those who did receive rituximab tended to be younger 40 (SD 11, range 20–56) versus 49 (SD 11, range 25–65) years old, ($P = 0.02$), have a lower percent predicted TLC 62.3% (SD 18.5, range 34–102) versus 73.8% (SD 14.4, range 51–107), ($P = 0.04$), a lower percent predicted DLCO (hemoglobin corrected) (38.7%, SD 12.6, range 24–66) versus 53.8%, (SD 15.8, range 30–89.6), ($P = 0.005$), and more patients had a mPAP > 30 mmHg after fluid challenge (8/14 versus 6/28, $P = 0.04$) (Table 1).

Safety

One of 42 patients (2.4%) died during transplant hospitalization from acute myocardial infarction. The patient had coronary artery disease (65% occlusion of the right coronary artery) but refused percutaneous coronary intervention with coronary stent implantation due to the procedure delaying HSCT for 1 year while on post-stent antiplatelet therapy. There was one grade 4 toxicity (pericardial effusion with tamponade). Grade 3 toxicities and number of patients affected were: hypophosphatemia [4], hyperglycemia [3], hyponatremia [2] culture negative febrile neutropenia [2], atrial fibrillation [2], dyspnea [1], hyperphosphatemia [1], and asymptomatic hypotension [1] (Table 2).

The mean number of days of neutropenia (ANC < 500) was 5.2 (SD 2.2, range 1–10), the mean number of platelet and red blood cell transfusions was 0.3 (SD 0.97, range 0–5) and 1.85 (SD 2, range 0–9), respectively, and the mean day of discharge was day 10 (SD 1.3, range 8–14). The mean number of days with fever was 0.85 (SD 1.6, range 0–6). In the first-year post HSCT, five patients had upper respiratory tract infections (URTI), bronchitis, or pneumonia. During the first year after HSCT, two viral infections occurred: BK virus uremia and influenza.

Over the first year, more secondary autoimmune diseases (AD) occurred post-transplantation in patients treated without rituximab 21% (3 AD in 14 patients) versus with rituximab 0% (0 in 28 patients) ($P = 0.03$) Two patients developed hypothyroidism and one developed rheumatoid arthritis. During the first year, more URTIs occurred in patients not receiving rituximab 28% (4/14) versus those who received rituximab 3.6% (1/28) ($P = 0.04$).

1-year outcome between fludarabine based and rituximab-fludarabine based regimens

In total, six of 42 patients relapsed within the first year and continued or restarted mycophenolate mofetil. Relapse occurred in 36% (5/14) who did not receive rituximab in the

Table 2 Transplantation inpatient and 1-year toxicity.

Parameter: where indicated includes mean/ standard deviation (range)	All patients	Flu/Cy/ATG	Flu/Cy/ATG with rituximab ± IVIG	*P* value between regimens with and without rituximab
Transplant-related deaths	1/42	0/14	1/28	0.99
All deaths	4/42	2/14	2/18	0.99
Infections during inpatient hospitalization	0/42	0/14	0/28	NA
Day of discharge	10/1.3 (8–14)	9/1 (8–12)	10/1.3 (8–14)	0.03
Days absolute neutrophil count <500/ul	5.2/2.2 (1–10)	4.4/2 (1–8)	5.6/2.2 (2–10)	0.07
Number PRBC transfusion	1.85/2 (0–9)	1.64/1.3 (0–4)	1.96/2.3 (0–9)	0.90
Number Platelet transfusions	0.3/0.97 (0–5)	0.07/0.3 (0–1)	0.46/1.2 (0–5)	0.31
Number of patients with fever (>38.0 °C)	10/42	4/14	6/28	0.71
Number of days with fever	0.85/1.6 (0–6)	0.57/1.1 (0–4)	1/1.8 (0–6)	0.99
Grade 3 inpatient toxicity				
Hypophosphatemia	4/42	1/14	3/28	0.99
Hyperglycemia	4/42	2/14	2/28	0.59
Hyponatremia	2/42	0/14	2/28	0.54
Febrile neutropenia	2/42	1/14	1/28	0.99
Atrial fibrillation	2/42	0/14	2/28	0.48
Dyspnea	1/42	1/14	0/28	0.33
Hyperphosphatemia	1/42	0/14	1/28	0.99
Asymptomatic hypotension	1/42	0/14	1/28	0.99
Grade 4 inpatient toxicity				
Pericardial effusion/tamponade	1/42	1/14	0/28	0.33
Post discharge relapse deaths	2/42	2/14	0/28	0.11
Late post HSCT infections				
Upper respiratory tract infections (sinusitis, bronchitis, bacterial pneumonia)	5/42	4/14 3 restart MMF	1/28	0.04
Viral (BK uremia, influenza)	2/42	0/14	2/28	0.54
Number of secondary autoimmune diseases hypothyroidism (2), rheumatoid arthritis (1)	3/42	3/14	0/28	0.03

