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A Single-Center Pilot Prospective Study of Topical Application of Platelet-Derived Eye Drops for Patients with Ocular Chronic Graft-versus-Host Disease

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A B S T R A C T

Ocular involvement of chronic graft-versus-host disease (cGVHD) is a complication that occurs in up to 60% of patients after allogeneic hematopoietic stem cell transplantation. Conventional therapeutic options include medical and surgical procedures that are administered depending on the severity of the condition, but most of them have provided unsatisfactory results and, to date, there is no consensus about treatment. We considered that topical application of a platelet lysate, administered as eye drops, might be considered an alternative worthwhile of investigation to treat ocular surface disorders in patients suffering from cGVHD. Therefore, we conducted a single-center prospective pilot study to assess the efficacy and safety of using eye drops made from reconstituted lysed platelet concentrate. Twenty-six patients with ocular cGVHD were eligible for the study; all but 2 completed their scheduled 1-year treatment and complied with the hematologic and ophthalmic regimen. At their first assessment interviews, after 30 days of treatment, 91% of patients reported an improvement in their symptoms and for 32%, substantive objective differences were measured. Remission of corneal damage was seen for 86% of our cohort, and improved National Institutes of Health scores for 73%, of whom 8% achieved the best score of 0 (ie, non–dry eye). Similar results were seen at later time points. Comparing outcomes for our patient cohort to those determined retrospectively for patients in our institutional database revealed a 5-year overall survival (OS) of 65%. This OS is comparable to patients with limited cGVHD (75%) and is superior to that of patients with nonocular extensive cGVHD or without cGVHD (30% and 59%, respectively) ($P = .013$). Our results suggest that platelet-derived eye drops are a safe, practical, and well-tolerated therapeutic option that offers substantial benefits for most patients affected by ocular cGVHD at onset. The favorable OS of our patient cohort suggests that this topical therapy, rather than systemic immunosuppression, may be the treatment of choice.

INTRODUCTION

Allogeneic (allo) hematopoietic stem cell transplantation (HSCT) is a potentially curative approach for several hematologic neoplasms [1]. According to most registries, the number of patients is growing mostly because of an increase of transplantations from unrelated and haplo-identical donors. Less aggressive conditioning regimens and improved supportive care have led to a reduction in treatment-related mortality, with patients ages 60 to 65 years routinely able to undergo this procedure [2].

Despite significant advances in the allo-HSCT field, graft-versus-host disease (GVHD) remains the most important cause of morbidity and mortality after transplantation. Although acute GVHD (aGVHD) has a considerable impact on nonrelapse mortality (NRM), chronic GVHD (cGVHD) is the

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most common long-term complication and occurs in 30% to 70% of adults who survive for more than 100 days after receiving their transplantation [3]. cGVHD has been associated with a reduced risk of relapse, possibly because of a concomitant graft-versus-leukemia effect [4-6]. Despite this, cGVHD correlates with increased morbidity and mortality and has a major impact on the quality of life of subjects who have undergone allo-HSCT [7].

Ocular complications develop in a substantial percentage of patients after allo-HSCT as part of aGVHD or cGVHD. Ocular manifestations of aGVHD, ranging from conjunctival hyperemia to pseudomembraneous conjunctivitis, are uncommon but reported in literature because they might be associated with irreversible ocular damage and very poor prognosis [8]; ocular complications are more frequently associated with the occurrence of systemic cGVHD and arise in up to 60% of such cases [9,10]. This pathology results from the infiltration of the lacrimal gland by fibroblasts and T lymphocytes; these infiltrates provoke impaired secretory function and corneal damage [11,12]. Common symptoms of ocular cGVHD include inflammation and ocular discomfort, such as photophobia, pain, foreign body sensation, and dry eyes (xerophthalmia). Infectious conjunctivitis is also a frequent complication. Treatment usually involves steroids and artificial tears, neither of which are satisfactory. Ordinarily, the patient will continue to experience considerable discomfort and suffer reduced visual acuity and poorer quality of life [13].

