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Response to Immunosuppressive Treatment Predicts Outcome in Patients with Chronic Graft-versus-Host Disease: A Single-Center Analysis of Longitudinal Data

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It has been reported that chronic graft-versus-host disease (cGVHD) is associated with significant morbidity and mortality after allogeneic stem cell transplantation (allo-SCT). The risk of relapse is generally reduced when cGVHD is present, but prognosis may be affected by increased toxicity and/or risk of infection associated with immunosuppressive treatment (IST). We performed a longitudinal data analysis of cGVHD, including the evolution of cGVHD itself over time in response to IST, in a single-center cohort of 313 consecutive patients undergoing allo-SCT. We found that lack of sustained response without withdrawal of IST within 6 months of cGVHD development was associated with higher transplantation-related mortality (hazard ratio, 2.32; 95% confidence interval, 1.24-4.33) compared with cGVHD-free patients. Conversely, response conferred better overall survival (hazard ratio, 0.42; 95% confidence interval, 0.18-0.95). Our analytical approach allowed us to integrate the evolution of cGVHD in a predictive model of transplantation outcome; notably, remission associated with permanent discontinuation of IST within the first 6 months from the occurrence of cGVHD seemed to correlate most closely with final outcome. Further confirmation from larger studies is needed.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-SCT) is an effective treatment for many hematologic malignancies [1]. Allogeneic lymphocytes can produce a graft-versus-malignancy (GVM) effect; thus cure after allo-SCT may be stem from the elimination of malignant stem cells or the establishment of a durable immune control on their progeny [2]. Unfortunately, this beneficial effect is limited by graft-versus-host disease (GVHD), which is responsible for most of the early and delayed mortality occurring after allo-SCT.

Chronic GVHD (cGVHD) affects 30% to 70% of all allo-SCT recipients who survive for 100 days, with a median onset of 4 to 6 months after transplantation [3]. cGVHD is associated with very high morbidity and mortality and thus carries a significant medical burden in both affected patients and society as a whole. As an example, the median time of initiation of immunosuppressive treatment (IST) is 2 to 3 years after diagnosis of cGVHD, with 15% of patients requiring IST even 7 years after diagnosis [4].

Patient outcomes related to the development of cGVHD depend on disease severity and presentation. Limited forms of cGVHD have been associated with prolonged survival owing to the GVM effect, even if GVHD persists for several months or even years after allo-SCT, whereas extensive forms are associated mainly with increased transplantation-related mortality (TRM) from infectious complications occurring after IST [5,6]. In general, patients who develop GVHD are at less risk for relapse, but this does not translate into better survival in patients with acute GVHD (aGVHD) or severe cGVHD, in whom the lower relapse risk is overcome by the higher TRM due to GVHD itself [5,7].

As a late event, from a statistical standpoint, cGVHD is usually analyzed as a time-dependent covariate to take into account the temporal pattern of this late event and the dynamic patient population at risk [8]; however, even in the context of a time-dependent analysis of cGVHD, some limitations exist. In fact, the event "chronic GVHD" is usually considered single and invariable, occurring at a landmark day from allo-SCT (ie, 6 months post transplantation) and with a certain degree of severity (limited or extensive form, according to the Seattle classification scheme [9]). Actually, cGVHD is a dynamic event that may persist for several months or even years after allo-SCT and changes its features along the time in terms of organ involved, specific treatments, and global severity. Unfortunately, all of these changes and the dynamic nature of cGVHD are not taken into account by the current analytical methods, which still do not make complete use of data.

The aim of the present analysis was to overcome current analytical limitations by using longitudinal data in the analysis of cGVHD to better predict transplantation outcomes by capturing information on the evolution of cGVHD over time. Internal preliminary analyses have suggested that the initial presentation and severity of cGVHD, as

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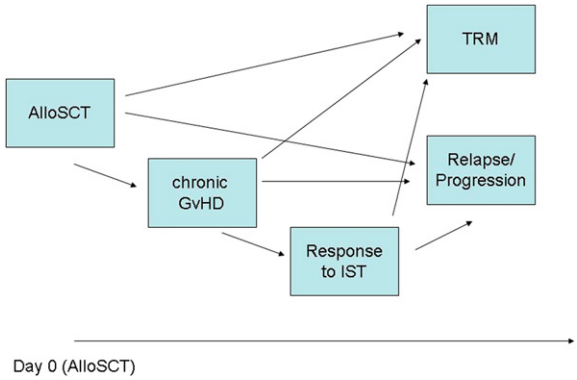

Figure 1. Multistep process after allo-SCT. AlloSCT indicates allogeneic stem cell transplantation; TRM, transplant-related mortality.

well as its response to treatment within 12 months of diagnosis, are predictive of final outcome [10]. Here, we translated these elements into an analytical method capable of capturing the event "sustained response to IST" in the post-transplantation setting. Figure 1 shows the multistep processes occurring during the post-transplantation period.

