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Title: **Autologous non-myeloablative hematopoietic stem cell transplantation for diffuse cutaneous systemic sclerosis: identifying disease risk factors for toxicity and long-term outcomes in a prospective, single-arm trial**

Running title: Stem cell transplantation for systemic sclerosis

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**Abstract**

Objective: Two randomized trials for patients with diffuse systemic sclerosis (SSc) demonstrated an overall survival (OS) and event-free survival (EFS) advantage of autologous hematopoietic stem cell transplantation (AHSCT) using CD34+ selected peripheral blood stem cells (PBSC) compared to monthly cyclophosphamide. We asked if an unmodified PBSC graft followed by maintenance mycophenolate mofetil (MMF) after AHSCT, instead of CD34+ selected graft, could provide comparable AHSCT outcomes.

Methods: 20 patients with high-risk SSc were enrolled in a prospective, single-arm trial with cyclophosphamide 200 mg/kg and horse anti-thymocyte globulin (CY200/ATG), followed by unmanipulated autologous PBSC, then MMF maintenance starting at 2 months after AHSCT.

Results: Point estimates of OS and EFS at 5 years after AHSCT were 85% (95% CI, 60.4%-94.9%) and 75% (95% CI, 50%-88.7%), respectively. Median follow-up was 7.5 years (range, 5.6-11.6) after transplant for living patients. Eight patients (40%) required intensive care unit treatment early after transplant. Early transplant-related mortality occurred in 2 patients (10%). Five patients developed relapse/progression of SSc after AHSCT. Four of 9 patients with anti-RNA polymerase-III antibody had both prior scleroderma renal crisis and the lowest quartile of estimated glomerular filtration rate (eGFR) upon study entry; all 4 patients developed prolonged organ failure/death early post-transplant.

Conclusion: We observed favorable OS and EFS after AHSCT for SSc patients, using CY200/ATG, unmanipulated PBSC and MMF post-transplant maintenance, that was comparable to trials

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with CD34+ graft selection. We identified a possible risk factor, pretransplant low eGFR, for adverse outcome after AHSCT.

**Introduction**

Diffuse cutaneous systemic sclerosis (dcSSc) is a devastating autoimmune disorder that is often fatal due to progressive internal organ involvement.[1-3] Although disease modifying anti-rheumatic drugs (DMARDs) and biologics have been studied, none have shown sustained survival benefit.[4-6] Two large randomized clinical trials demonstrated an overall survival (OS) and event free survival (EFS) advantage of autologous hematopoietic stem cell transplantation (AHSCT) compared to an effective DMARD, consisting of monthly cyclophosphamide (750 mg/m$^2$x 12 months).[7-8] The non-myeloablative conditioning regimen for the first trial (ASTIS) consisted of cyclophosphamide 200 mg/kg plus rabbit anti-thymocyte globulin (ATG) 7.5 mg/kg followed by CD34+ immunomagnetically selected autologous peripheral blood stem cells (PBSC).[7] In the AHSCT arm, the transplant related mortality (TRM) was 10.1% and the 5-year OS and EFS was 81% and 78%, respectively.[7] The second trial (SCOT) consisted of myeloablative fractionated total body irradiation (TBI) 8 Gray ([Gy], with lung, kidney shielding limiting the radiation dose to 2 Gy), cyclophosphamide 120 mg/kg, horse ATG 90 mg/kg (TBI/CY/ATG), followed by infusion of CD34+ selected autologous PBSC.[8] In the per protocol AHSCT arm, the TRM was 6% at 72 months (due to myelodysplastic syndrome [MDS]), the 72-month OS and EFS was 86% and 74%, respectively.[8]

Between the publication of the ASTIS and SCOT trial, we conducted a multi-center, single-arm trial evaluating the efficacy of an irradiation-free, non-myeloablative cyclophosphamide 200

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mg/kg plus horse ATG conditioning (CY200/ATG), followed by unselected autologous PBSC infusion and maintenance mycophenolate mofetil (MMF), for patients with high-risk dcSSc, using the eligibility criteria of the SCOT trial. CD34+ selection was not used primarily because of limited availability, regulatory burden, added cost, and potential increased risk of opportunistic infections. MMF was given after AHSCT to prevent disease relapse. The primary outcome measure was EFS, defined as survival without prolonged organ damage. Secondary objectives included safety and time to disease relapse requiring treatment with additional DMARDs. We sought to evaluate the overall safety and potential efficacy of the conditioning regimen with post-transplant MMF maintenance therapy given in place of CD34+ selection of the autologous graft.

## Patients and Methods

### *Trail design, interventions, and oversight*

This investigator-initiated phase 2 trial was conducted at 6 paired United States rheumatology and transplant centers. The trial ended when the last participant completed 5 years of follow-up, with a maximum follow-up of 11.6 years. Participants were followed annually for 5 years after AHSCT and at study completion. The trial was registered with ClinicalTrials.gov (NCT01413100). An independent data safety monitoring board consisting of 2 rheumatologists, 1 transplant physician, and 1 biostatistician met every 6-12 months to review data. All participants provided informed written consent before trial participation. The trial was conducted in accordance with Helsinki Declaration and Good Clinical Practice guidelines. The

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protocol was approved by each site's institutional review board. Participant race and ethnicity was self-reported.

Participant inclusion and exclusion criteria were as follows: the diagnosis of SSc (ACR/EULAR 2013 criteria[9]) with diffuse subset and disease duration of ≤5 years from first non-Raynaud sign or symptom, with either pulmonary/ renal involvement or rapidly progressive, extensive, severe skin involvement for ≤2 years. Pulmonary involvement required active interstitial lung disease (ILD) determined by chest computed tomography plus forced vital capacity (FVC) or lung diffusing capacity for carbon monoxide (DLCO, adjusted for hemoglobin) <70% predicted value. Renal involvement required previous scleroderma-related renal disease. Patients had received either MMF, mycophenolic acid (MPA), or cyclophosphamide for ≥4 months with no clinical benefit. Exclusion criteria included active gastric antral vascular ectasia, DLCO and FVC <40% and <45% predicted value, respectively, left ventricular ejection fraction (LVEF) <50%, serum creatinine ≥2.0 mg/dL. Patients with evidence of SSc cardiac involvement or pulmonary arterial hypertension (mean PA pressure >40 mmHg) were excluded.

Mobilization of autologous PBSC used granulocyte-colony stimulating factor (G-CSF, filgrastim) 16 μg/kg/day subcutaneous for 5 days. Prednisone 0.5 mg/kg/day was administered during G-CSF and for 5 days afterwards to prevent flare of autoimmune disease. Apheresis and cryopreservation were completed in compliance with certified institutional practice.

All DMARDs were stopped prior to conditioning. Between 6-21 days after completion of apheresis, patients were admitted to the HSCT unit and began conditioning with intravenous cyclophosphamide 50 mg/kg/day (days -5 to -2, total dose 200 mg/kg), an equal Mesna dose,

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and horse ATG 15 mg/kg/day (days -5, -3, -1, +1, +3, +5). Intravenous methylprednisolone 1 mg/kg was administered before each ATG dose. Autologous PBSC (cell dose >2.5 x$10^6$ CD34/kg) were thawed and infused on day 0. Prophylactic antibiotics, antiviral, and anti-fungal medications were administered per standard institutional practice. Lisinopril was administered to maintain systolic blood pressure between 90-110 mmHg. Maintenance MMF/MPA was started 2-4 months after HSCT; dose was titrated to tolerance (target dose MMF 1 g or MPA 720 mg, twice daily), with a plan to continue maintenance treatment for ≥2 years.