ATG anti-thymocyte globulin, *Cy* cyclophosphamide, *Flu* fludarabine, *HSCT* hematopoietic stem cell transplantation, *IVIG* intravenous immunoglobulin, *MMF* mycophenolate mofetil, *PRBC* packed red blood cell

conditioning regimen compared with 3.6 % (1/28) of patients who received rituximab in the conditioning regimen ($P = 0.01$).

The mean mRSS improved from 17.8 (standard deviation (SD) 9.9) to 6.1 (SD 3.6) and from 17.9 (SD 13) to 8.5 (SD 10.1) in the fludarabine-based and fludarabine-rituximab based regimens, respectively (Table 3). For patients treated with a rituximab containing regimen, the mean FVC improved from pre-HSCT of 65.8 (SD 15.6) to 69.6 (SD 17.5) and the mean TLC improved from 73.8 (SD 14.4) to 77.7 (SD 15.9). The mean DLCO (Hgb corrected) declined from 53.8 (SD 15.8) to 51.8 (SD 11.9). For patients treated without rituximab the mean FVC improved from 56.3 (SD 16.1) to 62.3 (SD 13.3), TLC improved from 62.3 (SD 18.5) to 71.4 (SD 13.8), and DLCO (hemoglobin corrected) declined from 38.3 (SD 12.6) to 37.8 (SD 12.4). The changes in FVC, TLC, or DLCO (hemoglobin corrected) between the rituximab and non-rituximab containing regimens was not significant (Table 3). Differences in patient immunoglobulin levels (IgG, IgA, and IgM) were not significantly different one year after HSCT between conditioning regimens without versus with rituximab (Table 3).

Differences between the two rituximab-based regimens

For patients treated with 1000 mg of rituximab the mean FVC, TLC, and DLCO (hemoglobin corrected) changed by +4.6%, +5.5%, and −0.9% respectively (refer to Table 4 for SD and range). For patient treated with 500 mg of rituximab and IVIG, the mean FVC, TLC, and DLCO (hemoglobin corrected) changed by +3.2%, +2.4%, and −2.8%, respectively (refer to Table 4 for SD and range). When comparing the two rituximab-based

Table 3 Outcome after transplantation between regimens with and without rituximab.

Parameter	Flu/Cy/ATG mean/SD (range)	Flu/Cy/ATG rituximab ± IVIG Mean/SD (range)	*P* value between regimens with and without rituximab
Relapse at 1 year	5/14 (36%)	1/28 (4%)	0.01
Overall survival at 1 year	12/14 (86%)	27/28 (96.4%)	0.25
mRSS pre-HSCT	17.8/9.9 (5–36)	17.9/13 (3–48)	
mRSS change at 12 months	6.1/3.6 (1–13)	8.5/10.1 (0–34)	
mRSS net change after 1 year	−11.7[a]	−9.4[b]	0.28
FVC pre-HSCT (percent predicted)	56.3/16.1 (32–94)	65.8/15.6 (41–95)	
FVC at 6 months	62.3/13.3 (41–86)	69.6/17.5 (42–98	
FVC net change after 6 months	+6	+3.8	0.48
TLC pre–HSCT (percent predicted)	62.3/18.5 (24–102)	73.8/14.4 (51–107)	
TLC at 6 months	71.4/13.8 (52–92)	77.7/15.9 (52–112)	
TLC net change after 6 months	+9.1	+3.9	0.30
DLCO (Hgb corrected) pre-HSCT (percent predicted)	38.3/12.6 (24–66)	53.8/15.8 (30–89.6)	
DLCO (Hgb corrected) at 6 months	37.8/12.4 (25–61)	51.8/11.9 (31–77)	
DLCO (Hgb corrected) net change after 6 months	–0.5	–2.0	0.98
IgG (mg/dl) pre-HSCT	1133/227 (915–1550)	1194/474 (565–2790)	
IgG (mg/dl) one year post HSCT	978/185 (74.3–127)	898/302 (573–1500)	
Net change in IgG (mg/dl)	−155	−296	0.08
IgA (mg/dl) pre-HSCT	228/105 (97–405)	241/147 (24–580)	
IgA (mg/dl) one year post HSCT	187/74 (127–327)	177/116 (34–354)	
Net change in IgA (mg/dl)	−41	−64	0.79
IgM (mg/dl) pre-HSCT	109/57.8 (35–203)	95/48.2 (32–187)	
IgM (mg/dl) one year post HSCT	89/51.9 (38–152)	111/68.9 (31–300)	
Net change in IgM (mg/dl)	–20	+16	0.25