PATIENTS AND METHODS

Study Population

The study population comprised adult patients undergoing allo-SCT for a hematologic malignancy after conditioning with a fludarabine-busulfan-antithymocyte globulin regimen as reported elsewhere [11,12] between May 1999 and June 2010 at Institut Paoli-Calmettes (Marseille, France). Indications for such a conditioning regimen in younger patients relied on previous autologous SCT and/or presence of comorbidities precluding administration of a myeloablative regimen owing to expected high regimen-related toxicity. Cyclosporine alone, or in combination with mycophenolate mofetil in cases with a mismatched unrelated donor, was administered as GVHD prophylaxis, without methotrexate in accordance with local protocol [11,12]. cGVHD was classified retrospectively according to National Institutes of Health (NIH) criteria as mild, moderate, or severe [13]. The duration of IST was determined, and any changes in cGVHD severity over time were documented at 6-month intervals for up to 36 months, then annually thereafter. Clinical and biological information allowed to calculate the organ-specific and the global score at each timepoint. Information on cGVHD presentation was collected: progressive, quiescent or de novo, as well as classic, overlap, or late-onset acute manifestations. An example of longitudinal data collection and NIH-defined classification is provided in Appendix 1.

First-line systemic treatment of cGVHD was methylprednisolone 1 mg/kg/day with or without cyclosporine. IST was initiated in the presence of an extensive cGVHD according to the Seattle criteria [9]. Local steroids and/or other treatments have been used for limited forms (eg, isolated oral, ocular, or mild skin involvement). Various agents were used beyond the first-line treatment, according to clinical or biological manifestations, including mycophenolate mofetil, methotrexate, extracorporeal photopheresis, rituximab, total lymphoid irradiation, and imatinib [14-21]. Patients were evaluated at the transplantation center at least every 3 to 6 months; a further IST line was administered if no improvement of cGVHD occurred within 6 months or progression was observed within 3 months from the start of the previous IST line. An algorithm for choosing the proper drug therapy is shown in Figure 2. The number of IST lines administered to each patient was recorded as well.

Sustained response to IST was defined as the complete disappearance of all reversible clinical and/or biological manifestations of cGVHD together with full and permanent IST withdrawal (ie, from severe form, coded as 3, to resolution of cGVHD, coded as 0; see Appendix 1). If improvement or even resolution was observed but the patient was still under IST, then cGVHD was coded as the previous time point, until definitive IST withdrawal occurred; in that case, the patient was considered a complete responder and coded as 0.

Data on other important patient and transplantation characteristics were collected, including patient age, diagnosis, disease risk at the time of allo-SCT, donor type, stem cell source, comorbidity index [22], GVHD prophylaxis, and platelet count at development of cGVHD. Data were collected retrospectively from electronic clinical records. Data collection started at the time of allo-SCT and continued until the last observation, death from any cause, or relapse/progression of the original disease. The study design was approved by the local Institutional Review Board.

Statistical Analysis

Values are expressed as median (range) or number (percentage) as applicable. Overall survival (OS) was estimated using the Kaplan-Meier method [23]; cumulative incidence in the presence of competing risks was calculated for TRM and relapse or progression [24]. cGVHD was considered a time-dependent variable, and subsequent classification was assigned according to the response to IST at 6, 12, 18, and 24 months after the occurrence of cGVHD.

Differences in OS and cause-specific risk for TRM and relapse/progression between responders and nonresponders after cGVHD were assessed using Cox regression with time-dependent covariates [25]. Patients were considered "without cGVHD" until cGVHD occurred, at which point they were switched to the classification "cGVHD with nonresponse to IST." Patients who responded to IST were switched to "cGVHD with response to IST" at the time point of interest (6, 12, 18, or 24 months after the occurrence of cGVHD).

The combined role of form and presentation of cGVHD at baseline and response status was assessed, and interaction was created and evaluated by means of the likelihood ratio test, comparing models without interaction and with interaction. Patients not evaluable for cGVHD owing to censoring or occurrence before day +100 post-HSCT were not considered in the analysis for comparison of subsequent response to IST in patients with and without cGVHD.

