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Chronic graft-versus-host disease in the era of post-transplant cyclophosphamide

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DECLARATIONS

Ethics approval and consent to participate

This project was approved by an ethical committee (Comité de Ética de la Investigación con Medicamentos – Hospital Universitario y Politécnico La Fe). Registry number: 09/2019-465.

Consent for publication

Not applicable for individual patient data. This is a pooled analysis.

Availability of data and material

Anonymized data are available for researchers upon e-mail request to the corresponding author.

Competing interests

No conflicts of interest to disclose.

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Authors' contributions

PA, MAS, and JS conceptualization and writing original draft. JM, ABR, MV, PC, AL, PG, JE, AB, IGS, PS, GL, BL, LE, NV, AH, JL, JPR, MG, MDR, CS, JDR review and editing.

ABSTRACT

Chronic graft-versus-host disease (cGVHD) remains a leading cause of late morbidity after allogeneic hematopoietic cell transplantation (HCT), but its phenotype under modern prophylaxis with post-transplant cyclophosphamide (PTCy) is not well characterized. We conducted a prospective, single-center study of 600 consecutive adults undergoing HCT with PTCy-based prophylaxis to assess incidence, clinical manifestations, treatment response, prognostic factors, and outcomes. Donors included matched siblings (36%), matched unrelated (34%), haploidentical (24%), and mismatched unrelated (6%). The 1-year cumulative incidence of moderate-to-severe cGVHD was 22% (95% CI, 19–26%). The mouth was the most frequently involved organ (64%), with lichen planus-like changes as the predominant diagnostic feature, whereas sclerotic forms were uncommon. Notably, 27% of moderate-to-severe cases were managed successfully without systemic corticosteroids. The cumulative incidence of systemic therapy requirement was 15% at 1 year, with risk significantly higher in donors ≥30 years and in female-to-male transplants. Among 105 patients requiring systemic steroids, 64% achieved complete response, 32% discontinued immunosuppression, yet 18% developed cGVHD-related sequelae. Mouth ulcers and erythema, as well as a lung score ≥2 at steroid initiation independently predicted shorter failure-free survival. At 2 years, overall survival, cGVHD-free relapse-free survival, and GVHD-free relapse-free survival were 76% (95% CI, 72–79), 63% (95% CI, 60–68), and 57% (95% CI, 53–62), respectively. In conclusion, after HCT with PTCy-based prophylaxis, systemic therapy was required in only a minority of patients, with risk influenced by donor age and sex mismatch rather than donor type. While corticosteroids were generally effective, a substantial subset required salvage therapy, underscoring the burden of refractory cGVHD and the need for steroid-sparing approaches and novel interventions.

INTRODUCTION

Despite advancements in the prevention (1–5) and treatment (6–8) of chronic graft-versus-host disease (cGVHD), it continues to be a leading cause of morbidity and mortality after allogeneic hematopoietic cell transplantation (HCT), with a substantial impact on survivors' quality of life (9–11). Post-transplant cyclophosphamide (PTCy) is a widely adopted strategy for GVHD prophylaxis, extending beyond haploidentical transplantation to all donor types due to its demonstrated efficacy in reducing GVHD compared to standard regimens combining a calcineurin inhibitor and an antimetabolite (4,5,12–14).

While numerous studies have assessed the incidence and severity of cGVHD following PTCy-based HCT (4,5,15–19), only two small single-center studies have provided detailed descriptions of the clinical phenotype of cGVHD (20,21). One early study observed a distinct pattern of organ involvement and a more favorable response to treatment in patients undergoing haploidentical HCT with PTCy compared to those receiving HLA-matched unrelated donor transplants with calcineurin inhibitor-based prophylaxis (20). These findings were subsequently confirmed by another study reporting reduced visceral organ involvement after PTCy (21). Nonetheless, comprehensive data characterizing cGVHD in the context of PTCy-based prophylaxis remain scarce.

This single-center study aims to evaluate the incidence, clinical manifestations, prognostic factors, treatment response, and outcomes of cGVHD in a large series of patients receiving GVHD prophylaxis with PTCy, sirolimus or tacrolimus, and mycophenolate mofetil (MMF) across different donor types.

PATIENTS AND METHODS

Patients, Study Design, and Data Collection

This single-center, prospective, observational study included all consecutive adult patients who underwent a first allogeneic HCT with PTCy using matched sibling donors (MSD), matched unrelated donors (MUD), mismatched unrelated donors (MMUD), or haploidentical donors at the Hospital Universitari i Politècnic La Fe (Valencia, Spain) between January 2017 and February 2025. Patient data, transplant procedures, cGVHD clinical presentation, grading and treatment response were prospectively collected and recorded in a computerized database. Clinical charts were additionally reviewed to resolve any inconsistencies or missing data. The study was registered by the Spanish Agency of Medicines and Health Products with the reference code IIF-SIR-2019-01. According to the Declaration of Helsinki, the protocol was

approved by the Research Ethics Board of Hospital Universitari i Politècnic La Fe with reference code 09/2019-465.

Transplant Procedures and Graft-versus-Host Disease Management

Details on transplantation procedures, including patient eligibility criteria, donor selection, conditioning regimen, and supportive measures, have been previously described (22). GVHD prophylaxis consisted of PTCY, sirolimus, and MMF (23). Tacrolimus was used instead of sirolimus in cases of previous exposure to inotuzumab ozogamicin (n = 20) or when required by specific prospective clinical trials (n = 25).

Management of cGVHD was done following international recommendations (24–26). Patients with mild cGVHD, together with those with moderate or severe forms showing predominantly mucocutaneous involvement, were initially managed with topical therapies and/or reintroduction of prophylactic immunosuppression (sirolimus or tacrolimus, always restarting the same agent used in the initial prophylaxis) in an attempt to avoid systemic steroids. For the remaining patients requiring systemic therapy, prednisone was administered at a dosage of 1 mg/kg/day. Subsequent therapy for patients with steroid-refractory cGVHD or steroid-dependent cGVHD was individualized, typically prioritizing extracorporeal photopheresis (ECP) for skin-limited disease and ruxolitinib for extra cutaneous manifestations (27,28).

Definitions

cGVHD diagnosis and staging was based on the National Institutes of Health (NIH) consensus criteria (29). cGVHD was graded according to maximum cGVHD severity at any point during patient follow-up. Visceral cGVHD was defined as involvement of at least one of the following organs: liver, lungs, gastrointestinal tract, serous membranes (21). Sclerotic features included cutaneous sclerosis, fasciitis, or joint contractures (30). cGVHD-related death was defined as deaths occurring in patients with moderate or severe during cGVHD therapy, in which cGVHD was the primary or secondary cause of death, including deaths due to infection in the course of cGVHD treatment (31). Response to steroids was assessed as best response according to NIH Consensus criteria (32). cGVHD-relapse-free survival (CRFS) was analyzed in the entire transplanted cohort and defined as survival without cGVHD requiring systemic therapy, relapse, or death, with time calculated from transplantation (33). GVHD-free, relapse-free survival (GRFS) was defined as the absence of grade III-IV acute GVHD, cGVHD requiring systemic therapy, relapse, graft failure, or death, measured from transplantation. Chronic GVHD failure-free survival (cGVHD-FFS) was

analyzed only among patients who started a first-line of systemic therapy for cGVHD and was defined as a composite outcome including death, relapse, or the need for next-line systemic immunosuppressive therapy, with time calculated from the start of systemic treatment for cGVHD (34).