Kaplan-Meier estimates of OS, EFS, and additional DMARD-free EFS (DEFS) were obtained with a focus on point estimates at 5- and 10-years. EFS was defined as survival without meeting the protocol defined endpoint of organ injury (kidney injury requiring renal replacement dialysis for >6 months, sustained LVEF <30%, or sustained decline of FVC >20%), similar to prior trials.[7-8] DEFS was defined as survival without meeting EFS endpoint and without additional DMARD treatment. Patients were for evaluable for disease response if they survived >3 months after HSCT. TRM was defined as death before day +90 after HSCT. Data were censored at last follow-up. Analysis used Prism software, v10.0. Changes in least squares (LS) means were based on the linear mixed effect model, where change from baseline was the outcome, baseline value and study time were the covariates. Models were fitted with random effect of intercept. Local institutional funding supported the collection and reporting of data. Data cut-off was 12/01/2023. Data will be shared in a public repository.

**Results**

*Patient Characteristics*

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Twenty patients with dcSSc meeting entry criteria were enrolled and completed autologous HSCT between January 2012 to September 2018. Baseline characteristics are shown in **Table 1**. The mean age of study participants was 46 years (range, 13-70), with 15 females, 5 males. The median time from disease onset to HSCT was 18 months (range, 6-59). The mean number of prior (failed) DMARDs for SSc was 2 (range, 1-5). At pre-transplant evaluation, the mean modified Rodnan skin score (mRSS) was 34 (range, 8-46). Eighteen patients (90%) had ILD, and 5 patients (25%) had a history of scleroderma renal crisis (SRC) prior to enrollment. The mean baseline adjusted DLCO was 62% predicted value (range, 41-91%), the mean FVC was 73% (range, 50-111%). Four (20%) participants had a prior history of tobacco smoking. The median estimated glomerular filtration rate (eGFR, ml/min/1.73m$^2$) was 105 (range, 33-150, CKD-EPI 2021 equation[10]).

All participants had baseline anti-nuclear antibody (ANA) titer of ≥1:320. Eight participants (40%) were anti-topoisomerase 1 (Scl-70) positive (ATA+), 9 (45%) were anti-RNA polymerase-III positive (ARA+), one patient was positive for both. No one was anti-centromere positive.

*Engraftment*

Median CD34+ cell dose of the unmodified autologous PBSC infused at transplant was 5.57 (range, 2.8-15.56) x10$^6$/kg. All patients had sustained neutrophil engraftment (range, 8-13) days after transplant followed by sustained platelet engraftment.

*Overall and Event-Free Survival*

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Sixteen patients were alive at last follow-up (**Figure 1**). The median follow-up of surviving patients was 7.5 years after transplant (range, 5.6-11.6 years). The 5- and 10-year OS estimates were 85% (95% confidence interval [CI], 60.4%-94.9%) and 77% (95% CI, 49%-91%), respectively (**Fig. 2a)**. There were 6 events (4 deaths and 2 patients developed prolonged organ failure). The 5- and 10-year EFS estimates were 75% (95% CI, 50%-88.7%) and 67% (95% CI, 40.5%-84.3%), respectively (**Fig. 2b**).

*Transplant-Related Mortality (TRM) and Adverse Events*

TRM occurred in 2 patients (**Fig. 1**), on days +37 and +44 after HSCT, indicating a 1-year TRM of 10% (95% CI, 2.6%-34.6%). The patient (#14) who had TRM at day +37, developed fever, hypoxemia, skin rash, pulmonary infiltrates on day +10 after transplant, consistent with engraftment syndrome[11]. Despite therapy, respiratory symptoms worsened by day +13, requiring intubation and mechanical ventilation. Acute kidney injury (AKI) and anuria developed, requiring daily renal replacement therapy beginning on day +14. Echocardiogram showed global hypokinesis with LVEF of 25%. After transient stabilization, the patient developed recurrent fevers with hypotension. Despite maximal pressor support and anti-infective agents, the clinical status worsened with development of multiple brain infarcts, acute hepatic injury, coagulopathy, followed by progressive multi-organ failure, leading to death on day +37. An autopsy showed diffuse pulmonary fibrosis, diffuse alveolar damage, small vessel sclerosis, patchy fibrin exudates, and airways with extensive mucous plugging. The heart showed remote infero-posterior LV myocardial infarction. Kidneys showed medullary casts, focal glomerulosclerosis, focal neointimal formation, but no microthrombi. The brain cortex

showed acute ischemic injury of the left parietal and occipital regions. The liver showed centrilobular atrophy/dropout and fibrosis.

The patient (#9) who had TRM on day +44 developed progressive pulmonary edema with mild cardiac hypokinesis starting on day +1 after HSCT with concurrent worsening renal function, initially stabilized with diuresis. On day +13, cardiac failure worsened, with LVEF 20-25%, combined with volume overload and anuria, and renal replacement therapy was initiated. Symptoms gradually improved and the patient was discharged from hospital on day +43 with planned thrice-weekly hemodialysis. Within 20 minutes of the first outpatient hemodialysis, the patient developed fever and hypotension, then acute respiratory failure and cardiac arrest. Despite a prolonged resuscitation effort, the patient expired. Death was due to sepsis in the setting of AKI with SRC.

Both patients with early TRM developed evidence of SRC with AKI progressively developing between 7 to 14 days after HSCT with concurrent engraftment syndrome and evidence of cyclophosphamide-induced cardiomyopathy. Terminal events included acute respiratory failure, sepsis physiology, and multi-organ injury.

Thirteen patients (65%) developed at least one grade ≥3 adverse event, which were outside of the expected, time-limited hematological/gastrointestinal toxicity of HSCT. In addition to the 2 patients with early TRM, 3 patients (#7, #12, #15) developed at least two grade-4 adverse events after HSCT requiring intensive care unit (ICU) stay for the treatment of combined AKI requiring renal replacement therapy and limited-duration intubation with mechanical ventilation for alveolar hemorrhage (n=1) or respiratory distress due to volume overload (n=2)



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during the first month post-transplant. The patient (#12) with alveolar hemorrhage recovered lung and renal function by 3 and 4 months after HSCT, respectively. The remaining 2 patients promptly recovered lung function but required ongoing hemodialysis for >6 months due to prolonged renal injury. One patient (#7) with prolonged renal injury also developed ruptured left renal artery aneurysm day +77 post-transplant, treated with embolization. This patient proceeded to a successful living donor kidney transplant 2 years after HSCT. The second patient (#15) recovered renal function by 18 months and discontinued hemodialysis, however they had persistent cardiomyopathy after transplant with LVEF of 25-30%. The 2 patients with renal failure >6 months after HSCT met the protocol endpoint of organ failure.

One patient (#8) with RSR' right ventricular conduction delay on electrocardiogram prior to HSCT developed persistent, refractory ventricular tachycardia at day +10 post-transplant requiring ICU and intravenous anti-arrhythmia drug treatment. This patient underwent successful right cardiac ablation 1-month post-transplant, followed with oral diltiazem, with no recurrence of significant arrhythmia beyond 2 months after HSCT. In total, 8 patients (40%, #7, #8, #9, #10, #11, #12, #14, #15) received ICU treatment early after transplant for a median of 27 (range, 4-50) days.