The impact of responders and nonresponders on cumulative incidence of TRM or relapse/progression was estimated using two different landmark

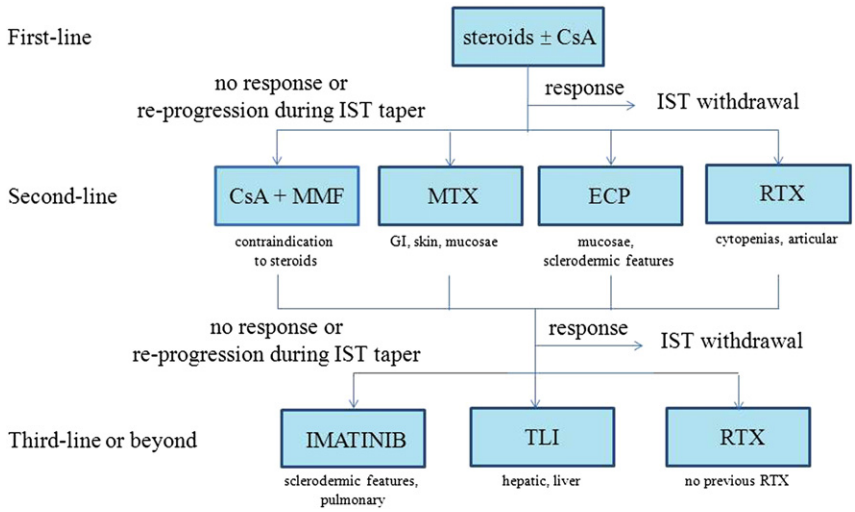

Figure 2. Algorithm applied on IST lines. IST indicates immunosuppressive treatment; MTX, methorexate; CsA, cyclosporine; MMF, mycophenolate mofetil; ECP, extracorporeal photoapheresis; RTX, rituximab; TLI, total lymphoid irradiation; GI, gastroinestinal.

analyses. First, 2 time points, 180 days and 260 days post-SCT, were separately fixed to detect patients with cGVHD. Then, second landmark points, fixed at 365 and 450 days post-SCT, respectively, were fixed to allow assessment of response/nonresponse within 6 months of cGVHD onset. These landmark points were chosen to capture a sufficient number of patients without losing an excessive number of events, potentially leading to loss of statistical power. TRM and relapse were considered competing events with one another, and a Fine and Gray competing-risks regression model was used [26]. Hazard ratio (HR) for the Cox model and subhazard ratio (SHR) for the FG model with respective 95% confidence intervals (CI) were provided for responders and nonresponders, with patients without cGVHD serving as the reference group.

Adjustment for patient age (continuous variable), HLA matching between patient and donor (HLA-identical vs HLA-mismatched), stem cell source (bone marrow vs peripheral blood stem cells), disease risk at time of allo-SCT, and comorbidity index [22] (above vs below the median) was performed by stepwise selection of variables with a P value $< .15$ on univariate analysis. Disease risk at allo-SCT was considered standard for patients with acute leukemia in first complete remission and chronic myelogenous leukemia in chronic phase, and high for all other patients.

To the illustrate the probability of OS and TRM or relapse/progression according to response status after cGVHD, the multistate approach [27-29] was used to obtain nonparametric transition probabilities among different intermediate states (eg, cGVHD, response to IST) and final states (eg, OS, TRM/relapse).

The probability of singular final events was calculated using the Aalen-Johansen estimator for transition probabilities [30]. For nonresponders, probability was calculated based on the status of "cGVHD without response," whereas for responders, probability was calculated as the likelihood of transition from the state of responder within 6 months of cGVHD onset to the state of the final event of interest.

Statistical analyses were performed using Stata version 11.0 (StataCorp, College Station, TX) and R version 2.12.3 (*mstate* package; R Institute for Statistical Computing, Vienna, Austria). A P value of .05 was considered statistically significant.

RESULTS

Patient and Transplantation Outcomes

A total of 313 patients meeting our inclusion criteria underwent allo-SCT at our institution between May 1999 and June 2010. One-hundred twenty-nine of these patients developed cGVHD, 128 of whom were evaluable for complete data; 184 patients did not develop cGVHD. Median patient age at allo-SCT was 52 years (range, 18-70 years). Patient and transplantation characteristics are summarized in Table 1. Median follow-up after allo-SCT was 30 months (range, 7-120 months). Data on comorbidity score were available for 271 patients (86%); median score was 2 (range, 0-8).

OS was 59% (95% CI, 53%-65%) at 3 years. Cumulative incidences of TRM and relapse/progression at 3 years were 21% (95% CI, 17%-27%) and 22% (95% CI, 17%-27%), respectively. A total of 159 patients (51%) developed aGVHD (cumulative incidence, 50% [95% CI, 44%-55%]; median onset, 38 days post-SCT [range, 8-99 days]).

cGVHD

Among the 128 evaluable patients with cGVHD, 76 patients had previous aGVHD; 41 of these 76 patients presented with progressive cGVHD. Progressive cGVHD indicated the onset of cGVHD without resolution of previous existing aGVHD. Seventy-two patients developed aGVHD but not cGVHD, 10 patients developed aGVHD and died before day +100 post-SCT, and 2 patients were censored before day +100. A total of 124 deaths were registered; 50 of these patients had developed cGVHD before dying. Before day +100, 19 patients died, and 6 were censored. The median interval from allo-SCT to onset of cGVHD was 132 days (range, 80-599 days); cumulative incidence was 45% (95% CI, 39%-51%).