Statistical Analyses

Categorical variables were compared using the Chi-square test, while continuous variables were analyzed with the Wilcoxon rank-sum or the Kruskal-Wallis rank-sum test. Unadjusted time-to-event analyses were performed using the Kaplan-Meier estimate, and, for comparisons, the log-rank tests. Competing risk analyses were performed using cumulative incidence, and comparisons were made using Gray's test. A 95% confidence interval (95% CI) was applied. Pre-HCT risk factors for the cumulative incidence of developing clinically relevant outcomes were analyzed including moderate-severe cGVHD, cGVHD requiring systemic therapy and CRFS. The univariable analyses included patient-, disease-, and transplant-related characteristics, as presented in Table 1. In addition, the univariable analysis for cGVHD-FFS incorporated the 2014 NIH clinician-assessment items recorded at the initiation of systemic steroids. All variables reaching statistical signification ($p \leq 0.05$) in the univariable analysis were included in the multivariable analysis. In the multivariable analysis, no significant interaction was detected between donor type and donor age (all p-value > 0.4). For multivariable analysis, the Cox proportional hazard model or the Fine and Gray method for competing events was used. Statistical analyses were conducted using R statistical software version 4.5.2.

RESULTS

Patients, disease, and transplant characteristics

The baseline patient, disease, and transplant characteristics of the 600 adult patients included in the study are shown in Table 1. The median age was 55 years (range, 15–72), and 58% were male. Most of recipients had acute leukemia (n = 371, 62%). A high or very high disease risk index was observed in 38% of the series (n = 218) and 14% had received a prior autologous HCT. Allografts were obtained from MSD (n = 213, 36%), MUD (n = 206, 34%), haploidentical donors (n = 143, 24%), and MMUD (n = 38, 6%). Most patients (92%) received peripheral blood grafts. GVHD prophylaxis consisted of PTCy, sirolimus, and MMF in 555 patients (93%), while the remaining 45 patients (7%) received PTCy, tacrolimus, and MMF.

Incidence, severity, and clinical profile of cGVHD

cGVHD occurred in 239 patients (40%) and was classified as mild in 94 (16%), moderate in 90 (15%), and severe in 55 (9%). The median time to onset was 5.4 months (interquartile range [IQR], 3.9 to 7.4). The 1-year cumulative incidence of cGVHD was 37% (95% CI, 33 – 41) for any grade, 22% (95% CI, 19 – 26%) for moderate or severe, and 8% (95% CI, 6 – 11%) for severe cases (Figure 1).

Univariable analysis for cumulative incidence of moderate or severe cGVHD is detailed in Table S1. In the multivariable analysis, as shown in Table 2, the risk of moderate-to-severe cGVHD was significantly increased when the donor was older than 30 years (subdistribution hazard ratio [SHR] 1.87; 95% CI,1.19 – 2.96), and in female donors to a male recipients (SHR 1.58; 95% CI, 1.03 – 2.41). These results remained consistent when we included donor type in the multivariable model.

As shown in Table 3, the most frequently affected organ was the mouth (n = 153, 64%), followed by the skin (n = 124, 52%), eyes (n = 121, 51%), liver (n = 105, 44%), genital tract (n = 49, 21%), joints and fascia (n = 40, 17%), and gastrointestinal tract (n = 36, 15%), with involvement lungs and other organs in fewer than 10% of cases. Severe grade 3 involvement was most common in the skin (n = 26, 11%), and mouth (n = 14, 6%), with other organs affected in fewer than 5% of cases. Patients receiving unrelated grafts had a significantly lower incidence of hepatic (13% vs. 24% MSD and 17% haploidentical, P = 0.007) and visceral cGVHD (16% vs. 27% MSD and 21% haploidentical, P = 0.013). The number of organs affected did not vary across donor types.

Clinical phenotype of moderate to severe cGVHD

Table 4 summarizes NIH diagnostic, distinctive, common, and other signs and symptoms detected in the 145 patients with moderate or severe cGVHD. Diagnostic, distinctive, common, and other features were present in 81%, 63%, 60%, and 46% of patients, respectively. Lichen planus-like changes in the mouth were the most frequent diagnostic feature (n = 107). Fourteen signs and symptoms listed in the 2014 NIH Diagnosis and Staging Working Group report were absent in this cohort. Those include sweat impairment, loss of body hair, scaling, premature gray hair, periorbital hyperpigmentation, esophageal web, exocrine pancreatic insufficiency, myositis, hypergammaglobulinemia, autoantibodies (autoimmune hematolytic anemia, immune thrombocytopenia), Raynaud's phenomenon, myasthenia gravis, and cardiac conduction abnormality or cardiomyopathy. Representative organ-specific findings are shown in Figure 2.

Sclerotic features were observed in 19 out of 145 patients with moderate or severe cGVHD (13%), including cutaneous sclerosis (n = 12), joint stiffness or contractures secondary to fasciitis or sclerosis (n = 9), and isolated fasciitis (n = 1), with three patients presenting more than one sclerotic manifestation. Sclerotic cGVHD was higher in patients with a diagnosis of myelodysplastic and myeloproliferative neoplasms (Odds ratio [OR] 6.2, 95% CI 1.4 – 26.7).

Twelve patients (2%) developed lung cGVHD, with an even distribution across NIH severity grades: mild (n=4), moderate (n=4), and severe (n=4). The clinical phenotype, detailed in Table 4, was characterized primarily by air trapping, observed in 8 patients. Three patients, all with grade 3 lung cGVHD, underwent lung transplantation at 15, 16, and 29 months after HCT. One patient died 24 months after lung transplantation due to multiorgan failure, while the remaining two are alive at 1 and 6 years after lung transplantation.

Treatment of moderate to severe cGVHD

Figure 3 depicts the patient flow through the different lines of treatment.

Patients not requiring systemic therapy

Overall, 40 of the 145 patients (27%) were successfully managed without systemic corticosteroids, including 34 of 90 (38%) with moderate cGVHD and 6 of 55 (11%) with severe cGVHD, none of whom required systemic therapy thereafter. In moderate cases, disease activity was primarily localized to the skin (n = 19), mouth (n = 19), liver (n = 17), or eyes (n = 16), with less frequent involvement of joints (n = 8), genital tract (n = 7), or gastrointestinal tract (n = 3). The six patients with severe cGVHD had a score of 3 in either the skin (n = 3) or genital tract (n = 3), with a maximum score of 1 at all other sites. This conservative approach, applied at physician discretion, consisted of topical therapies (n = 9), resumption of GVHD prophylaxis (n = 10, sirolimus in 9 cases and tacrolimus in 1), or both (n = 21, sirolimus plus topical treatment in 20 and tacrolimus plus topical treatment in 1). All patients responded, with 31 patients (77%) achieving complete response (CR) and 9 (23%) partial response (PR).

Patients requiring systemic therapy

One hundred and five patients were treated with systemic corticosteroids. The cumulative incidence of cGVHD requiring systemic therapy was 15% (95% CI, 13 – 18%) at 1 year. Univariable analysis for cumulative incidence of cGVHD requiring systemic therapy is detailed in Table S2. In multivariable analysis (Table 2), the cumulative incidence of cGVHD requiring systemic therapy was higher in transplants

from donors aged ≥ 30 years (SHR 1.65, 95% CI 1.13 – 2.41) and in female-to-male transplants (SHR 1.49, 95% CI 1.03 – 2.15). Seventy-six patients (64%) achieved CR, 17 (16%) PR, and 21 (20%) had steroid treatment failure, including 7 (7%) with stable disease, 11 (10%) with mixed response, and 3 (3%) with progression. Of the 67 patients achieving CR, at a median time to response of 59 days (IQR, 24 to 143 days), 57 did not require further treatment, and 34 were able to discontinue immunosuppressive therapy at a median of 164 days (IQR, 77 to 381 days). Ten patients experienced cGVHD exacerbation after initial CR. The 12-month cGVHD-FFS after first-line systemic corticosteroids was 58% (95% CI: 50 – 69). Univariable analysis for cumulative incidence of cGVHD-FFS is detailed in Table S3. In multivariable analysis (Table 2), shorter cGVHD-FFS was independently associated with the presence of a lung score ≥2 (hazard ratio [HR] 2.65, 95% CI 1.14 – 6.17), mouth erythema (HR 2.01, 95% CI 1.06 – 3.81), and mouth ulcers (HR1.82, 95% CI 1.00 – 3.30 at the initiation of systemic steroids.