ATG anti-thymocyte globulin, *Cy* cyclophosphamide, *DLCO* diffusing capacity of the lung for carbon dioxide, *Flu* fludarabine, *FVC* forced vital capacity, *Hgb* hemoglobin, *HSCT* hematopoietic stem cell transplantation, *IgA* immunoglobulin A, *IgG* immunoglobulin G, *IVIG* intravenous immunoglobulin, *IgM* immunoglobulin M, *mRSS* modified Rodnan skin score, *SD* standard deviation, *TLC* total lung capacity

[a]5 patients stayed on or restarted mycophenolate mofitel.

[b]none remained on while one restarted mycophenolate mofitel.

regimens, there were no significant differences in skin score, lung function (FVC, TLC, DLCO), or immunoglobulin levels (Table 4).

Discussion

Primary cardiac involvement in systemic sclerosis lacks a standardized definition [11]. Autopsy studies have demonstrated more than 50% of SSc patients have fibrosis, lymphocytic inflammation, or band necrosis despite normal coronary arteries [12]. However, this definition of primary cardiac SSc can only be obtained premortem via multiple random cardiac biopsies and as a result the diagnosis of primary cardiac involvement is under appreciated. A less invasive approach is cardiac MRI, which can detect expansion of the myocardial interstitial space visually by myocardial late gadolinium enhancement, or quantitatively by extracellular volume fraction (ECV) – an index that is elevated in the presence of myocardial amyloidosis, edema, or fibrosis [13]. In this cohort of patients, 24% (10 of 42) and 69% (29 of 42) had gadolinium enhancement or an elevated ECV, respectively. Other markers indicative of scleroderma-related stressed myocardium, when non-ischemic in origin, are wall hypokinesis, diastolic dysfunction, elevated BNP, conduction blocks, delays, arrhythmias, flipped P or T waves, elevated PASP or mPAP or intraventricular septal flattening or septal bounce [11]. Of note, left ventricular ejection fraction is not a good indicator of scleroderma-related myocardial dysfunction as it was normal in all patients. All of the patients described herein had more than one of these markers of stressed myocardium present before HSCT (Table 1). Cyclophosphamide infused at high doses (200 mg/kg) may cause acute myocardial

Table 4 Differences in outcome between the two rituximab regimens.