According to the Seattle classification scheme [9], 26 patients had limited cGVHD, and 102 patients had extensive cGVHD. Among the 26 patients with limited cGVHD, 16 patients had a moderate form, and 10 patients had a severe form, according to NIH definitions [13]. The 102 patients with extensive cGVHD included 14 with a mild form, 40 with a moderate form, and 48 with a severe form. The 14 patients with NIH-defined mild cGVHD who were originally coded as Seattle-defined extensive vGVHD were reclassified as previous aGVHD favorably evolving into progressive, late acute, or overlap mild cGVHD after revision of patient records. At presentation, features of cGVHD were "classic" in 75 patients and "overlap" in 10 patients. Forty-three patients had late-onset aGVHD. Organs affected, as well as respective scores at the onset of cGVHD, are detailed in Figure 3A. Skin, liver, and mouth were most involved, whereas other organs, such as lungs and articulations, were rarely involved at onset (Figure 3A).The median number of administered IST lines was 1 (range, 0-6), and the median duration of IST among the living patients was 21 months (range, 3-105 months). Involved organs accounted for distinct patterns of NIH-defined severity scores at 6, 12, 18, and 24 months after onset of cGVHD. Skin, mucosa, liver, and gastrointestinal tract involvement declined over time, whereas eye and lung involvement appeared to increase, especially between 6 and 12 months (Figure 3B).

Table 1
Patient and Transplantation Characteristics

Characteristic	Value
Patients, n (%)	313 (100)
Age, years, median (range)	52 (18-70)
Diagnosis, n (%)	
Acute leukemia	121 (39)
Myelodysplastic syndrome	22 (7)
Chronic myelogenous leukemia	16 (5)
Non-Hodgkin lymphoma	69 (22)
Hodgkin lymphoma	10 (3)
Multiple myeloma	48 (15)
Other	27 (9)
Comorbidity score, median (range)	2 (0-8)
Antithymocyte globulin total dose, n (%)	
2.5 mg/kg	140 (45)
5 mg/kg	163 (52)
>5 mg/kg	10 (3)
Donor, n (%)	
HLA-identical sibling	237 (76)
Unrelated donor	76 (24)
Stem cell source, n (%)	
Bone marrow	18 (6)
Peripheral blood stem cells	295 (94)
GVHD prophylaxis, n (%)	
Cyclosporine alone	270 (86)
Cyclosporine + mycophenolate mofetil	43 (14)
IST lines, n, median (range)	1 (0-6)
Duration of IST, months, median (range)	21 (3-105)

Impact of cGVHD on Transplantation Outcomes

OS

The patients with cGVHD were divided into 2 groups according to whether remission with permanent discontinuation of IST was obtained (responders; n = 60) or not (nonresponders; n = 68). Of note, 12 of the 68 nonresponders died before 6 months after the onset of cGVHD, 11 due to cGVHD itself and 1 due to relapse of underlying disease, despite severe cGVHD. These 11 patients were considered nonresponders. Among the 60 responders, 41 patients responded within 6 months of cGVHD onset, 8 responded within 12 months, and 11 responded beyond 18 months. The response rate within 6 months was slightly higher in patients with classic cGVHD