Three patients (15%) had transient, low-level reactivation of Epstein-Barr virus during the first 100 days after transplant, but none required treatment. The two patients with early TRM had cytomegalovirus reactivation after HSCT that responded to ganciclovir treatment.

In addition to the 2 patients with early TRM, 1 patient (#20) died at 40 months after transplant due to sepsis/infection, unrelated to HSCT. This patient did not take MMF maintenance therapy



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and initially had an excellent response in skin tightness symptoms. However, beginning at 34 months post-transplant, the patient reported increasing diffuse skin tightness, but was not started on DMARD due to patient preference. They had a non-ST- elevation myocardial infarct with concurrent pericardial effusion at month 39. The pericardial effusion cytology was hypocellular, containing histiocytes, mesothelial cells and few lymphocytes. At month 40 post-transplant, the patient was found unresponsive and died shortly after arrival to hospital due to overwhelming sepsis. Recurrence of SSc disease activity likely contributed to this patient's death; no autopsy was obtained.

There was one late death at 8 years after transplant. This patient (#3) developed low-grade MDS with complex cytogenetics at 6.2 years after HSCT attributable to late effects of the conditioning regimen or prior cyclophosphamide. The malignancy progressed to high-grade MDS by 7 years after HSCT and did not respond to 3 cycles of decitabine/venetoclax. The MDS progressed to secondary acute myelogenous leukemia (AML) and the patient died of AML complications without recurrence of SSc disease.

*SSc Response to Treatment*

Eighteen of 20 patients were evaluable for disease response to HSCT. Thirteen of 18 patients (72%) initiated and were compliant with MMF/MPA maintenance therapy with treatment duration ranging from 9 months to ≥5 years after HSCT (**Fig. 1**). Reasons for not starting post-transplant MMF/MPA included patient/physician preference (n=3) or AKI (n=2). There were no significant adverse events reported among the patients treated with MMF/MPA maintenance other than mild upper respiratory infection ([URI], n=2). Reasons for discontinuation of

MMF/MPA <2 years post-transplant included SSc progression/relapse (n=1) and URI (n=2). Seven (39%) patients continued MMF/MPA for ≥5 years after HSCT.

Excluding the 4 patients who met an early post-transplant EFS endpoint, renal function was stable or improved compared to baseline among all surviving patients at time points >3 months post-transplant. In addition, available follow-up echocardiograms and electrocardiograms for the 14 patients with long-term EFS were stable/unchanged.

Among the 18 evaluable patients, 12 (67%) achieved a sustained, ≥50% reduction in skin scores at a median of 12 (range, 2-60) months after HSCT without additional DMARD treatment. Eight (44%) of 18 patients achieved a sustained, ≥10% improvement in predicted FVC at a median of 18 (range, 12-60) months post-transplant. Seven (39%) of 18 patients achieved a sustained, ≥15% improvement in predicted DLCO at a median of 4 (range, 2 -5) years post-transplant. No patient met the lung-function failure endpoint. **Table 2, Fig. 3** show the LS mean change in 4 outcome measures: mRSS, FVC, DLCO, and HAQ-DI. There were significant improvements in all 4 outcome measures over the course of the trial with improvements seen at ≤1 year after AHSCT with continuing improvement/ stabilization.

A minority of patients who were positive for SSc-autoantibodies at pre-transplant evaluation became negative (below the clinically positive range) post-transplant. Of the 8 patients who were anti-topoisomerase-1 positive (ATA+), 3 (38%) became autoantibody-negative by 1-4 years post-transplant. Of the 9 patients who were anti-RNA polymerase-III positive (ARA+), 4 (44%) had decreased levels to negative range by 2 years post-transplant (using the same assay platform). Of the 20 patients who were ANA positive pre-transplant, 7 (35%) became ANA-



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negative, 7 patients had decreased or unchanged ANA titer, and 6 patients (30%) had no follow-up testing.

*SSc Relapse/ Progression*

Five patients developed SSc relapse/progression after AHSCT. This included 4 patients that developed SSc disease relapse/progression requiring treatment with additional DMARDs (tocilizumab [#18, #10], pulse cyclophosphamide [#16], abatacept [ #6]) at 8, 17, 9, 53 months post-transplant, respectively, despite compliance with MMF/MPA maintenance therapy (**Fig. 1**). Skin tightness (n=2) or decline in DLCO% (n=2) prompted the initiation of additional DMARD (1 patient [#18] had skin worsening and DLCO% decrease at month 8). One patient (#10) switched from MMF to tocilizumab at month 17 due to stable skin tightness symptoms. The addition of tocilizumab met the protocol definition for relapse/progression. Treatment with a new DMARD improved the disease status for all 4 patients. Two patients (#5, #13) received weekly methotrexate for treatment of inflammatory arthritis, starting at 2 and 4 years, respectively, post-transplant. As described above, one patient not taking MMF maintenance had SSc relapse, did not start DMARD, and died at month 40.

*Additional DMARD- and Event-Free Survival (DEFS)*

For the entire cohort, the combined additional DMARD-free and event-free survival (DEFS) was 55% (95% CI, 31.3%-73.5%) and 48% (95% CI, 24.6%-68.3%) at 5 and 10 years, respectively (**Fig. 2c**). DEFS excluded the protocol-prescribed MMF maintenance (n=13) and weekly methotrexate (n=2) given for arthritis. Four of the 5 patients (80%) who did not receive post-transplant

MMF/MPA did not experience disease relapse/progression (mean follow-up, 7.3 years). Nine of the 13 patients (69%) who took MMF/MPA maintenance therapy did not experience relapse/progression (mean follow-up, 8.7 years, **Fig. 1**).

*Impact of Pre-transplant Scleroderma Renal Crisis with Impaired Renal Function*

Among the 5 patients with a pre-transplant history of SRC (**Table 1, Fig.1**), 4 patients (#7, #9, #14, #15) were ARA+ and had the lowest quartile of eGFR pre-transplant (< 65 ml/min/1.73m$^2$). These 4 patients developed AKI with evidence of SRC early post-transplant, and then had TRM or sustained organ injury. All other 16 patients had baseline eGFR ≥75 mL/min/1.73m$^2$, none of these 16 patients experienced a sustained organ injury event/death early post-transplant.

*Impact of Anti-RNA Polymerase III (ARA+) and Scl-70 (ATA+) Autoantibody*

There were 9 ARA+ patients (45%) at study entry (**Table 1, Fig. 1**). This included the 4 patients with a pre-transplant history of SRC and eGFR <65mL/min/1.73m$^2$. Among these 9 patients, 2 died of TRM, 2 met an early EFS endpoint (prolonged organ failure), and 3 experienced SSc relapse requiring initiation of DMARD at 8, 9, 17 months, respectively, post-transplant (all patients described above). In contrast, among the 11 ARA- patients, as described above, 1 died with scleroderma relapse at 40 months, 1 had disease progression at 53 months successfully treated with abatacept, and 1 died of AML at 8 years. The 5-year point-estimate of EFS for ARA+ and ARA- patients was 56% (95% CI, 20.4%-80.5%) and 91% (95% CI, 50.8%-98.7%), respectively (**Fig. 4a**). The 5-year point-estimate of DEFS for ARA+ and ARA- patients was 22% (95% CI, 3.4%-51.3%) and 82% (95% CI, 44.7%-98.7%), respectively (**Fig. 4b**). The 5-year point estimate of EFS



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and DEFS for ATA+ (Scl-70+) patients (n=8) was 87.5% (95% CI, 38.7%-98.1%) and 75% (95% CI, 31.5%-93%), respectively.