Parameter	Flu/Cy/ATG/Rituximab 1000 mg	Flu/Cy/ATG/ Rituximab 500 mg + IVIG	*P* value between rituximab 1000 mg vs rituximab 500 mg + IVIG
Relapse at 1 year	1/14 (7%)	0/14 (0%)	NS
Post HSCT deaths at 1 year	1/14	0/14	NS
mRSS pre-HSCT	16.1/11.9 (4–42)	19.7/14.2 (3–48)	
mRSS change at 6 months	10.7/11.7 (0–38)	11.2/9.6 (2–30)	
mRSS net change after 6 months	–5.4	–8.5	0.83
FVC pre–HSCT (percent predicted)	65/14.1 (41–87)	66.5/17.5 (44–95)	
FVC at 6 months	69.6/17.3 (42–97)	69.7/18.6 (46–98)	
FVC net change after 6 months	+4.6	+3.2	0.77
TLC pre-HSCT (percent predicted)	72.5/15.4 (54–106)	75/13.9 (51–107)	
TLC at 6 months	78/17.8 (52–112)	77.4/14.3 (55–102)	
TLC net change after 6 months	+5.5	+2.4	0.32
DLCO (Hgb corrected) pre-HSCT (percent predicted)	50.6/16.8 (30–84)	57.1/14.7 (38–89.6)	
DLCO (hHgb corrected) at 6 months	49.7/13.7 (31–77)	54.3/9.2 (42–69)	
DLCO (hHgb corrected) net change after 6 months	–0.9	–2.8	0.97
# patients with URTI during 1 year post HSCT	0/14	1/14	NS
IgG pre-HSCT	1226/539 (730–2790)	1164/422 (565–1820)	
IgG 6 months post HSCT	946/336 (483–1440)	792/183 (566–1170)	
Net change in IgG after 6 months	–280	–372	0.51
IgA pre-HSCT	259/158 (50–580)	225/140 (24–560)	
IgA 6 months post HSCT	198/96.6 (90–401)	114/77.7 (50–255)	
Net change in IgA after 6 months	–61	–111	0.37
IgM pre-HSCT	86/46.8 (34–185)	100/50.3 (32–187)	
IgM 6 months post HSCT	73/56.6 (20–171)	67/38 (20–115)	
Net change in IgM after 6 months	–13	–33	0.09

ATG anti-thymocyte globulin, *Cy* cyclophosphamide, *DLCO* diffusing capacity of the lung for carbon dioxide, *Flu* fludarabine, *FVC* forced vital capacity, *GAVE* gastric antral vascular ectasia, *Hgb* hemoglobin, *HSCT* hematopoietic stem cell transplantation, *IgA* immunoglobulin A, *IgG* immunoglobulin G, *IgM* immunoglobulin M, *IVIG* immunoglobulin, *mPAP* mean pulmonary artery pressure, *mRSS* modified Rodnan skin score, *MRI* magnetic resonance imaging, *PASP* pulmonary artery systolic pressure, *TLC* total lung capacity, *URTI* upper respiratory tract infection

necrosis and or acute cardiomyocyte dysfunction and, as we have previously reported, is poorly tolerated in patients with systemic sclerosis who have an elevated PASP, mPAP, or interventricular septal flattening or bounce [1, 6–8]

We report, herein, that HSCT using a regimen composed of fludarabine (120 mg/m^2), cyclophosphamide (60 mg/kg), and ATG (6.0 mg/kg) with or without rituximab (500 or 1000 mg) was well tolerated in patients with SSc and compromised cardiac function. These regimens were designed for cardiac parameters that the EBMT guidelines consider contraindications to receive a high dose cyclophosphamide-based regimen [8]. Despite selecting only high risk cardiac-compromised patients, TRM using a fludarabine based regimen was 2.4%, (i.e., one of 42 patients treated), which is below the 6–10% TRM from the traditional high dose cyclophosphamide (ASTIS, ASSIST) or total body irradiation (SCOT) based regimens [14]. The lower mortality, despite selection of more severely compromised cardiac candidates may be secondary to the lower total dose of cyclophosphamide (60 mg/kg) compared with 120 mg/kg for SCOT and 200 mg/kg for ASTIS and ASSIST.

The shorter neutropenic interval of 5.2 days compared with 9 to 10 days in the ASSIST, ASTIS, and SCOT trials may also contribute to lower mortality by decreasing the days at risk for neutropenic related infections. The period of neutropenia (5.2 days) was approximately one half as long as the traditional high dose cyclophosphamide (200 mg/kg)/ ATG (ASSIST and ASTIS) [1–3] or radiation-based (SCOT) [4] protocols. The numbers of platelet and PRBC transfusions (0.3 and 1.85, respectively) were also significantly less than the reported mean of 5.3 units of

platelets and 4.4 units of PRBCs required for patients treated with a high dose cyclophosphamide conditioning regimen [1, 2]. Ten patients (24%) had fever (temperature always ≥38 °C) which was significantly lower than the number of patients who developed fever on a high dose cyclophosphamide regimen (~70% developed fever >38 °C) [1–3].