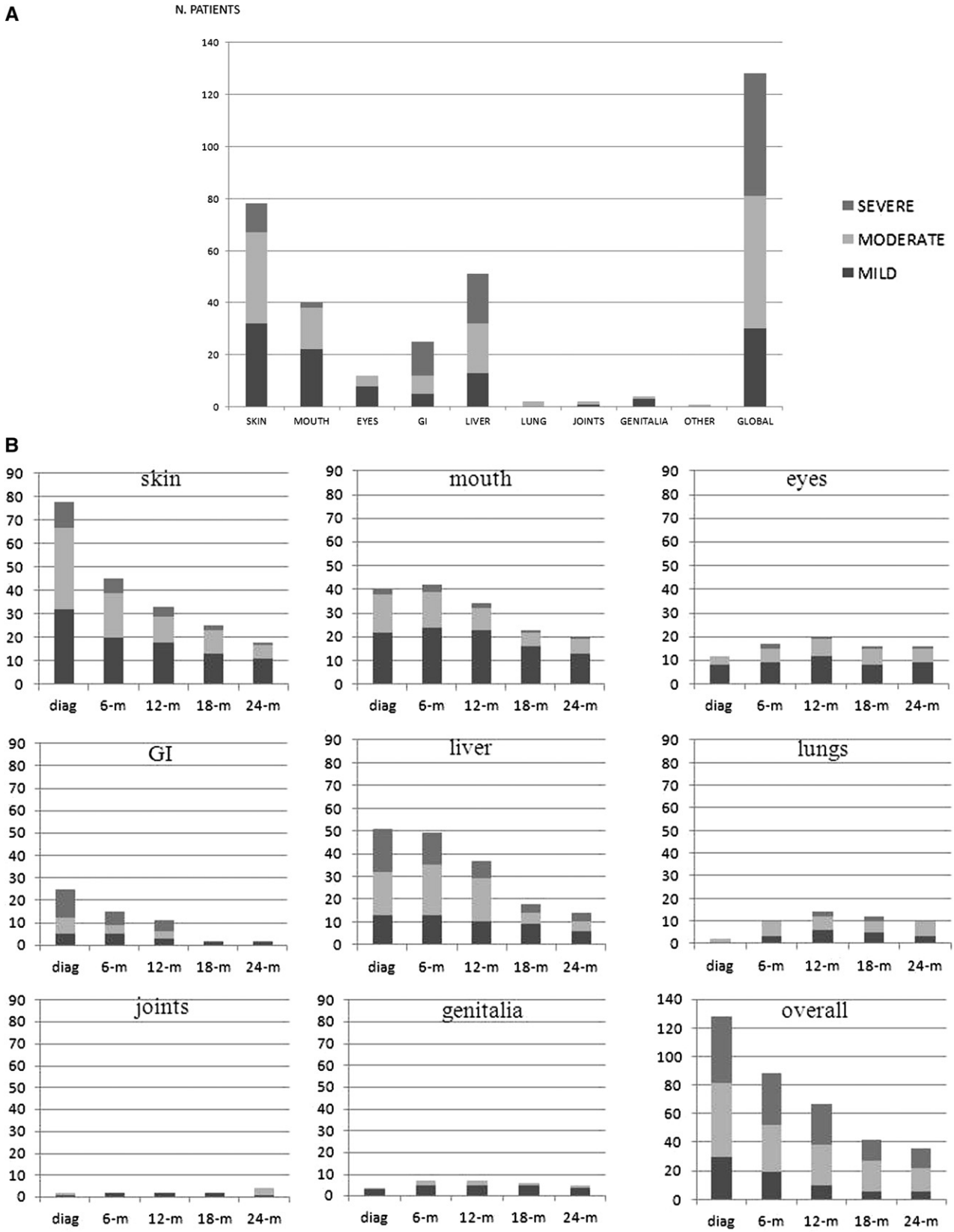

Figure 3. (A) Global and organ-specific scores at onset of cGVHD. (B) Organ-specific NIH-defined severity at diagnosis of cGVHD and up to 24 months thereafter.

features (35%, vs 30% in patients with overlap cGVHD and 28% in those with late-onset aGVHD), although the difference was not statistically significant ($P = .68$).

In the OS model for cGVHD, the unadjusted HR was 0.79 (95% CI, 0.52-1.19) for patients with cGVHD compared with those without cGVHD ($P = .25$). Only age and aGVHD were

significantly associated with OS. Results did not change after adjustment for these 2 variables. Unexpectedly, disease risk did not affect OS in our series, possibly related to the relatively high 3-year OS in our high-risk patients (58% [95% CI, 50%-65%] versus 60% [95% CI, 49%-70%] in standard-risk patients; $P = .79$). We found no significant correlation between comorbidity index score [22] and OS; however, it must be noted that the score was calculated prospectively for only the 122 patients (39%) who underwent allo-SCT in our center since 2008.

When analyzing platelet count at the onset of cGVHD, we found that a count $<100 \times 10^9$/L was associated with higher mortality risk (HR, 2.25; 95% CI, 1.01-5.01; $P = .05$). Low platelet count was associated with more aggressive features of cGVHD; in fact, 65% of cGVHD events in patients presenting with a platelet count $<100 \times 10^9$/L were progressive, compared with only 22% of events in patients with a platelet count $>100 \times 10^9$/L ($P < .0001$). Similarly, we found that NIH-defined severity at cGVHD onset was predictive of OS; mortality rate was 23% in patients with a mild form, 30% in those with a moderate form, and 59% in those with a severe form ($P = .002$). Progressive-onset cGVHD was associated with a 46% mortality rate, compared with 35% of quiescent and de novo presentations, a non statistically significant difference ($P = .28$).

In the model for response/nonresponse considering only the patients who developed cGVHD, unadjusted HR was 0.46 (95% CI, 0.20-1.06) for responders at 6 months, 0.81 (95% CI, 0.19-3.55) for responders at 12 months, and 0.75 (95% CI, 0.17-3.30) for responders at 18 months or later compared with the reference group of patients with cGVHD but with no response over time. Globally, response status was not significantly associated with OS ($P = .28$); only when considering response at 6 months did we observe a trend toward better OS among responders ($P = .09$). In addition, responders did better at 6 months than patients without cGVHD (HR, 0.42; 95% CI, 0.18-0.95). Similar results were obtained on multivariate analysis. The probability of death according to response at 6 months is shown in Figure 4A.

Including the interaction term between form or presentation and response status into the model for OS did not significantly change our results ($P = .24$, likelihood ratio test). Of note, the beneficial effect of response at 6 months was comparable among the distinct types of cGVHD presentation ($P = .83$). Adjusted HRs are presented in Table 2.