Of the 4 patients who became ARA seronegative after transplant, 1 (#10) received DMARD (tocilizumab) at month 17, and all 4 eventually had sustained improvements in skin scores and lung function. One patient (#19) who remained ARA seropositive post-transplant had a very favorable clinical response to transplant.

**Discussion**

This single-arm, multi-center study confirmed the efficacy of non-myeloablative CY200/ATG conditioning and autologous PBSC transplantation with post-transplant MMF for patients with severe dcSSc. Similar to the ASTIS trial which used CY200/ATG conditioning, transplant-related mortality was 10%.[7] With 20 patients in this trial, the median follow-up was 7.5 years; the 5-year OS and EFS of 85% and 75%, respectively, numerically similar to that observed in the ASTIS trial, 81% and 78%, respectivley.[7] Because there was detailed clinical information available for each patient in this trial, we identified potential disease-associated factors that may contribute to an adverse outcome after HSCT. A prior history of SRC and decreased eGFR were factors that appeared to lead to early post-transplant events including death or prolonged organ injury. In addition, a high proportion of ARA+ patients reached endpoints of death, organ injury, or disease progression after HSCT. Previous trials identified cardiac involvement of SSc and pulmonary arterial hypertension as risk factors for early adverse outcome.[7,12] As patients with these risk factors were excluded in this trial, we have identified additional disease factors potentially associated with adverse outcome when using CY200/ATG conditioning.



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DcSSc with ARA+ autoantibody is associated with more extensive skin disease, renal crisis, and pulmonary hypertension compared to other SSc-associated autoantibodies.[13-16] Compared to the general SSc population, there was a high proportion of ARA+ patients in this trial.[13-16] ARA+ SSc is associated with SRC and patients with SRC frequently develop chronic kidney disease (CKD), with impaired eGFR that does not necessarily recover.[17] For this trial, no dose adjustment of cyclophosphamide in the setting of stage 2/3a CKD was made, and this factor very likely contributed to the observed early TRM and cyclophosphamide-associated toxicity. A key observation is that CY200/ATG conditioning may be too toxic for dcSSc patients with reduced eGFR. This observation may be relevant for explaining the 0% early TRM observed in the SCOT trial which limited the conditioning regimen total cyclophosphamide dose to 120 mg/kg and enrolled patients with better renal function.[8] Recent studies have shown increased potential for cyclophosphamide induced toxicity in patients with even mildly impaired renal function. [18] The exact recommended lower limit of eGFR eligible for CY200/ATG is uncertain due to the limited number of patients studied but may be in the range of <65 to 75 ml/min/1.73m$^2$. For SSc patients with reduced eGFR, cyclophosphamide dose-adjustment should be explored in future clinical trials. No methylprednisolone dose adjustment was made for ARA+ patients. Moderate-high dose methylprednisolone has been associated with SRC in early SSc patients who are ARA+.[19,20] Although prophylactic lisinopril was given to reduce the risk of SRC after AHSCT, this did not appear to prevent SRC progression in these patients, highlighting that this population should be carefully considered for HSCT.

The relatively low DMARD- and event-free survival among ARA+ patients is a new and potentially important finding. Patients who are ARA+ may have a more aggressive SSc disease

course. [13-16] Furthermore, compared to the SCOT trial TBI/CY/ATG conditioning, we speculate that the non-myeloablative CY200/ATG conditioning may be insufficient to completely eradicate the underlying autoimmune pathology in some dcSSc patients. For patients who were ATA+ (Scl-70+), the 5-year EFS and DEFS were favorable, 87.5% and 75%, respectively. The patients with ARA+ had worse outcomes but the data were confounded by the presence of pretransplant SRC and decreased eGFR (in 4 of 9 ARA+ patients). The incidence of SSc relapse after AHSCT was not significantly different between the ATA+ versus ARA+ groups, but the number of evaluable patients was low. Future studies should compare the transplant outcome of ARA+ versus ATA+ SSc patients.

Eight patients (40%) required ICU treatment early after transplant. This reflected the toxicity of the high-dose cyclophosphamide in SSc patients in this trial. Other myeloablative conditioning regimens appear to be associated with less toxicity, such as the SCOT trial TBI/CY/ATG regimen[8]. It is likely that the disease-related factors of recent SRC and decreased eGFR (stage 2/3a CKD) contributed to the adverse events after CY200/ATG. With a limited number of enrolled patients, we were unable to identify other variables such as age or prior treatment as significant risk factors for adverse outcomes. For those patients with follow-up data, there were significant improvements in mRSS, FVC%, DLCO%, and HAQ-DI. These findings are consistent with published AHSCT data[7,8] and compares favorably to non-transplant treatment outcomes.[21]

Thirteen patients were compliant with MMF maintenance therapy for ≥9 months duration. No significant adverse events were reported among the patients taking MMF maintenance other than 2 mild URI cases. Overall, post-transplant MMF maintenance was well-tolerated and



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appeared safe. Two retrospective, non-randomized, SSc transplant registry studies suggested that CD34+ selection was associated with a better response to HSCT therapy; however, patients who received unmanipulated grafts in those trials did not receive MMF maintenance.[22,23] Future randomized trials are needed to definitively assess the impact of MMF maintenance versus CD34+ selection on post-transplant disease relapse.

TBI-based conditioning has been associated with a risk of post-transplant secondary malignancies.[24,25] One reason for avoiding TBI in this trial was to assess if there was a reduced incidence of MDS/AML using the CY200/ATG regimen. There was one patient (5%) that developed secondary MDS/AML at 6.2 years after HSCT. This is consistent with the 6% incidence of secondary MDS after TBI/CY/ATG conditioning in the SCOT trial[8]. The patient with post-transplant MDS in our study was older and received pre-transplant cyclophosphamide. Cyclophosphamide is an alkylating agent with a known risk for development of secondary malignancies.[25,26] The risk of secondary malignancy after autologous HSCT is low, but non-negligible. Further studies are needed to compare the difference in secondary malignancy incidence between TBI- and CY-based conditioning regimens.

It was noteworthy that a minority of patients (38%) who were seropositive for SSc-associated autoantibodies at pre-transplant evaluation became seronegative post-transplant. It took 1 to 4 years for these patients to become seronegative, but a clinically favorable response to transplant did not require conversion to seronegativity. Future studies are needed to assess if conversion to autoantibody seronegative status is related to more durable clinical responses.

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Limitations of this study include the limited number of patients, the single-arm trial design, inadequate representation from underrepresented populations, the incomplete adherence to MMF/MPA maintenance therapy, which may result in over-estimation of the significance of study findings. The study was not designed to detect differences between groups.