Excluding three patients who relapsed with their primary disease and one who died before second-line therapy, the remaining 44 patients with PR or steroid-refractory disease received second-line treatment: ECP (n=24), ruxolitinib (n=15), MMF (n=3), and one each with belumosudil or rituximab. The patient receiving belumosudil had grade 3 lung cGVHD and, after receiving corticosteroids, MMF and FAM, was started on belumosudil at physician discretion; however, he subsequently required ruxolitinib and lung transplantation. The patient treated with rituximab had nephrotic syndrome as the sole manifestation of cGVHD, but proteinuria persisted despite rituximab and subsequent treatment lines with ruxolitinib and tacrolimus, and the patient is now awaiting inclusion in a clinical trial. Overall, 25 of 44 patients (57%) responded to second-line therapy, including CR in 15 patients (34%), PR in 10 (23%). Nineteen patients (43%) had treatment failure, including stable disease in 11 patients (25%), mixed response in 4 (9%) and progression in 4 patients (9%). CR was maintained in 11 patients, 6 of whom discontinued all immunosuppression. Among partial responders, 8 of 10 did not require a third line of therapy. ECP produced 12 CR (50%) and 6 PR (25%), whereas ruxolitinib yielded 3 CR (20%) and 3 PR (20%). The 12-month cGVHD-FFS after second-line treatment was 55% (95% CI: 42 – 72).

Among the 25 patients who were candidates for third-line therapy (4 with unsustained CR, 2 PR, and 19 second-line failures), 3 patients died, 1 relapsed with primary disease, and 2 were awaiting treatment at data cutoff. The remaining 19 patients initiated third-line therapy, consisting of ruxolitinib (n = 9), ECP (n = 5), lung transplantation (n = 2), or axatilimab, tacrolimus, or steroid re-treatment (n = 1 each). Fifteen patients (79%) responded, including 7 CR (2 who discontinued immunosuppression) and 8 PR. The remaining 4 patients had stable disease and

proceeded to fourth-line treatment with belumosudil, lung transplantation, MMF, or dupilumab. The patient treated with belumosudil experienced cGVHD progression, whereas the other three patients achieved CR.

Outcomes

At last follow-up, among the 105 patients treated with systemic corticosteroids 44 (42%) were in CR, off immunosuppression and without sequelae; 27 patients (26%) had active cGVHD and remained on treatment, 15 patients (14%) were in CR but still required immunosuppressive therapy; and 19 patients (18%) had cGVHD-related sequelae. The median duration of immunosuppressive treatment in patients who were able to discontinue all immunosuppression was 13.4 months (range 1.7 - 96.6). This duration was similar in patients with sclerotic cGVHD (median, 14.0 months; range, 8.3–66.3).

In the overall cohort, 90 patients died without prior relapse at a median of 98 days (IQR, 57 – 184) after transplantation. The 2-year cumulative incidence of non-relapse mortality (NRM) was 13% (95% CI, 11 – 16). Causes of NRM included infections (n = 31, 34%), acute GVHD (n = 27, 30%), chronic GVHD (n = 8, 9%), and other causes, each accounting for less than 5%. For patients with cGVHD, the 2-year cumulative incidence of NRM was 4% (95% CI, 2 – 7). When analyzed as a time-dependent variable, cGVHD was not associated with NRM (HR 0.72; 95% CI, 0.4 – 1.4) or relapse risk (HR 0.78; 95% CI, 0.6 – 1.1).

The median follow-up for survivors was 37 months (IQR, 19 – 58). The 2-year overall survival (OS) was 76% (95% CI, 72 – 79), CRFS was 63% (95% CI, 60 – 68), and GRFS was 57% (95% CI, 53 – 62). Univariable analysis for CRFS is provided in Table S4. In multivariate analysis, bone marrow grafts were associated with a higher CRFS (HR 0.56; 95% CI, 0.33 – 0.95), whereas female-to-male transplantation had an adverse impact on this outcome (HR 1.52; 95% CI, 1.16 – 1.99) (Table 3).

Healthcare burden of cGVHD

As shown in Table 5, median treatment duration was 82, 178, and 154 days for the first, second, and third line, respectively. Outpatient follow-up remained intensive throughout the course of therapy, with patients maintaining approximately weekly visits during first-, second-, and third-line treatment, and hospitalization was not uncommon, particularly in the third-line setting. Hematologic toxicity was frequent, with all-grade neutropenia reported in 32–40% of patients and grade 3–4 neutropenia in 8–16%.

DISCUSSION

This study provides a comprehensive real-world characterization of cGVHD after PTCy-based prophylaxis. Only a minority of patients required systemic therapy for cGVHD, with risk driven primarily by donor age and female-to-male sex mismatch rather than donor type. The phenotype was dominated by mucocutaneous involvement, often controllable with topical or prophylaxis-based approaches, while sclerotic forms were uncommon. Although systemic therapy was generally effective, a substantial subset required salvage treatment, underscoring the need for steroid-sparing strategies and novel interventions. These findings challenge the routine use of systemic corticosteroids in all moderate-to-severe cases and instead support individualized, risk-adapted management that redefines therapeutic priorities and informs donor selection in the PTCy era.

The main limitation of this non-interventional prospective study is that treatment practices evolved during the study period and novel therapeutic options were only introduced in the most recent years, leading to heterogeneous implementation depending on approval timing and clinical adoption. Strengths include the prospective, uniform data collection within a single-center cohort treated with a standardized transplant strategy, complemented by multidisciplinary evaluation from organ-specific specialists (dermatologists, rheumatologists, ophthalmologists, gastroenterologists, pulmonologists, and gynecologists). This approach ensured detailed phenotyping, optimized management, especially regarding topical and steroid-sparing strategies, and precise recording of organ-specific manifestations, thereby enhancing the consistency and reliability of the findings.

The incidence of cGVHD in our cohort was comparable to that reported in the ALLG BMT12 CAST and BMT CTN 1703 trials using PTCy-based GVHD prophylaxis (4,5), as well as in other real-world studies (13,14,35–38). However, few studies in cGVHD have provided a detailed description of clinical phenotypes and organ-specific manifestations (21,34). In our series, identifying the most common manifestations (Figure 2) provides actionable guidance for clinicians, whereas the absence of 14 NIH 2014 items points to unnecessary complexity in the criteria. Predominant skin or mucosal involvement identified patients most likely to benefit from conservative, steroid-sparing strategies. Within this spectrum, sclerotic cGVHD emerged as a distinctive and clinically relevant phenotype, often associated with considerable disability and morbidity. Compared with prior studies using calcineurin inhibitor plus antimetabolite prophylaxis (30), sclerotic cGVHD in our cohort appeared less frequent (20% vs. 10% at 3 years), without evidence of delayed immunosuppression withdrawal, though such cross-study comparisons warrant caution. Taken together, these observations suggest that refining phenotypic profiling could streamline the application

of NIH criteria and support more individualized management strategies in the PTCy setting.