A subanalysis looking at organ-specific response at 6 months detected a 26% organ-specific response rate, that is, 59 organs out of 225 affected at onset of cGVHD (Figure 3A). In no specific organ was response significantly associated with overall response within 6 months ($P > .05$ for each organ compared with average response of all organs), but organ-specific responses were proportional to the absolute number of organs involved at the onset of cGVHD (skin: 25 of 88; mouth: 9 of 40; eyes: 6 of 12; GI: 8 of 25; liver: 11 of 51; lungs: 0 of 2; joints: 0 of 2; genitalia: 0 of 4; other: 0 of 1). Consequently, no significant association was found between organ-specific response and OS.

TRM

Globally, the impact of response status was statistically significant ($P = .006$). A higher risk for nonresponders (HR, 2.32; 95% CI, 1.24-4.33) compared with cGVHD-free patients was observed, along with a trend toward decreased risk in responders within 6 months (HR, 0.30; 95% CI, 0.04-2.36); the difference between responders at 6 months and nonresponders was significant ($P = .047$).

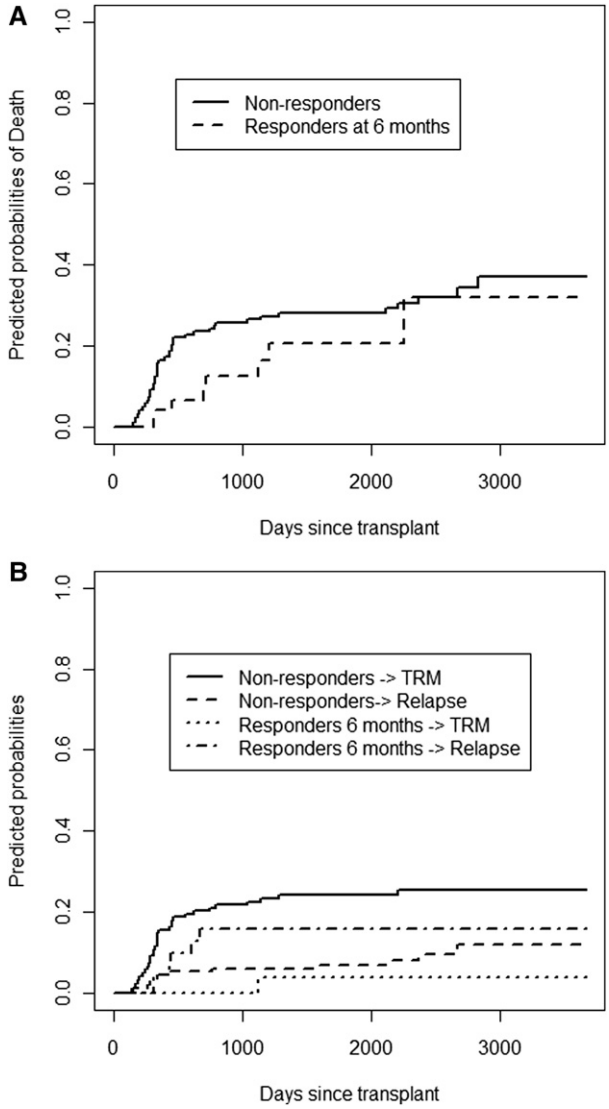

Figure 4. (A) Predicted probability of death according to response at 6 months after onset of cGVHD. (B) Predicted probabilities of TRM and relapse/progression according to response at any time or at 6 months. Probabilities were evaluated from the day of response to IST (for responders) or from the day of development of cGVHD (for nonresponders).

After adjustment for age and aGVHD, the P value was .06 for the difference between nonresponders versus cGVHD-free patients and .04 for the difference between nonresponders and responders at 6 months. The risk for TRM was greater in nonresponders with moderate/severe cGVHD compared with cGVHD-free patients (HR, 2.17; 95% CI, 1.12-4.19; $P = .02$). In contrast, the lack of sustained response to IST at 6 months in patients with mild cGVHD did not result in worse TRM in these patients compared with responders ($P = .51$). A platelet count $<100 \times 10^9$/L was associated with higher TRM (HR, 3.29; 95% CI, 1.41-7.70; $P = .006$). Regarding cGVHD presentation, progressive-onset cGVHD was associated with a 35% TRM compared with 21% for quiescent and de novo forms ($P = .13$).

Results of our landmark analysis are consistent with the foregoing findings, as detailed in Appendix 2. Similar to OS, we found no significant association between organ-specific response within 6 months post-SCT and TRM.