In summary, this study suggests that non-myeloablative CY200/ATG (without TBI) conditioning is an effective autologous HSCT regimen for many patients with dcSSc. Importantly, we identified a factor for adverse outcome after transplant: decreased pre-transplant eGFR (approximately <65-75ml/min/1.73m$^2$), which occurred in patients with a prior history of SRC with ARA+ dcSSc. For patients with reduced eGFR, we advise to avoid AHSCT using CY200/ATG conditioning and consider an alternative regimen such as TBI/CY/ATG (SCOT trial[8]) or a clinical trial with dose-reduced cyclophosphamide. For patients without these factors (no prior SRC and normal eGFR), the CY200/ATG regimen with MMF maintenance appears to be a highly effective treatment, however, there was a moderate incidence of ICU admission. We showed that MMF maintenance after AHSCT appeared safe and was well tolerated. Whether MMF maintenance therapy is an effective substitute for CD34+ selection remains unknown, however, the OS and EFS in this trial was consistent with the ASTIS trial that employed CD34+ selection.[7,8] Finally, we observed a 5% incidence of secondary MDS late after transplant, highlighting the need for close follow-up monitoring of patients after transplant and consideration for more aggressive interventions early in the course of this complication.

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**Author Contributions**: GEG, RAN, MDM, DK, MHW, KMS, DEF designed the study. GEG, TG, DK, SH analyzed the data. GEG, DK, MHW, MGM, MDM, RWS, VS, CH, SJ, AP, LAH, JK, DEF, RAN recruited and enrolled patients, conducted study visits, reported data and served as site principal investigators of the trial, GEG wrote the initial manuscript draft, and all authors reviewed and contributed to the final draft of the manuscript.

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**References**

1. Mayes MD, Lacey JV Jr, Beebe-Dimmer J, et al. Prevalence, incidence, survival, and disease characteristics of systemic sclerosis in a large US population. Arthritis Rheum 2003; 48:2246-55.

2. Elhai M, Meune C, Avouac J, Kahan A, Allanore Y. Trends in mortality in patients with systemic sclerosis over 40 years: a systematic review and meta-analysis of cohort studies. Rheumatology (Oxford) 2012; 51:1017-26.

3. Nihtyanova SI, Schreiber BE, Ong VH, et al. Prediction of pulmonary complications and long-term survival in systemic sclerosis. Arthritis Rheumatol 2014; 66:1625-35.

4. Tashkin DP, Elashoff R, Clements PJ, et al. Effects of 1-year treatment with cyclophosphamide on outcomes at 2 years in scleroderma lung disease. Am J Respir Crit Care Med 2007; 176:1026-34.

5. Khanna D, Denton CP, Jahreis A, et al. Safety and efficacy of subcutaneous tocilizumab in adults with systemic sclerosis (faSScinate): a phase 2, randomised, controlled trial. Lancet 2016; 387:2630-40.

6. Tashkin DP, Roth MD, Clements PJ, et al. Mycophenolate mofetil versus oral cyclophosphamide in scleroderma-related interstitial lung disease (SLS II): a randomized controlled, double-blind, parallel group trial. Lancet Respir Med 2016; 4:708-19.

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7. van Laar JM, Farge D, Sont JK, et al. Autologous hematopoietic stem cell transplantation vs intravenous pulse cyclophosphamide in diffuse cutaneous systemic sclerosis: a randomized clinical trial. JAMA 2014; 311:2490-8.

8. Sullivan KM, Goldmuntz EA, Keyes-Elstein L, et al. Myeloablative autologous stem-cell transplantation for severe scleroderma. N Engl J Med 2018; 378:35-47.

9. van den Hoogen F, Khanna D, Fransen J, et al. 2013 classification criteria for systemic sclerosis: an American college of rheumatology/European league against rheumatism collaborative initiative. Ann Rheum Dis 2013; 72:1747-1755.

10. Inker LA, Eneanya ND, Coresh J, et al. New creatinine and cystatin C-based equations to estimate GFR without race. N Engl J Med 2021; 385:1737–1749.

11. Cornell RF, Hari P, Drobyski WR. Engraftment syndrome after autologous stem cell transplantation: an update unifying the definition and management approach. Biol Blood Marrow Transplant. 2015 Dec; 21:2061-2068.

12. Farge D, Burt RK, Oliveira M-C, et al. Cardiopulmonary assessment of patients with systemic sclerosis for hematopoietic stem cell transplantation: recommendations from the European Society for Blood and Marrow Transplantation Autoimmune Diseases Working Party and collaborating partners. Bone Marrow Transplantation 2017; 52:1495–1503.

13 Nihtyanova SI, Denton CP. Autoantibodies as predictive tools in systemic sclerosis. Nature Rev Rheumatol 2010; 6:112–6.

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14. Nihtyanova SI, Schreiber BE, Ong VH, et al. Prediction of pulmonary complications and long-term survival in systemic sclerosis. Arthritis Rheumatol 2014; 66:1625–35.

15. Motegi S, Toki S, Yamada K, et al. Demographic and clinical features of systemic sclerosis patients with anti-RNA polymerase III antibodies. J Dermatol 2015; 42:189–92.

16. Hoffmann-Vold AM, Midtvedt Ø, Tennøe AH, et al. Cardiopulmonary disease development in anti-RNA polymerase III positive systemic sclerosis; comparative analyses from an unselected, prospective patient cohort. J Rheumatol 2017; 44:459–65.

17. Iliopoulos G, Daoussis D. Renal dysfunction in systemic sclerosis beyond scleroderma renal crisis. Rheumatol Int 2021; 41:1203-1208.

18. Jabber MM, Takahashi T, Kirstein MN, et al., Influence of renal function on phosphoramide mustard exposure: a nonlinear mixed-effects analysis. J Clin Pharm 2023; 63:135-142.

19. Penn H, Howie AJ, Kingdon EJ, et al., Scleroderma renal crisis: patient characteristics and long-term outcomes. QJM 2007; 100(8):485-94.

20. Guillevin L, Bérezné A, Seror R, et al., Scleroderma renal crisis: a retrospective multicentre study on 91 patients and 427 controls. Rheumatology 2012; 51:460–467.

21. Gregory K, Hansen D, Penglase R, et al., Outcomes of patients with diffuse systemic sclerosis eligible for autologous stem cell transplantation treated with conventional therapy. Arthritis Rheumatol. 2024; 76:1294-1302.

Author Manuscript

22. Henes J, Oliveira MC, Labopin M, et al., Autologous stem cell transplantation for progressive systemic sclerosis: a prospective non-interventional study from the European Society for Blood and Marrow Transplantation Autoimmune Disease Working Party. Haematologica 2021; 106:375-383.

23. Ayano M, Tsukamoto H, Mitoma H, et al. CD34-selected versus unmanipulated autologous haematopoietic stem cell transplantation in the treatment of severe systemic sclerosis: a post hoc analysis of a phase I/II clinical trial conducted in Japan. Arthritis Res Ther 2019; 21(1):30.

24. Rohatiner AZ, Nadler L, Davies AJ, et al., Myeloablative therapy with autologous bone marrow transplantation for follicular lymphoma at the time of second or subsequent remission: long-term follow-up. J Clin Oncol 2007; 25:2554-9.

25. Pedersen-Bjergaard J, Andersen MK, Christiansen DH. Therapy-related acute myeloid leukemia and myelodysplasia after high-dose chemotherapy and autologous stem cell transplantation. Blood 2000; 95:3273-3279.

26. Akhtari M, Bhatt VR, Tandra PK, et al., Therapy-related myeloid neoplasms after autologous hematopoietic stem cell transplantation in lymphoma patients, Cancer Biology & Therapy 2013; 14:1077-1088.