Based on these unsatisfactory responses, there is considerable interest in developing alternative treatments with which to control autoimmunity, suppress inflammation, and promote tissue regeneration. We reasoned that an attractive strategy that could reverse the underlying pathological processes of cGVHD could be the topical application of an autologous platelet concentrate, lysed, and then reconstituted as eyes drops (PClys) [14-16]. The rationale for this approach is the capacity of blood components to enhance healing and stimulate tissue regeneration. In particular, platelet concentrates have a demonstrable positive impact on wound healing by modulating its different phases, especially re-epithelialization and tissue remodeling [17]. The bioactive components are likely vitamins, growth factors (GF), and fibronectin, all of which are required for corneal and conjunctival integrity; modulators of the inflammatory response are possibly also involved [18,19].

In ophthalmology, eye drops comprising platelet-rich plasma have been successfully used to treat dry eye syndrome for patients with Sjogren's disease [20]; the adoption of this approach for patients with ocular cGVHD has been suggested [21]. A caveat to this approach is that reports of platelet-rich plasma's success were retrospective and involved small numbers of patients [22]. We therefore designed a phase II clinical study to offer PClys eye drops to patients who had undergone allogeneic transplantation and were diagnosed with ocular cGVHD. We now report the results of this trial with a focus on the following: (1) the characteristics of patients with ocular cGVHD in terms of general clinical outcome and (2) feasibility and efficacy of PClys eye drops for the treatment of ocular cGVHD.

METHODS

Patients

This study was conducted at the SS Antonio e Biagio e Cesare Arrigo Hospital, Alessandria, by a consortium of the hematology, ophthalmology, and transfusion divisions. Since 2005, follow-up had been provided by local ambulatory services for patients who survived for more than 100 days after allo-HSCT. Follow-up involved systematic cGVHD screening, based on the Seattle [23] and National Institutes of Health (NIH) criteria [24] (the latter assigned retrospectively). A subjective activities of daily living score was also collated. All patients receiving allo-HSCT for a hematologic malignancy were requested to complete a self-administered, subjective, questionnaire about their ocular disability (the Ocular Surface Disease Index) [25]. This was used to identify patients with suspected ocular cGVHD. Patients scoring greater than 15 were referred to an ophthalmologist for a formal diagnosis and assessment for study eligibility.

Treatment with the PClys eye drops was offered from January 2007 to January 2014. Eligibility criteria for enrollment were (1) recent diagnosis of ocular cGVHD, (2) no active systemic or ophthalmic disease other than cGVHD, (3) absence of major systemic comorbidity other than those related to systemic cGVHD, (4) adequate control of primary hematological neoplasm with life expectancy $>$ 3 months, and (5) a platelet count in excess of $100 \times 10^3/\mu L$. After their enrollment in the study, all patients underwent a scheduled ophthalmic evaluation at days +30, +180, and +360. Additional evaluations where conducted on an ad hoc basic. Ophthalmic analyses included both subjective and objective tests. Subjective symptoms as pain, photophobia, and eye dryness were assessed according to the NIH Eye Score and Ocular Surface Disease Index questionnaire. Objective tests included Schirmer's test to assess the ability of the eye to produce sufficient lachrymal fluid to stay moist; a tear film break-up time test; and a best visual acuity score. In Schirmer's test, the ability to moisten a 15-mm paper strip was considered our reference (normal) value and a 5-mm increase was rated as improved; for the tear film break-up time test, $>$ 10 seconds was the reference time and a 5-second increase was considered improved. Finally, there was an assessment of corneal damage, which comprised fluorescein score and lissamine score. The study was planned to be of exploratory nature without definition of a formal hypothesis of efficacy. Enrollment was allowed for 7 years with the aim of enrolling at least 28 patients with ocular cGVHD. All patients gave written consent before inclusion in the study; their treatment was authorized by our local ethical committee (Prot. n° ASO.-Tras07.04 del CE 18/04/2007).

Preparation and Administration of the CP Lysate Eye Drops

The preparation of PClys eye drops has been described previously [26]. Briefly, 60 mL of anticoagulated peripheral blood was collected from each patient and centrifuged to obtain an autologous platelet concentrate. The concentrate (at $.7 \times 10^6$/mL) was frozen and thawed (−80°C/37°C) to lyse platelets. The lysate was then diluted with sterile balanced saline solution (30% V/V) and aliquoted as 30 ready-to-use, sterile doses (Col System, Biomed Device Modena, Italy). The eye drops were stored frozen (−20°C) by the study participants, who used them daily. All samples were subject to microbiological control to verify sterility. Patients were provided with a monthly supply of eye drops. Patient training was provided for storage (the sterile vials were frozen in home freezers for up to 30 days), the thawing protocol (vials were thawed and then stored for the day at 4°C), and topical application (2 eye drops per eye, 6 times each day for the duration of the 1-year treatment period).