As donor type may shape the organ-specific manifestations of cGVHD, we explored this issue and found that unrelated grafts were associated with lower hepatic and visceral involvement, while the overall number of affected organs was similar across donor types. This analysis was motivated by previous evidence showing that donor-related factors can influence immune reconstitution, alloreactivity, and target-organ tropism, potentially predisposing to distinct cGVHD phenotypes (39). Prior reports are more difficult to interpret in this regard, as PTCy was restricted to the haploidentical setting, while MSD and MUD transplants received calcineurin inhibitor–based prophylaxis (20,21). In that context, haploidentical recipients showed reduced ocular, joint/fascia (20), or visceral involvement (21). By contrast, our study allows for a more direct assessment of donor type, since all patients received PTCy-based prophylaxis. Overall, these findings suggest that donor type may influence specific organ manifestations of cGVHD, although the overall burden of multi-organ involvement appears largely unaffected. Differences in transplant platforms, GVHD prophylaxis, and cohort characteristics likely contribute to variability across studies. Other donor-related factors such as age and sex have been shown to significantly influence the risk of cGVHD and may represent modifiable determinants in donor selection.

The predominant use of sirolimus-based GVHD prophylaxis in our cohort is an important contextual factor. Recent studies have suggested that tacrolimus may interfere with T-cell exhaustion and could predispose to cGVHD (40), whereas sirolimus has distinct immunomodulatory effects and is even used in the treatment of cGVHD. These differences may have influenced the incidence, severity, or phenotype observed in our study and should be considered when comparing our findings with cohorts using other immunosuppressive regimens.

A substantial proportion of patients with moderate or severe cGVHD in our cohort were managed successfully without systemic corticosteroids, reflecting real-world clinical practice rather than protocol-driven treatment. While this challenges the conventional recommendation to initiate full-dose systemic corticosteroids in all such cases (24,29), it aligns with real-world experiences (41,42). Identifying which patients may achieve satisfactory disease control with limited systemic exposure remains an important goal, and future studies should explore clinical predictors (43), biomarkers (44), or dynamic monitoring tools to guide more personalized treatment strategies.

Concerning those who were treated with systemic steroids, slightly more than half of patients achieved a satisfactory sustained response, which is consistent with the

results reported in one study (45) and higher than the response rates described in other publications (46,47). This trend needs to be confirmed in future, prospective trials. In our cohort, second-line treatment achieved meaningful disease control in more than half of patients with steroid-refractory or steroid-dependent cGVHD, where responses rates were somewhat higher with ECP, likely reflecting its preferential use in mucocutaneous disease, which is known to be more responsive and carries a favorable safety profile (27,28). By contrast, ruxolitinib was often reserved for patients with more severe or less typical organ involvement, which may partly explain its lower CR rates compared with the REACH3 trial (6).

Successful discontinuation of immunosuppression has historically been modest, occurring in 20–30% of patients receiving calcineurin inhibitor-based GVHD prophylaxis (48,49). In cohorts receiving PTCy prophylaxis, including our study and the BMT CTN 1703 (4), rates of successful discontinuation were above 40%. These observations underscore the potential long-term protective effect of PTCy and highlight the opportunity to tailor immunosuppression more safely.

In conclusion, our study provides a comprehensive real-world characterization of cGVHD in the era of PTCy-based prophylaxis, highlighting that individualized and steroid-sparing strategies are feasible and effective, particularly for patients with skin or mucous membrane-predominant disease. Multidisciplinary management is pivotal for accurate phenotyping, optimal treatment selection, and minimization of complications. Detailed organ-specific characterization analysis provide valuable insights for future clinical trials, and refinement of diagnostic criteria. Continued research is warranted to identify biomarkers predicting response to conservative management, optimize salvage therapy sequencing, and further improve outcomes while reducing the clinical burden of cGVHD.

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TABLES

Table 1. Patient, disease, and transplant characteristics

Characteristic	Overall n = 600
Sex, n (%)	
Male	347 (58)
Female	253 (42)
Age, yrs, median (range)	55 (15-72)
HCT-CI, n (%)	
Low (0)	168 (28)
Intermediate (1-2)	182 (30)
High (≥3)	250 (42)
Prior Autologous HCT, n (%)	82 (14)
Diagnosis, n (%)	
Acute leukemia	371 (62)
Myelodisplastic and myeloproliferative neoplasms	116 (19)
Lymphoma and myeloma	92 (15)
Non-malignant disease	21 (3.5)
Disease risk index, n (%)	
Low	71 (12)
Intermediate	290 (50)
High and very high	218 (38)
Donor age, yrs, median (range)	35 (8-74)
Donor type, n (%)	
MSD	213 (36)
MUD	206 (34)
MMUD	38 (6)
Haplo	143 (24)
Donor-patient gender combination, n (%)	
Female – Male	115 (19)
Other	485 (81)
Donor-patient CMV status, n (%)	
Positive/Positive	313 (52)
Negative/Positive	159 (27)
Positive/Negative	57 (10)
Negative/Negative	71 (12)
Stem cell source, n (%)	
Peripheral blood	554 (92)
Bone marrow	46 (8)
Graft cellularity, median (IQR)	
$x10^6$ CD34/kg	6.5 (4.7 – 8.6)
$x10^8$ total nucleated cells/kg	7.0 (5.3 – 9.4)
$x10^8$ CD3/kg	2.2 (1.7 – 2.9)
GVHD prophylaxis, n (%)	
PTCy + siro + MMF	555 (93)
PTCy + tacro + MMF	45 (7)

Table 2. Significant risk factors for cGVHD outcomes

Outcome	Variable	N	SHR* or HR** (95% CI)	p-value
Moderate or	**Donor age**			

Outcome	Variable	N	SHR* or HR** (95% CI)	p-value
severe cGVHD	≤ 30 years	244	Ref.	<0.01
	> 30 years	356	1.87 (1.19 – 2.96)	
	Donor-patient gender combination			
	No female to male	485	Ref.	0.03
	Female to male	115	1.58 (1.03 – 2.41)	
cGVHD requiring systemic therapy	**Donor age**			
	≤ 30 years	244	Ref.	0.01
	> 30 years	356	1.65 (1.13 – 2.41)	
	Donor-patient gender combination			
	No female to male	485	Ref.	0.04
	Female to male	115	1.49 (1.03 – 2.15)	
cGVHD-FFS event	**Lung score**			
	0-1	81	Ref.	0.02
	2-3	24	2.65 (1.14 – 6.17)	
	Mouth erythema			
	No	86	Ref.	0.03
	Yes	19	2.01 (1.06 – 3.81)	
	Mouth ulcers			
	No	91	Ref.	0.05
	Yes	14	1.82 (1.00 – 3.30)	
CRFS event	**Donor-patient gender combination**			
	No female to male	485	Ref.	0.003
	Female to male	115	1.52 (1.16 – 1.99)	
	Stem cell source			
	Peripheral blood	554	Ref.	0.03
	Bone marrow	56	0.56 (0.33 – 0.95)	

*Subdistribution hazard ratios (SHR) were obtained from Fine & Gray models and were applied to the analyses of the cumulative incidence of moderate or severe cGVHD and of cGVHD requiring systemic therapy.
**Hazard ratios (HR) were obtained from Cox proportional hazards models and were applied to the analyses of CRFS and cGVHD-FFS.