Table 2
Adjusted Hazard Ratios of OS, TRM and Relapse/Progression According to Response to IST and form of cGVHD

	OS		TRM		Relapse/Progression	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Response to IST		.17		.061		.18
No cGVHD	1.00		1.00		1.00	
cGVHD, nonresponse	0.77 (0.50-1.20)		1.92 (1.01-3.66)		0.52 (0.25-1.09)	
cGVHD, response at 6 months	0.42 (0.18-0.95)		0.24 (0.03-1.91)		0.82 (0.29-2.35)	
cGVHD, response after 6 months	0.57 (0.19-1.73)		1.33 (0.26-6.95)		1.01 (0.20-5.08)	
Response to IST and form of cGvHD		.24		.04		.32
No cGVHD	1.00		1.00		1.00	
Mild cGVHD, nonresponse	0.45 (0.18-1.14)		1.12 (0.36-3.41)		0.45 (0.11-1.94)	
Moderate/severe cGVHD, nonresponse	0.86 (0.55-1.36)		2.17 (1.12-4.19)		0.53 (0.24-1.18)	
Mild cGVHD, response at 6 months	0.38 (0.12-1.27)		0.53 (0.07-4.20)		0.41 (0.05-3.23)	
Moderate/severe cGVHD, response at 6 months	0.45 (0.16-1.28)		NE		1.09 (0.35-3.36)	
Mild cGVHD, response after 6 months	NE		NE		NE	
Moderate/severe cGVHD, response after 6 months	0.74 (0.24-2.23)		1.71 (0.33-8.96)		1.38 (0.28-6.91)	

NE indicates not evaluable owing to a lack of events in this category.

Relapse/progression

Response to IST at any time had no significant impact on relapse or progression in both univariate and multivariate analyses; the unadjusted HR was 0.49 (95% CI, 0.23-1.02) for nonresponders versus cGVHD-free patients. No differences were observed according to the form or presentation of cGVHD. Results of relapse/progression by landmark analysis were consistent, as detailed in Appendix 2. No associations were identified between disease, disease stage, donor type, or comorbidity score and relapse/progression. Probabilities of TRM and relapse/progression according to response to IST at 6 months post-SCT are shown in Figure 4B.

DISCUSSION

In this study, we specifically examined the impact of response to immunosuppressive therapy for cGVHD on transplantation outcomes. We found significantly higher TRM in patients who could not withdraw from IST within 6 months of onset of cGVHD compared with responders, which translated into worse survival (Figure 4A). In addition, nonresponders had significantly higher TRM compared with cGVHD-free patients (Table 2). No significant impact on relapse risk was seen in any group.

GVHD is associated with higher TRM and inferior survival, as well as reduced risk of relapse owing to a GVM effect [5,6,31-34]. In this study, we tried to overcome some previous analytical limitations related to lack of data on the evolution of cGVHD, as it appears in studies in which information about cGVHD is not captured as a longitudinal event and only data on occurrence, severity, and post transplantation day are reported. We integrated the transition state "response to IST" at distinct time points from the occurrence of cGVHD and found that remission associated with sustained withdrawal (or not) at 6 months from onset of cGVHD allowed us to create a predictive model capable of predicting prognosis for cGVHD according to response to IST.

We found a higher TRM at 6-months post-SCT in nonresponders compared with both responders and cGVHD-free patients, independent of the initial presentation of cGVHD. Importantly, the response to IST affected the prognosis dictated by the initial form or presentation of cGVHD, allowing us to improve and refine our model. Interestingly, lack of response to IST at 6 months in patients with mild cGVHD did not result in higher TRM compared with responders; most of these patients received topical rather than systemic IST, which might explain the comparable outcomes in the 2 groups. Moreover, the GVM effect was likely preserved as long as only topical IST was administered. In the transplantation setting, TRM is related to GVHD or infection; here, TRM in nonresponders was due to cGVHD itself, either directly or indirectly, given that the lack of response required a more prolonged duration of IST and/or the administration of further IST lines, thereby exposing patients to life-threatening infections even if a response to cGVHD was finally obtained. Here, the shorter duration of IST exposure among responders has proven beneficial in this sense and explains the lower TRM risk in this subset.

Although not the objective of the present study, our results confirm previous published findings on cGVHD and transplantation outcomes [5,6,31-34]; however, we found no statistically significant correlation between cGVHD and relapse or progression. This finding may be explained by our small study sample and the fact that patients who became responders disappeared from the cohort of nonresponders, thus contributing to the progressive loss of power of our comparisons. Another possible explanation is that diseases known to be highly sensitive to the GVM effect, such as chronic myelogenous leukemia or multiple myeloma, are represented by few patients in this series (eg, 16 [5%] for the former and 48 [15%] for the latter).

Our results are in line with a recent study by the Seattle group in which resolution of GVHD followed by withdrawal of IST was not associated with a subsequent increase in the risk of relapse compared with patients without GVHD (in whom withdrawal of IST was associated with a reduced risk of relapse during the first 18 months), but nonetheless was considerably higher than that in patients with GVHD [35]. Our relatively limited study sample does not allow us to draw definite conclusions about relapse/progression; however, we found no increase in relapse in patients with cGVHD and those who could withdraw from IST compared with patients without cGVHD or nonresponders with cGVHD (Table 2).