Statistical Analyses

Data were collected in a computerized database and analyzed using the SPSS version 18.0 statistical package. Response criteria for ocular cGVHD were measured based on improvement, stability, or worsening NIH score. Subjective and objective tests were analyzed using Wilcoxon signed-rank test.

Overall survival (OS) was defined as the probability of survival regardless of disease state; its distributions over time were estimated by the Kaplan-Meier product limit method with log-rank tests to assess differences between groups. It was calculated with 2 different time points. OS was calculated for all patients starting from day +100 after allo-HSCT to censor early death after allo-HSCT. OS was also calculated for patients with ocular GVHD from the date of onset of ocular symptoms and for the other patients from day +100 after allo-HSCT, after the analysis by Jacobs et al. [27] (Figure 1S).

NRM was defined as the probability of dying without previous occurrence of a relapse, which is a competing event. The cumulative incidence function for NRM was estimated by the Gray test (comparing the cumulative incidence curve of the main event [death without relapse] in the presence of the competing event [relapse]). All reported *P* values were obtained by the 2-sided exact method, at the conventional 5% significance level.

RESULTS

Patient Characteristics and Outcome

From March 2005 until August 2014, a total of 127 patients underwent allo-HSCT transplantation at our

institution for a hematologic neoplasm; their median follow-up was 654 days (range, 10 to 3486). The treated neoplasms included 53 (42%) acute myeloid leukemias, 10 (8%) myelodysplastic syndrome, 16 (13%) acute lymphoblastic leukemias, 24 (19%) non-Hodgkin lymphoma or chronic lymphocytic leukemias, 7 (5%) multiple myelomas, 5 (4%) Hodgkin lymphomas, and 12 (9%) other hematological malignancies. GVHD prophylaxis consisted of an inhibitor of calcineurin (cyclosporine or tacrolimus) combined with methotrexate or mycophenolate mofetil. Patient and disease characteristics, type of conditioning regimen, source of stem cells, and information about occurrence of GVHD and NRM events are illustrated in details in Table 1.

Occurrence of Ocular cGVHD

Twenty-nine patients (23% of the allo-HSCT population) developed ocular cGVHD; these patients were treated for acute myeloid leukemia (10 patients, 34% of the cohort), myelodysplastic syndrome (6 patients, 20%), acute lymphoblastic leukemia (3 patients, 7%), and non-Hodgkin lymphoma (3 patients, 10%). Seven patients (24%) were treated for other hematological malignancies. Seventeen patients (58%) received a myeloablative conditioning and 12 received (42%) a reduced-intensity conditioning. The median follow-up was 1087 days after allo-HSCT, with a range of 241 to 2947 days. Median time of onset of ocular GVHD was 218 days (range, 90 to 1750 days). Clinical characteristics of the 29 patients who developed ocular cGVHD are illustrated in Table 2; a detailed description of ocular manifestations at baseline is reported in Table 3.

Table 1
Patient Characteristics

Characteristic	Value
Gender	
Male	69 (54%)
Female	58 (46%)
Diagnosis	
AML	53 (42%)
NHL	18 (14%)
ALL	16 (13%)
MDS	10 (8%)
Other	30 (23%)
Conditioning regimen	
Myeloablative	77 (61%)
Reduced intensity	50 (39%)
Source of stem cells	
PB	111 (87%)
BM	16 (13%)
Type of donor	
Sibling donors	56 (44%)
MUD	68 (53%)
Haploidentical	4 (3%)
T cell depletion	
ATG	64 (50%)
Campath	1 (<1%)
GVHD	
Acute	19 (15%)
Chronic	72 (57%)
Extent of cGVHD	
Limited	13 (10%)
Extensive	59 (47%)
Ocular GVHD	
No	98 (77%)
Yes	29 (23%)
Relapse after HSCT	
No	93 (73%)
Yes	34 (27%)
Death	
No	78 (61%)
Yes	49 (39%)
Cause of death (before day 100)	
Progression of disease	9
Viral encephalitis (suspected)	1
aGVHD	1
Refractory hemolytic anemia	1
Multiorgan failure	1

AML indicates acute myeloid leukemia; NHL, non-Hodgkin lymphoma; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; PB, peripheral blood; BM, bone marrow; MUD, matched unrelated donor; ATG, antithymocyte globulin.