Table 3. Organ and maximum severity distribution of cGVHD

Organ	Grade 1, n (%)	Grade 2, n (%)	Grade 3, n (%)	Total, n (%)
Skin	60 (25)	38 (16)	26 (11)	124 (52)
Mouth	108 (45)	31 (13)	14 (6)	153 (64)
Eyes	93 (39)	19 (8)	9 (4)	121 (51)
Gastrointestinal tract	19 (8)	12 (5)	5 (2)	36 (15)
Liver	47 (20)	56 (23)	2 (<1)	105 (44)
Lungs	4 (2)	4 (2)	4 (2)	12 (5)
Joints and fascia	33 (14)	6 (3)	1 (<1)	40 (17)
Genital tract	18 (8)	21 (9)	10 (4)	49 (21)
Other	3 (1)	1 (<1)	1 (<1)	5 (2)

Table 4. NIH diagnostic, distinctive, common, and other signs and symptoms detected at any point in patients with moderate or severe cGVHD

Organ or site	Diagnostic (n=116, 81%)	Distinctive (n=91, 63%)	Other (n=66, 46%)	Common (n=87, 60%)
Skin n = 104 72%	Poikiloderma (n=6, 6%) Lichen planus-like features (n=12, 12%) Sclerotic features (n=11, 11%) Morphea-like features (n=2, 2%) Lichen sclerosus-like features (n=1, 1%)	Depigmentation (n=3, 3%) Papulosquamous lesions (n=1, 1%)	Ichthyosis (n=4, 4%) Hypopigmentation (n=5, 5%) Hyperpigmentation (n=33, 32%)	Erythema (n=32, 31%) Maculopapular rash (n=66, 63%) Pruritus (n=52, 50%)
Nails n = 24 17%	-	Dystrophy (n=19, 79%) Longitudinal ridging/splitting (n=1, 4%) Nail loss (n=1, 4%)	-	-
Scalp and body hair n = 3 2%	-	New onset alopecia (n=2, 67%)	Thinning scalp hair (n=1, 33%)	-
Mouth n = 115 80%	Lichen planus-like changes (n=107, 93%)	Mucosal atrophy (n=1, 1%) Ulcers (n=28, 24%) Pseudomembranes (n=5, 4%)	-	-
Eyes n = 67 47%	-	Pseudomembranes (n=4, 6%) New onset dry, gritty, or painful eyes (n=6, 9%) Cicatricial conjuntivitis (n=2, 3%) KCS (n=55, 82%) Confluent areas of punctate keratopathy (n=6, 9%)	Photophobia (n=3, 4%) Blepharitis (n=3, 4%)	Pain (n=11, 10%) Erythema (n=10, 9%)
Genitalia n = 22 15%	Lichen planus-like features (n=4, 18%) Lichen sclerosus-like features (n=2, 9%) Vagginal scarring or clitoral/labial agglutination (n=11, 50%) Phimosis or urethral /meastus scarring or stenosis (n=5, 23%)	Ulcers (n=1, 5%)	-	-

Organ or site	Diagnostic (n=116, 81%)	Distinctive (n=91, 63%)	Other (n=66, 46%)	Common (n=87, 60%)
Gastrointestinal n = 25 17%	Strictures or stenosis in the upper to mid third of the esophagus (n=4, 16%)	-		Anorexia (n=13, 52%) Nausea (n=9, 36%) Vomiting (n=6, 24%) Diarrhea (n=8, 32%)
Lung n = 12 8%	Bronchiolitis obliterans diagnosed with lung biopsy (n=4, 33%)	Air trapping and bronchiectasis on chest CT (n=8, 67%)	Cryptogenetic organizing pneumonia (n=2, 17%) Restrictive lung disease (n=5, 42%)	-
Muscle, fascia, joints n = 24 17%	Fasciitis (n=1, 4%) Joint stiffness or contractures secondary to fasciitis or sclerosis (n=9, 38%)		Edema (n=2, 8%) Muscle cramps (n=5, 21%) Arthralgia or arthritis (n=18, 75%)	-
Hematopoietic and Immune n = 16 11%	-	-	Thrombocytopenia (n=7, 44%) Eosinophilia (n=10, 62%)	-
Other n = 7 5%	-	-	Pericardial or pleural effusions (n=4, 57%) Ascites (n=1, 14%) Peripheral neuropathy (n=1, 14%) Nephrotic syndrome (n=2, 29%)	-

Table 5. Resources utilization in the course of cGVHD treatment

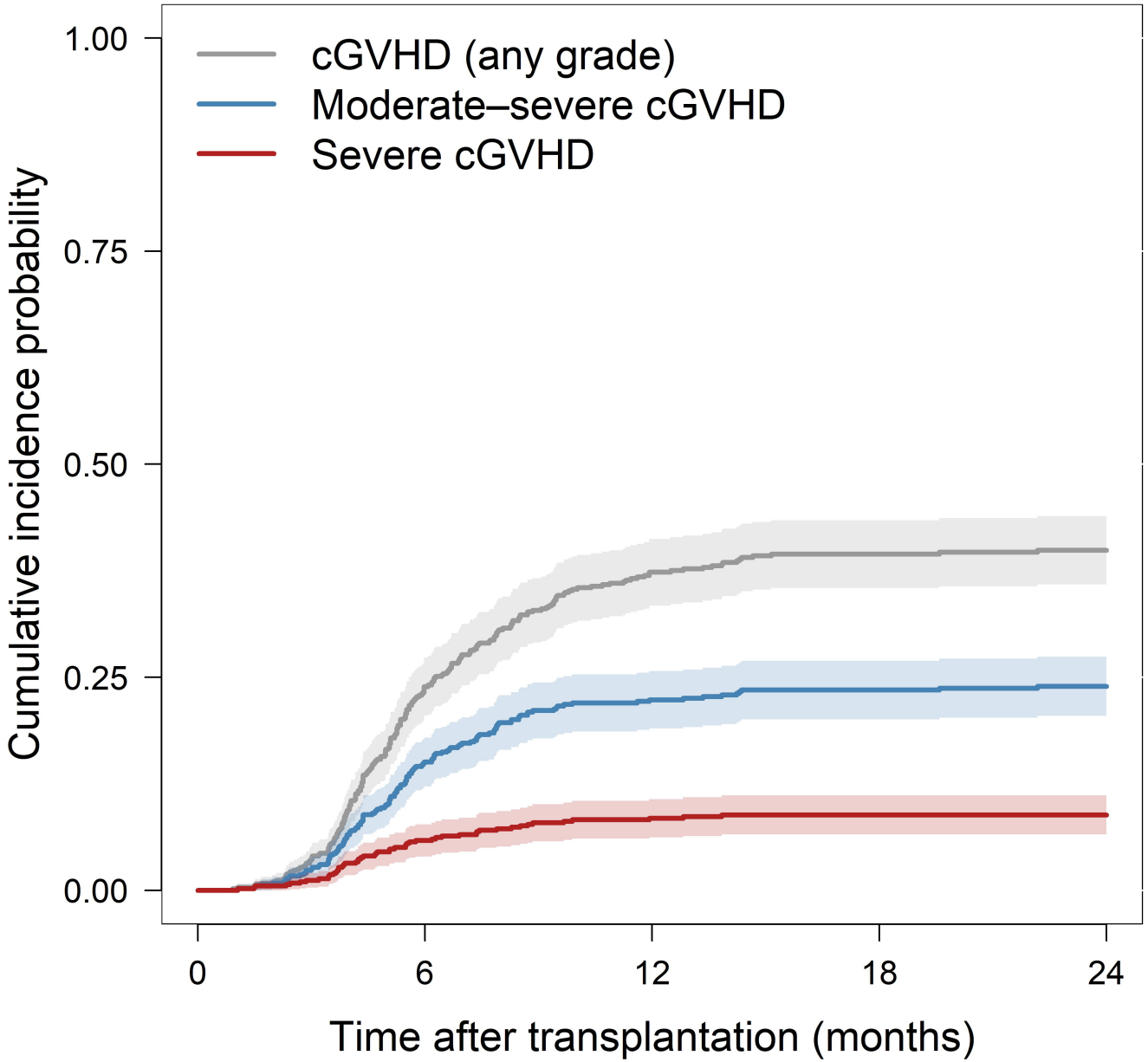
	First line	Second line	Third line
Patients n	105	44	19
Treatment Duration Days. Median (IQR)	83 (47 – 130)	178 (80 – 252)	157 (104 – 508)
Medical visits Visits per month. Median (IQR)	4 (3 – 6)	3 (2 – 4)	4 (1 – 4)
Hospitalization Days. Mean (range)	3 (0 – 48)	4 (0 – 53)	10 (0 – 155)
Neutropenia. % All grade Grade 3 and 4	 40 8	 38 14	 32 16
RBC transfusions Mean (range)	1 (0 – 11)	2 (0 – 20)	6 (0 – 42)
Platelets transfusions Mean (range)	1 (0 – 16)	2 (0 – 30)	4 (0 – 27)