In the cohort that did not receive rituximab, the pre-transplant TLC, DLCO, and mPAP were significantly worse than in the group who received rituximab (Table 1). Since all patients who meet criteria were enrolled sequentially and were not randomized, chance variations may arise between the cohort groups. Other variables such as duration of disease, requirement for nasal cannula oxygen, EKG or Holter monitor abnormalities, elevated BNP, diastolic dysfunction, wall hypokinesis, pericardial effusions, and MRI findings of interventricular flattening, septal bounce, elevated ECV, or myocardial enhancement were statistically identical between groups (Table 1).

Following HSCT the mRSS, FVC and TLC improved in both the rituximab and non-rituximab groups, and there were no statistically significant differences in the improvements in skin score or FVC or TLC between the groups (Table 3). In these patients selected for impaired cardiac function, the DLCO did not improve in any group. Failure of the DLCO to improve in systemic sclerosis with pre-HSCT impaired cardiac function are consistent with previous reports [1].

In the first year after transplantation, URTI were more common in the group treated with fludarabine, ATG, and cyclophosphamide (36%, 5 of 14), and were less frequent in the group that received rituximab along with fludarabine, ATG, and cyclophosphamide (7%, 1 of 28) $P = 0.04$ (Table 2). There was no significant difference in IgG, IgA, or IgM between the groups (Table 3). Therefore, a possible explanation for the increase in URTI for those who did not receive rituximab may be related to the increase in relapse rate and restarting post-transplant immune suppression in the non-rituximab group.

Fludarabine has been reported to increase the incidence of secondary autoimmune cytopenias [15], and within the first year, the incidence of secondary autoimmune diseases, hypothyroidism and rheumatoid arthritis, was higher in patients not receiving rituximab. In contrast, no patient who had rituximab added to the transplant regimen developed a secondary autoimmune disorder.

In patients with systemic sclerosis, rituximab has been reported to have a good safety profile and seems to improve skin score but not pulmonary function tests [16]. In this study, the addition of rituximab to a regimen of fludarabine, cyclophosphamide and ATG appeared to make the regimen safer with fewer relapses, less relapse related mortality, fewer URTIs, and fewer fludarabine-related secondary autoimmune diseases. This emphasizes that different conditioning regimens have different toxicities and outcome profiles.

There are limitations to this study. It was run sequentially and not randomized, the fludarabine, cyclophosphamide, ATG, group not treated with rituximab were significantly younger, had a lower FVC and TLC, and higher mPAP with fluid challenge (Table 1), and the study had a small number of patients with relatively short follow-up (1 year). However, the study was designed not to compare regimens but as an exploratory safety analysis to determine if patients with cardiac contraindications to conventional HSCT who cannot tolerate an intense cyclophosphamide-based conditioning regimen could tolerate a fludarabine-based transplant regimen. Although the number of patients undergoing HSCT was relatively small ($n = 42$), the numbers of patients who underwent HSCT in the randomized ASTIS and SCOT trials were also relatively small ($n = 72$ and 36, respectively).

Summary

A regimen of fludarabine (120 mg/m^2), ATG (6.0 mg/kg), and cyclophosphamide (60 mg/kg) while performed safely in systemic sclerosis patients with poor cardiac function was complicated within the first year after transplant by a high relapse rate, URTI, and secondary autoimmune diseases. The same fludarabine, ATG, and cyclophosphamide regimen that also included rituximab (500–1000 mg) appeared to be a safe effective regimen and over one year had a lower risk of relapse, URTI, and secondary autoimmune diseases. However, longer follow-up and comparison of late relapse rate, secondary autoimmune disorders, and opportunistic (especially upper respiratory tract and or viral) infections is warranted.

If remission rates are durable, due to lower treatment related mortality, shortened duration of neutropenia (5 days), and improvement in skin fibrosis and pulmonary function abnormalities (FVC and TLC), this low-risk regimen may be considered as a replacement for the traditional high dose cyclophosphamide-based and total body irradiation-based regimens for systemic sclerosis patients with normal creatinine clearance regardless of the presence or absence of a high-risk pretransplant cardiac profile.

Clinical trial registry: ClinicalTrials.gov Identifier: NCT03593902

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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