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**Table 1. Baseline characteristics of enrolled patients and select final outcomes.**

| Pt. # | Age at HSCT | Sex | Dx. to HSCT (mo) | Prior failed DMARDS (x duration in months) | SRC pre-HSCT? | S. creat | E-GFR | ILD? | FVC % | DLCO corr % | m RSS | Scl-70 | RNA Pol III | Final outcomes after HSCT | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | Organ Fail Sust.? | Re-lapse SSc? | Alive? | F/U (yrs) | Other |
| 1 | 64 | M | 11 | MMFx 7, pred | N | 1.1 | 75 | N | 95 | 83 | 31 | neg | neg | N | N | Y | 9.2 | |
| 2 | 53 | F | 59 | MTXx15, CYx6, MMFx30, CYx6 | N | 0.6 | 107 | Y | 64 | 63 | 8 | >8.0 | 46 | N | N | Y | 11.6 | |
| 3 | 55 | F | 18 | MTXx7, CYx6, pred | N | 0.7 | 101 | Y | 75 | 56 | 33 | 3.4 | neg | N | N | N | 8.0 | 2º AML |
| 4 | 55 | M | 10 | MMFx9, pred | N | 0.65 | 111 | Y | 67 | 46 | 31 | neg | neg | N | N | Y | 10.5 | |
| 5 | 19 | F | 6 | MMFx1, MPAx4 | N | 0.73 | 121 | Y | 63 | 77 | 41 | neg | neg | N | N | Y | 8.8 | |
| 6 | 13 | F | 43 | MTXx37, MMFx34, CYx4, RITx2, pred | N | 0.43 | 150 | Y | 66 | 47 | 46 | >8.0 | neg | N | Y | Y | 8.5 | |
| 7 | 53 | F | 18 | CYx5 | Y | 1.8 | 33 | Y | 69 | 70 | 32 | neg | >150 | Y | N | Y | 8.4 | Ren Tx |
| 8 | 38 | F | 15 | MTXx6, MMFx7, RITx2, pred | N | 0.52 | 121 | Y | 70 | 64 | 39 | >8.0 | neg | N | N | Y | 8.3 | |
| 9 | 60 | F | 22 | MMFx4.5 | Y | 1.1 | 57 | N | 91 | 91 | 41 | neg | 182 | Y | n/a | N | 0.1 | |
| 10 | 50 | M | 11 | MTXx2, MMFx6, CYx4 | N | 0.72 | 111 | Y | 68 | 70 | 46 | neg | 149 | N | Y | Y | 8.2 | |
| 11 | 34 | F | 18 | PIRFx9, TOCIx2, MMFx5, | N | 0.56 | 123 | Y | 50 | 61 | 26 | 165 | neg | N | N | Y | 8.3 | |
| 12 | 70 | F | 22 | MTXx1, CYx6, MMFx6, RITx2 | N | 0.53 | 99 | Y | 86 | 51 | 33 | 52 | neg | N | N | Y | 8.1 | |
| 13 | 35 | F | 36 | CYx12, RILOx3 | Y | 0.62 | 119 | Y | 61 | 43 | 26 | neg | neg | N | N | Y | 7.4 | |
| 14 | 52 | M | 14 | MMFx0.5, CYx6 | Y | 1.84 | 43 | Y | 61 | 41 | 39 | neg | 87 | Y | n/a | N | 0.1 | |
| 15 | 48 | M | 11 | MMFx4, CYx6 | Y | 1.4 | 62 | Y | 111 | 62 | 41 | neg | >80 | Y | N | Y | 7.0 | |
| 16 | 49 | F | 16 | MTXx4, MMFx10 | N | 0.71 | 104 | Y | 106 | 58 | 44 | neg | 127 | N | Y | Y | 6.8 | |
| 17 | 25 | F | 30 | MTXx6, MMFx18 | N | 0.84 | 99 | Y | 81 | 65 | 26 | >8.0 | neg | N | N | Y | 6.5 | |
| 18 | 41 | F | 15 | MTXx2, MMFx11, pred | N | 0.45 | 123 | Y | 97 | 67 | 32 | neg | 56 | N | Y | Y | 5.6 | |
| 19 | 43 | F | 36 | ETANx3, RILOx3, MMFx12 | N | 0.71 | 108 | Y | 72 | 68 | 29 | neg | 123 | N | N | Y | 5.8 | |
| 20 | 52 | F | 30 | MTXx6, MMFx1, MPAx10 | N | 0.73 | 99 | Y | 75 | 60 | 46 | >8.0 | neg | N | Y | N | 3.3 | |

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Abbreviations: Pt.: patient, #: number, HSCT: hematopoietic stem cell transplantation, M: male, F: female, Dx: diagnosis, interval time from scleroderma diagnosis, mo.: months, DMARDS: disease modifying anti-rheumatic drugs (shown in sequence administered), MMF: mycophenolate mofetil, pred: prednisone at various doses above 20 milligram per day for varying duration, MTX: methotrexate, CY: cyclophosphamide, MPA: mycophenolic acid, RIT: rituximab, PIRF: pirfenadone, TOCI: tocilizumab, RILO: rilonacept, ETAN: etanercept, SRC: scleroderma renal crisis, N: no, Y: yes, S. Creat: serum creatinine prior to HSCT, E-GFR: estimated glomerular filtration rate based on CKD-EPI 2021 equation (formula including sex and age), patient #6 used Schwartz pediatric bedside eGFR equation, ILD: interstitial lung disease, FVC: forced vital capacity percent predicted value, DLCOcorr%: diffusion lung capacity carbon monoxide corrected for hemoglobin percent predicted value, mRSS: modified Rodnan skin score (maximum score 51), Scl-70: scleroderma-70 autoantibody measured in arbitrary units per milliliter (values in positive range are shown and are dependent on lab-specific clinical test), neg: negative clinical test range autoantibody value, RNA Pol III: anti-ribonucleic acid polymerase- 3 (values in positive range are shown in units per milliliter), Fail Sust: sustained organ failure >6 months (if alive), n/a: not applicable, F/U: follow-up after HSCT at last contact, $2^{o}$ AML: secondary acute myeloid leukemia, Ren Tx: renal transplant.

Eighteen patients had ILD progression (in addition to increasing mRSS) as the primary indication for autologous HSCT. Two patients (#1, #9) had rapidly increasing mRSS (one patient [#9] also had a prior history of SRC) as the primary indication for HSCT. Thirteen participants were non-Hispanic White, 5 non-Hispanic other, 1 non-Hispanic Black and 1 Hispanic.