FIGURE LEGENDS

Figure 1. Cumulative incidence of any grade, moderate-severe, and severe cGVHD with 95% confidence intervals (shadowed area).

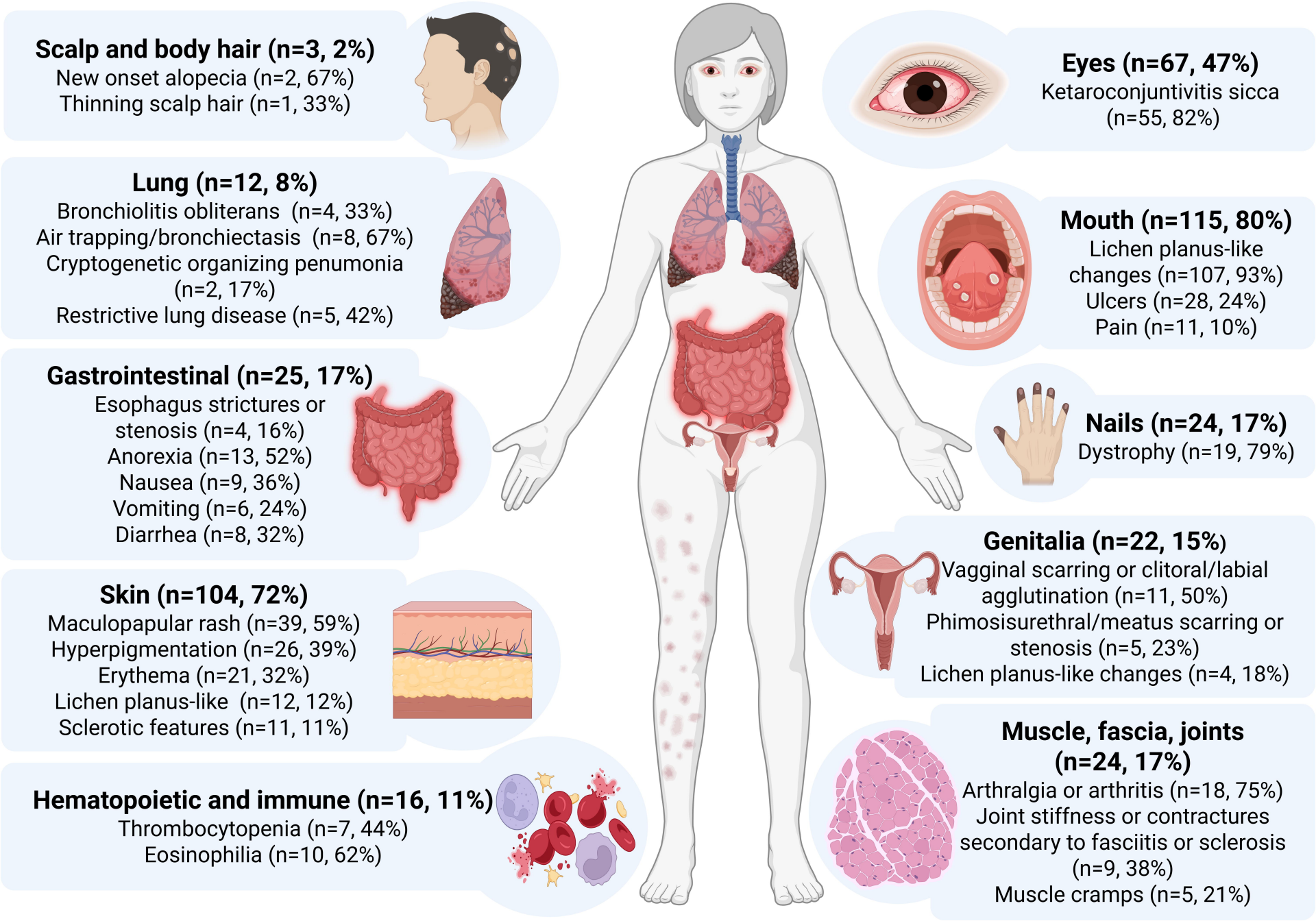
Figure 2. Clinical phenotype of cGVHD.

Figure 3. Patients flow chart.



Patients at risk

600	363	224	184	154
600	411	297	250	213
600	464	367	312	268


Scalp and body hair (n=3, 2%)
New onset alopecia (n=2, 67%)
Thinning scalp hair (n=1, 33%)
Lung (n=12, 8%)
Bronchiolitis obliterans (n=4, 33%)
Air trapping/bronchiectasis (n=8, 67%)
Cryptogenetic organizing penumonia (n=2, 17%)
Restrictive lung disease (n=5, 42%)
Gastrointestinal (n=25, 17%)
Esophagus strictures or stenosis (n=4, 16%)
Anorexia (n=13, 52%)
Nausea (n=9, 36%)
Vomiting (n=6, 24%)
Diarrhea (n=8, 32%)
Skin (n=104, 72%)
Maculopapular rash (n=39, 59%)
Hyperpigmentation (n=26, 39%)
Erythema (n=21, 32%)
Lichen planus-like (n=12, 12%)
Sclerotic features (n=11, 11%)
Hematopoietic and immune (n=16, 11%)
Thrombocytopenia (n=7, 44%)
Eosinophilia (n=10, 62%)
Eyes (n=67, 47%)
Ketaroconjuntivitis sicca (n=55, 82%)
Mouth (n=115, 80%)
Lichen planus-like changes (n=107, 93%)
Ulcers (n=28, 24%)
Pain (n=11, 10%)
Nails (n=24, 17%)
Dystrophy (n=19, 79%)
Genitalia (n=22, 15%)
Vagginal scarring or clitoral/labial agglutination (n=11, 50%)
Phimosisurethral/meatus scarring or stenosis (n=5, 23%)
Lichen planus-like changes (n=4, 18%)
Muscle, fascia, joints (n=24, 17%)
Arthralgia or arthritis (n=18, 75%)
Joint stiffness or contractures secondary to fasciitis or sclerosis (n=9, 38%)
Muscle cramps (n=5, 21%)