Here, we propose a more comprehensive statistical approach to the multifaceted and evolving behavior of cGVHD, in an attempt to use the available data more completely. Most published works have used the Kaplan-Meier method [31], Cox regression with time-dependent covariates [6,32,33], or competing-risk analysis without time-dependent covariates [34]. We acknowledge that the relatively small number of patients included in our analysis represents a limitation, as does the study's retrospective nature, potentially leading to intrinsic bias in the grading of cGVHD according to NIH criteria and/or in the evaluation of response to IST. We cannot exclude the possibility of a relative

underestimation of the involvement of some organs, such as lungs and genitalia (Figure 3B), given that pulmonary function testing and physical examination of the genitalia were not performed unless guided to do so by clinical complaints. On the other hand, our cohort appeared to be quite homogeneous in terms of cGVHD treatments and response assessments, owing mainly to the study's single-center design. Moreover, the methodological approach was rather conservative in its definition of "response to IST" and calculation of the time-dependent transitional probabilities, compensating for the relative narrowness of the study cohort.

Finally, our data suggest that evaluation of response to IST within the first 6 months after onset of cGVHD merits further exploration in larger series. It can be speculated that obtaining a response within this interval may be a target for future clinical studies in the cGVHD setting, with the objective of IST withdrawal (or at least a significant improvement with IST tapering) as soon as possible, 6 months at the latest. The "paradigm" of a steroid-based first-line treatment with or without a calcineurin inhibitor could be challenged in favor of a more effective treatment for patients with high-risk cGVHD. For this reason, the ability to identify features of cGVHD that are predictive of response to IST is of great interest.

In conclusion, the present study is the first work to specifically address the interpretation of cGVHD as a dynamic, multistate variable. Remission associated with permanent withdrawal of IST within 6 months of onset of GVHD predicted transplantation outcome in this homogeneous monocenter population of 313 patients. Present data may challenge current practices in managing cGVHD, with the aim of optimizing treatment and preventing the sometimes fatal complications that remain associated with this post-transplantation disease.

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APPENDIX 1. EXAMPLE OF LONGITUDINAL DATA COLLECTION AND NIH-DEFINED CLASSIFICATION

Timepoint	presentation	late aGvHD/classic/overlap	skin	mouth	eyes	GI	liver	lungs	joints	genital	other	global
diagnosis	progressive	overlap	1	1			2					2
6months			0	1			2					2
12months			0	1			3					3
18months												
24months												
diagnosis	progressive	late aGvHD	2				2					2
6months			2				2					2
12months			2				1					2
18months			2				0					2
24months			0				0					0
diagnosis	quiescent	late aGvHD	1				2					2
6months			0				2					2
12months			0				0					0
18months			0				0					0
24months			0				0					0
diagnosis	quiescent	classic	0			3	0	0				3
6months			0			1	0	2				3
12months			2			1	2	2				3
18months			2			1	2	2				3
24months			2			1	2	2				3
diagnosis	progressive	late aGvHD	2			3	2					3
6months			2			3	2					3
12months			2			3	2					3
18months												
24months												

APPENDIX 2. LANDMARK ANALYSIS

(A) Using a landmark point of day +180 from transplantation, 100 patients had at that time already developed cGvHD; response status at a landmark point of day +365 was considered.

An increase of cumulative incidence of TRM for patients who developed cGvHD within 180 days was observed ($SHR_{adjusted}$ (95% CI) = 1.99 (0.97-4.10); $P = .06$); when response status at subsequent 6 months was assessed, probability of TRM increased among non responders compared with patients free from cGvHD: $SHR_{adjusted}$ (95% CI) = 3.87 (1.18-12.70); $P = .02$. On the other hand, a trend toward less TRM was observed among responders compared with cGvHD-free patients: $SHR_{adjusted}$ (95% CI) = 0.53 (0.06-4.54); $P = .56$. Importantly, an almost significant difference between responders and non responders was observed ($P = .06$).

In the relapse/progression model, the presence of cGvHD was not associated with significant modification in cumulative incidence of relapse: $SHR_{adjusted}$ (95% CI) = 0.82 (0.41-1.63); $P = .56$. According to response to IST, no significant differences ($P = .63$) were noticed between responders (SHR [95% CI] = 0.83 [0.22-3.19]) and non responders (SHR [95% CI] = 1.16 [0.45-2.98]) when compared with cGvHD-free patients.

(B) Using a landmark point of day +260 from transplantation, 155 patients had at that time already developed cGvHD; response status at a landmark point of day +450 was considered.

Results on both TRM and relapse/progression were similar to (A) (data not shown); P value of the difference between responders and non responders was 0.10 for the TRM model and 0.50 for the relapse/progression model.