**Table 2. Least Squares Means changes in skin score, pulmonary function and disability index**

| Variable | Statistic | Value |
|---|---|---|
| Baseline mRSS score | N | 20 |
| | Mean (SD) | 34.5 (9.3) |
| | Median (Q1, Q3) | 33.0 (30.0, 41.0) |
| Change in mRSS at last assessment | N | 18 |
| | Mean (SD) | -20.3 (11.3) |
| | Median (Q1, Q3) | -22.5 (-29.0, -14.0) |
| | P-value | <0.0001 |
| LS Mean change in mRSS at year 5 | N | 18 |
| | LS mean | -25.1 |
| | 95% CI | -29.8, -20.4 |
| | P-value | <0.0001 |
| Baseline FVC% predicted | N | 20 |
| | Mean (SD) | 76.4 (16.3) |
| | Median (Q1, Q3) | 71.0 (65.0, 88.5) |
| Change in FVC% at last assessment | N | 17 |
| | Mean (SD) | 8.7 (11.9) |
| | Median (Q1, Q3) | 9.0 (0.0, 19.0) |
| | P-value | 0.0127 |
| LS Mean change in FVC% at year 5 | N | 17 |
| | LS mean | 10.5 |
| | 95% CI | 5.4, 15.6 |
| | P-value | 0.0001 |
| Baseline DLCO% predicted | N | 20 |
| | Mean (SD) | 61.7 (13.2) |
| | Median (Q1, Q3) | 62.5 (50.5, 69.0) |
| Change in DLCO% at last assessment | N | 17 |
| | Mean (SD) | 11.2 (14.4) |
| | Median (Q1, Q3) | 8.0 (2.0, 17.0) |
| | P-value | 0.0020 |
| LS Mean change in DLCO% at year 5 | N | 17 |
| | LS mean | 12.4 |
| | 95% CI | 5.5, 19.3 |
| | P-value | 0.0006 |
| Baseline HAQ-DI | N | 19 |
| | Mean (SD) | 2.1 (0.9) |
| | Median (Q1, Q3) | 2.4 (1.8, 2.8) |
| Change in HAQ-DI at last measure | N | 15 |
| | Mean (SD) | -0.8 (1.0) |
| | Median (Q1, Q3) | -0.5 (-1.9, -0.1) |
| | P-value | 0.0079 |
| LS Mean change in HAQ-DI at year 4 | N | 15 |
| | LS mean | -1.1 |
| | 95% CI | -1.6, -0.6 |
| | P-value | 0.0004 |

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Abbreviations: mRSS: modified Rodnan skin score, N: number of patients, SD: standard deviation, Q: quartile, LS: least square, 95% CI: 95% confidence interval, FVC%: forced vital capacity percent predicted value, DLCO%: diffusion lung capacity carbon monoxide corrected for hemoglobin percent predicted value, HAQ-DI: health assessment questionnaire disability index, (score range 0-3.0).

We assessed change from baseline in four outcome measures: mRSS, FVC%, DLCO%, HAQ-DI. LS mean change at year 5 was reported for mRSS, FVC%, DLCO%; LS mean change at year 4 was reported for HAQ-DI (as there was incomplete year 5 HAQ-DI data). There was no significant difference in the change in mRSS at last assessment between patients in the two autoantibody subgroups (Scl-70+ and RNA polymerase-III+).

**Figure Legends:**

**Figure 1.** Swimmer plot of survival and events for 20 patients with diffuse cutaneous systemic sclerosis (dcSSc) enrolled in this autologous hematopoietic stem cell transplant (HSCT) trial. Patient number (#) shown as identified in Table 1, in ascending sequence of trial enrollment. Red font patient # indicates ARA+ (anti-RNA-polymerase III seropositive) prior to HSCT. The symbol ! indicates prior history of scleroderma renal crisis (SRC), ↓ indicates baseline pre-HSCT decreased renal function (estimated glomerular filtration rate <65 mL/min/1.73m$^2$), * indicates death. Blue bar border indicates survival without event. Red bar border indicates survival after meeting protocol-defined organ failure event endpoint. Green bar border indicates survival after the addition of a disease-modifying anti-rheumatic drug (DMARD) for the treatment of clinical SSc progression or disease activity. Purple fill indicates treatment duration with protocol prescribed maintenance mycophenolate mofetil (MMF) or mycophenolic acid (MPA). All

patients received an unmodified (not CD34+ selected) autologous peripheral blood stem cell graft at time point 0, follow-up is shown in years.

**Figure 2**. Kaplan Meier survival curves of (**A**) overall survival (OS), (**B**) event-free survival (EFS), (**C**) composite of additional disease modifying anti-rheumatic drug (DMARD) and event-free survival (DEFS) for the entire cohort of 20 systemic sclerosis patients after hematopoietic stem cell transplantation (HSCT). EFS endpoint included death or meeting defined organ failure criteria for greater than 6 months. DEFS end-point included death, organ failure, or initiation of additional DMARD (indicator of disease relapse/ recurrence after transplant). Tick marks show time of censorship, status at time of last contact. Dotted lines indicate 95% confidence interval.

**Figure 3**. Rate of change in skin score, lung function and disability index score after non-myeloablative autologous hematopoietic stem cell transplantation (HSCT). Baseline (Bsl) values and statistical analysis of change over time is shown in **Table 2**. The colored lines are spaghetti plots of change from pre-transplant baseline for each patient, and the bold black lines are the overall change trend based on the linear mixed effect model. (**Upper left**) Change from pre-transplant baseline in modified Rodnan skin score (mRSS) reported at time points after autologous HSCT. (**Upper right**) Change in forced vital capacity (FVC) percent predicted value at time points after autologous HSCT. Eight of 18 evaluable patients had >10% increase in predicted value from the pre-transplant baseline. (**Lower left**) Change in diffusing capacity of the lung for carbon monoxide (DLCO) corrected for hemoglobin, percent predicted value at time points after autologous HSCT. Seven of 18 evaluable patients had >15% increase in



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predicted value from the pre-transplant baseline. (**Lower right**) Change in health assessment questionnaire disability index (HAQ-DI) value (range 0-3.0) from pre-transplant baseline up to 4 years after HSCT.

**Figure 4.** Kaplan Meier survival of patients based on anti-RNA polymerase III autoantibody (ARA) status (negative [neg] or positive [pos]) prior to hematopoietic stem cell transplantation (HSCT). (**A**) Event-free survival (EFS) of ARA neg (n=11) and ARA pos (n=9) patients (difference not significant). (**B**) Composite of additional disease modifying anti-rheumatic drug and EFS (DEFS): ARA neg and ARA pos. patients. Black lines indicate ARA neg, red lines indicate ARA pos. Tick marks show time of censorship, status at time of last contact. Dotted lines indicate respective 95% confidence interval.

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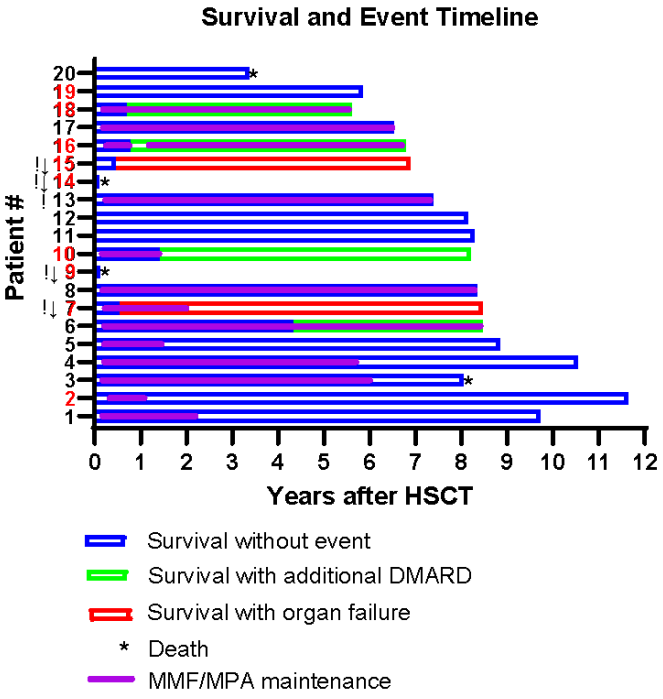