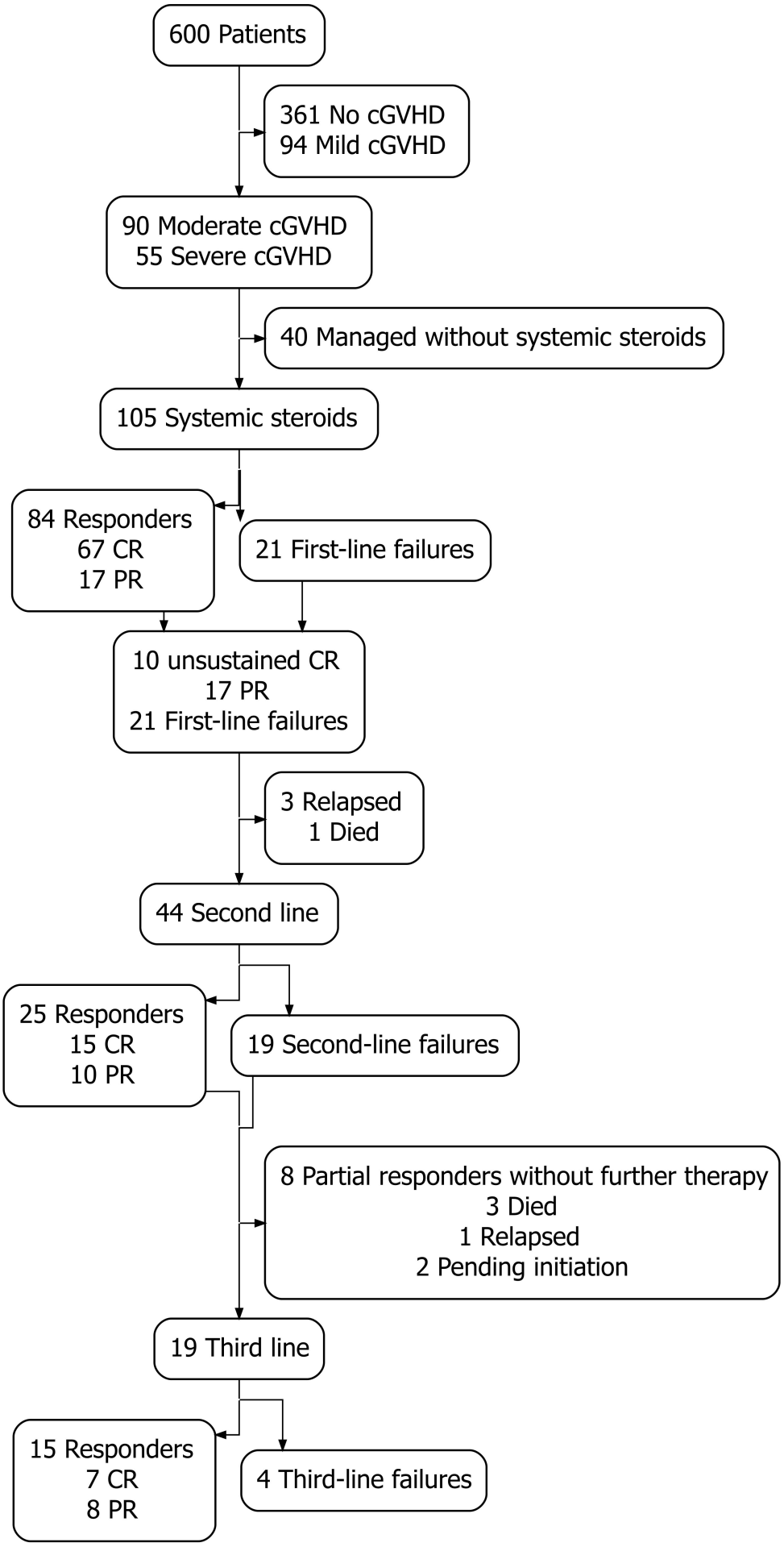

600 Patients
361 No cGVHD
94 Mild cGVHD
90 Moderate cGVHD
55 Severe cGVHD
40 Managed without systemic steroids
105 Systemic steroids
84 Responders
67 CR
17 PR
21 First-line failures
10 unsustained CR
17 PR
21 First-line failures
3 Relapsed
1 Died
44 Second line
25 Responders
15 CR
10 PR
19 Second-line failures
8 Partial responders without further therapy
3 Died
1 Relapsed
2 Pending initiation
19 Third line
15 Responders
7 CR
8 PR
4 Third-line failures

Table S1. Univariable analysis for cumulative incidence of moderate or severe cGVHD

Characteristic	n	12-month incidence (95% CI[1])	p-value[2]
Patient sex			0.5
Male	347	16 (13 - 20)	
Female	253	18 (14 - 24)	
Patient age			0.9
>55	295	17 (13 - 22)	
≤55	305	17 (13 - 22)	
HCT-CI			0.3
Low (0)	172	20 (14 - 27)	
Intermediate (1-2)	179	14 (8.9 - 19)	
High (≥3)	249	18 (13 - 23)	
Prior Autologous HCT			0.13
No	518	18 (15 - 21)	
Yes	82	13 (6.5 - 21)	
Diagnosis			0.10
Acute leukemia	371	19 (15 - 23)	
Myelodisplastic and myeloproliferative neoplasms	116	18 (12 - 26)	
Lymphoma and myeloma	92	12 (6.5 - 20)	
Non-malignant disease	21	0.00 (— - —)	
Disease Risk Index			0.062
Low	71	26 (16 - 37)	
Intermediate	290	15 (11 - 19)	
High and very high	218	19 (14 - 25)	
Donor age			**<0.001**
> 30	356	22 (18 - 27)	
≤ 30	244	10 (6.7 - 14)	
Donor type			0.4
MSD	213	19 (14 - 24)	
MUD	206	16 (11 - 22)	
MMUD	38	8.1 (2.0 - 20)	

Characteristic	n	12-month incidence (95% CI[1])	p-value[2]
Haplo	143	19 (13 - 26)	
Donor-patient gender combination			**0.006**
Female - Male	115	27 (19 - 36)	
Other	485	15 (12 - 18)	
Donor-patient CMV status			**0.036**
Positive/Positive	313	21 (16 - 25)	
Negative/Positive	159	10 (6.1 - 16)	
Positive/Negative	57	24 (14 - 36)	
Negative/Negative	71	12 (5.4 - 20)	
Stem cell source			0.4
Peripheral blood	554	18 (15 - 21)	
Bone marrow	46	11 (4.1 - 23)	
Infused CD34x10^6/kg			0.2
>7	241	20 (15 - 26)	
≤7	307	16 (12 - 21)	
Infused CD3x10^8/kg			0.4
>2	192	20 (15 - 26)	
≤2	134	16 (10 - 23)	
Infused TNCx10^8/kg			0.5
>7	267	19 (15 - 24)	
≤7	260	16 (12 - 21)	
GVHD prophylaxis			0.3
PTCy+Siro+MMF	555	18 (14 - 21)	
PTCy+Tacro+MMF	45	12 (4.2 - 23)	

[1]Confidence interval

[2]Gray's Test

Table S2. Univariable analysis for cumulative incidence of cGVHD requiring systemic therapy

Characteristic	n	12-month incidence (95% CI) [1]	p-value[2]
Patient sex			0.3
Male	347	16 (12 - 20)	
Female	253	18 (13 - 23)	
Patient age			0.8
>55	295	17 (13 - 21)	
≤55	305	16 (12 - 21)	
HCT-CI			0.2
Low (0)	172	19 (14 - 26)	
Intermediate (1-2)	179	13 (8.6 - 19)	
High (≥3)	249	17 (12 - 22)	
Prior Autologous HCT			0.063
No	518	17 (14 - 21)	
Yes	82	9.8 (4.6 - 18)	
Diagnosis			0.061
Acute leukemia	371	19 (16 - 24)	
Myelodisplastic and myeloproliferative neoplasms	116	17 (11 - 25)	
Lymphoma and myeloma	92	7.7 (3.4 - 14)	
Non-malignant disease	21	0.00 (— - —)	
Disease Risk Index			0.048
Low	71	25 (16 - 36)	
Intermediate	290	13 (9.1 - 17)	
High and very high	218	20 (15 - 26)	
Donor age			**<0.001**
> 30	356	21 (17 - 26)	
≤ 30	244	9.3 (6.0 - 13)	
Donor type			0.3
MSD	213	18 (13 - 23)	
MUD	206	16 (11 - 22)	
MMUD	38	8.2 (2.0 - 20)	

Characteristic	n	12-month incidence (95% CI) [1]	p-value[2]
Haplo	143	17 (11 - 24)	
Donor-patient gender combination			**0.005**
Female - Male	115	25 (18 - 34)	
Other	485	14 (11 - 18)	
Donor-patient CMV status			**0.016**
Positive/Positive	313	21 (16 - 25)	
Negative/Positive	159	9.7 (5.7 - 15)	
Positive/Negative	57	20 (11 - 32)	
Negative/Negative	71	10 (4.4 - 19)	
Stem cell source			0.8
Peripheral blood	554	17 (14 - 20)	
Bone marrow	46	11 (3.9 - 22)	
Infused CD34x10^6/kg			0.7
>7	241	18 (13 - 23)	
≤7	307	16 (12 - 20)	
Infused CD3x10^8/kg			0.9
>2	192	19 (14 - 25)	
≤2	134	14 (8.3 - 20)	
Infused TNCx10^8/kg			0.8
>7	267	18 (13 - 22)	
≤7	260	15 (11 - 19)	
GVHD prophylaxis			0.4
PTCy+Siro+MMF	555	17 (14 - 20)	
PTCy+Tacro+MMF	45	12 (4.2 - 23)	

[1]Confidence interval

[2]Gray's Test

Table S3. Univariable analysis for cGVHD failure free-survival

Characteristic	12-month probability (95% CI[1])	p-value[2]
Overall	58 (50 - 69)	
Patient Sex		0.5
Male	60 (48 - 74)	
Female	57 (44 - 73)	
Patient Age		0.7
>55	59 (47 - 74)	
≤55	58 (46 - 73)	
HCTCI		0.6
0	64 (49 - 82)	
1-2	66 (50 - 88)	
≥3	50 (37 - 67)	
Prior autologous HCT		0.8
No	58 (49 - 69)	
Yes	60 (36 - 99)	
Diagnosis		>0.9
Acute leukemia	60 (49 - 73)	
Myelodisplastic and myeloproliferative neoplasms	52 (34 - 78)	
Lymphoma and myeloma	61 (40 - 94)	
DRI		0.2
Low	60 (41 - 85)	
Intermediate	49 (36 - 66)	
High and very high	68 (55 - 85)	
Donor age		0.2
≤ 30	50 (34 - 73)	
> 30	61 (51 - 73)	
Donor type		0.4
MSD	60 (47 - 78)	
MUD	50 (36 - 70)	
MMUD	50 (12 - 100)	
Haplo	67 (52 - 87)	

Characteristic	12-month probability (95% CI[1])	p-value[2]
Donor-patient gender combination		0.8
Other	57 (46 - 69)	
Female - Male	63 (48 - 83)	
Donor-patient CMV status		>0.9
Positive/Positive	56 (45 - 70)	
Negative/Positive	57 (39 - 84)	
Positive/Negative	69 (48 - 99)	
Negative/Negative	63 (37 - 100)	
Stem cell source		0.8
Peripheral blood	59 (50 - 70)	
Bone marrow	50 (22 - 100)	
Infused CD34x10^6/kg		0.6
>7	57 (45 - 73)	
≤7	60 (48 - 75)	
Infused CD3x10^8/kg		>0.9
>2	64 (50 - 81)	
≤2	63 (46 - 87)	
Infused TNCx10^8/kg		0.4
>7	64 (52 - 79)	
≤7	54 (41 - 71)	
GVHD prophylaxis		0.6
PTCy+Siro+MMF	57 (48 - 67)	
PTCy+Tacro+MMF	100 (100 - 100)	
Skin at first line initiation		0.7
0-1	59 (48 - 72)	
2-3	58 (44 - 76)	
Eye at first line initiation		0.8
0-1	57 (47 - 68)	
2-3	70 (49 - 99)	
Mouth erythema at first line initiation		**<0.001**
No	64 (54 - 75)	
Yes	33 (17 - 64)	

Characteristic	12-month probability (95% CI[1])	p-value[2]
Mouth lichen at first line initiation		>0.9
No	56 (42 - 74)	
Yes	60 (49 - 74)	
Mouth ulcers at first line initiation		**<0.001**
No	66 (57 - 78)	
Yes	37 (22 - 60)	
Upper gastrointestinal grade at first line initiation		0.5
0-1	59 (50 - 70)	
2-3	50 (25 - 99)	
Lower gastrointestinal grade at first line initiation		0.2
0-1	60 (51 - 71)	
2-3	44 (21 - 92)	
Alanine aminotransferase at first line initiation		0.8
Normal	54 (41 - 71)	
Increased	62 (51 - 76)	
Alkaline phosphatase at first line initiation		0.8
Normal	56 (41 - 75)	
Increased	60 (49 - 73)	
FEV1 at first line initiation		**0.005**
>60	60 (51 - 71)	
≤59	14 (2 - 87)	

[1]Confidence interval

[2]Log-rank test

Table S4. Univariable analysis for chronic GVHD, relapse free-survival

Characteristic	12-month probability (95% CI[1])	p-value[2]
Overall	60 (57 - 65)	
Patient Sex		0.5
Male	62 (57 - 67)	
Female	58 (52 - 65)	
Patient Age		0.3
>55	59 (54 - 65)	
≤55	62 (57 - 68)	
HCTCI		0.4
0	65 (58 - 73)	
1-2	63 (56 - 70)	
≥3	55 (50 - 62)	
Prior autologous HCT		0.8
No	60 (56 - 65)	
Yes	60 (51 - 72)	
Donor age		0.061
≤ 30	67 (61 - 73)	
> 30	56 (51 - 61)	
Donor type		0.7
MSD	58 (52 - 65)	
MUD	63 (57 - 70)	
MMUD	72 (59 - 88)	
Haplo	57 (49 - 65)	
Donor-patient gender combination		**0.002**
Other	64 (60 - 68)	
Female - Male	46 (38 - 56)	
Donor-patient CMV status		**0.044**
Positive/Positive	58 (52 - 63)	
Negative/Positive	63 (56 - 71)	
Positive/Negative	50 (38 - 65)	
Negative/Negative	75 (66 - 86)	

Characteristic	12-month probability (95% CI[1])	p-value[2]
Stem cell source		**0.027**
Peripheral blood	59 (55 - 64)	
Bone marrow	74 (62 - 88)	
Infused CD34x10^6/kg		0.5
>7	60 (54 - 66)	
≤7	60 (55 - 66)	
Infused CD3x10^8/kg		0.052
>2	54 (47 - 61)	
≤2	64 (57 - 73)	
Infused TNCx10^8/kg		0.052
>7	56 (50 - 62)	
≤7	65 (60 - 71)	
GVHD prophylaxis		0.4
PTCy+Siro+MMF	61 (57 - 65)	
PTCy+Tacro+MMF	56 (43 - 73)	

[1]Confidence interval

[2]Log-rank test