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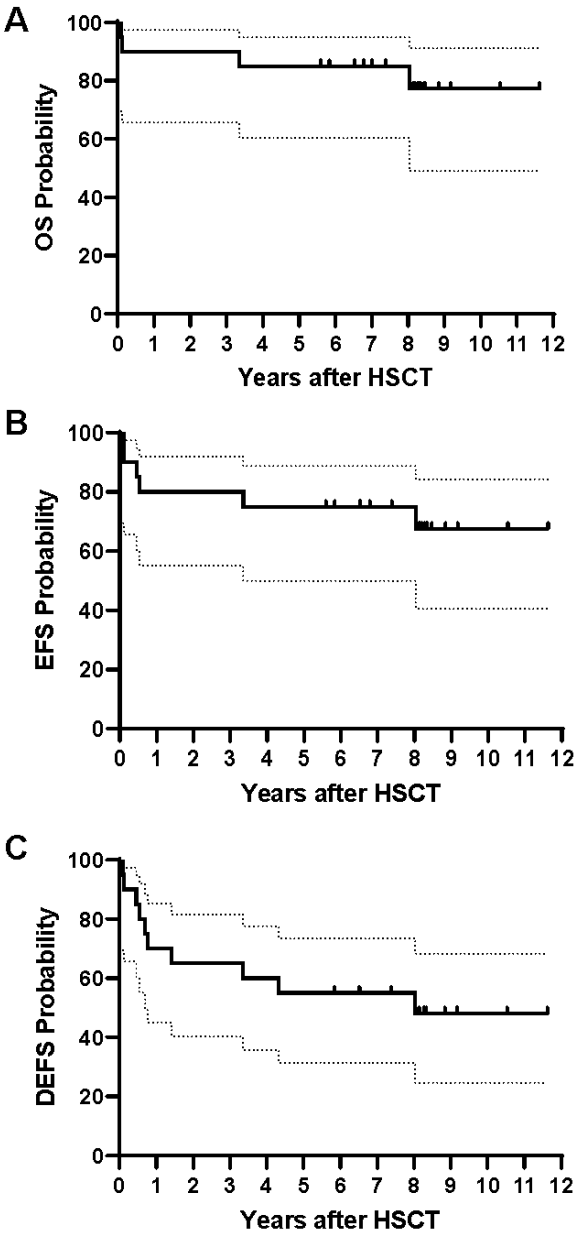


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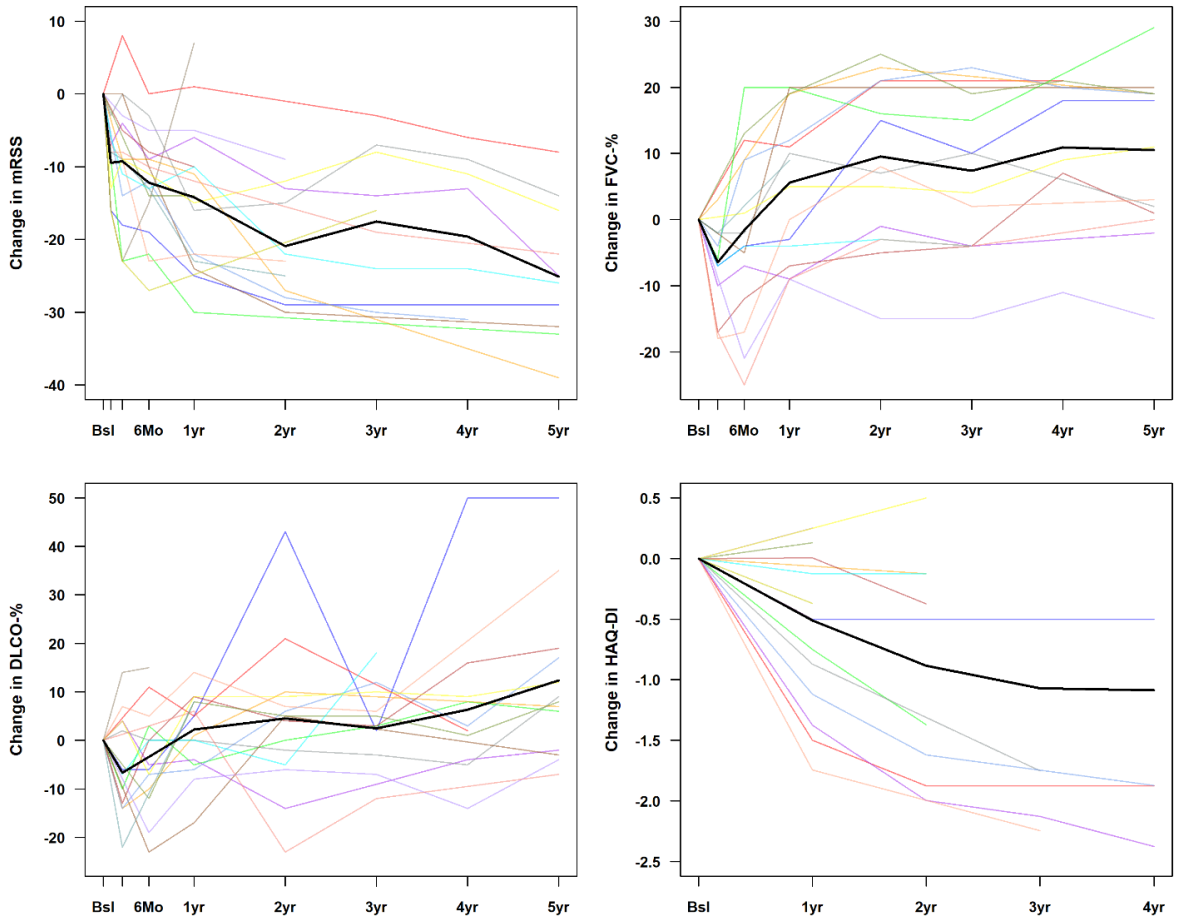


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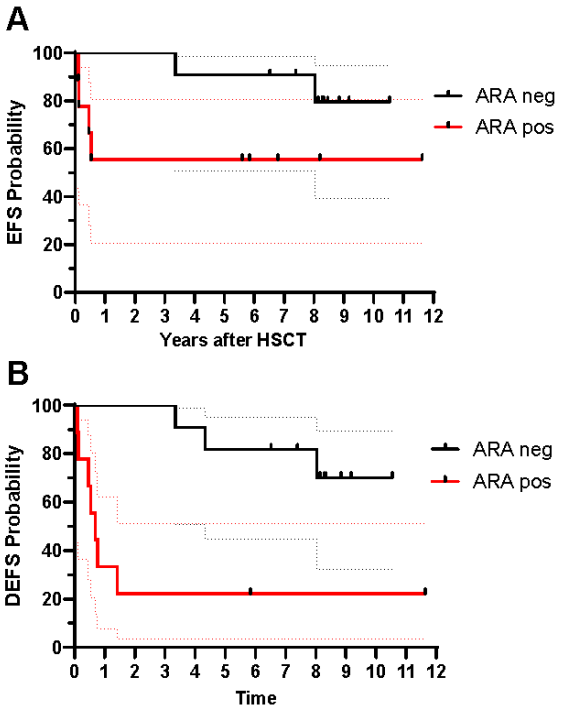


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