Treatment with PClys Eye Drops

Patient flow through our clinical study is shown in Figure 1. Of 29 patients diagnosed with ocular cGVHD, 26 were eligible for our study; 3 patients could not be enrolled for the following reasons: 1 patient with multiple myeloma was excluded because of rapidly progressing disease; 1 patients acute lymphoblastic leukemia patient was excluded because of severe, systemic concurrent symptoms; for the third patient, use of an antibiotic eyewash rapidly improved his ocular symptoms to a state where inclusion was no longer appropriate. Five of 26 patients (19%) were classified as NIH score 1, 18 patients (69%) scored 2, and 3 patients (12%) scored 3.

The median age of the cohort of 26 patients enrollment in the study was 60 (range, 24 to 67) and their median time from transplantation to enrollment was 258 days (range, 97 to 1750). Ocular cGVHD arose in 24 of 26 patients experiencing contemporary involvement of other organs; these patients restarted or increased immunosuppressive medications with the addition of PClys eye drops; 2 of 26 patients developed only ocular cGVHD and PClys eye drops were used as a stand-alone treatment. For 9 patients, ocular cGVHD was the sole manifestation defining extensive cGVHD.

All but 2 of our patients starting treatment completed their 1-year treatment schedule; 1 patient stopped his participation in the first few days because of treatment-associated ocular pain, burning, and conjunctival hyperemia; the episode resolved in few days with discontinuation of the eye drops. No evidence for infection was ever detected. Poor compliance was seen for the second patient, despite encouraging initial results. Among patients who underwent the planned treatment, the following results were observed: at day +30, 91% had an improvement of symptoms, 32% showed an improvement of objective criteria, and 86% demonstrated a remission of corneal damage. From a hematological point of view, 73% had an improved NIH score, with 8% attaining a 0 score. Positive results were also confirmed at +180 days: 86% reported continued subjective benefits, 59% experienced improved objective function, and 86% had remission of corneal damage. A further 8% improved their NIH score: 27% were now graded 0. These findings were confirmed at day +360. Results detailing these ophthalmological improvements are summarized in Figure 2 and a complete analysis of subjective and objective tests' results are shown in Table 3. None of the cohort had to further increase their systemic immunosuppressive therapy. It is worthwhile to note that during their eye drop therapy, 5 of the 9 patients manifesting ocular-only symptoms of extensive cGVHD (55%) were able to progressively taper and ultimately cease their systemic immunosuppressive therapies.

After 1 year of treatment, only 1 patient continued to use the eye drops because of persistent kerato-conjunctivitis. Results of treatment with PClys eye drops are detailed in Table 3.

Table 2
Clinical Characteristics at Enrollment

Patient	Disease	Gender	Age, yr	Source of Stem Cells	Type of Donor	cGVHD Other than Eyes	Ocular NIH Score	Global Scoring of GVHD	Ocular GVHD as the Sole Manifestation of Extensive cGvHD	Days from HSCT to Ocular GVHD
1	AML	M	55	PB	Related	Skin, mouth	2	Severe	No	1750
2	RAEB-2	F	53	PB	Related	Skin	2	Moderate	Yes	1738
3	AML	M	60	PB	Related	No	3	Moderate	Yes	324
4	NHL	M	51	PB	Related	Mouth, liver	1	Moderate	No	202
5	AML	M	23	PB	Related	Mouth	2	Mild	No	195
6	AML	M	36	BM	MUD	Skin, mouth	2	Moderate	No	1071
7	AML	M	46	PB	Related	Mouth	3	Moderate	No	210
8	RAEB-1	M	64	PB	Related	Skin	2	Mild	Yes	376
9	NHL	F	33	PB	Related	Skin, mouth, joint	3	Severe	No	206
10	CML	M	59	PB	Related	Skin, mouth, joint	2	Severe	No	502
11	MI	M	51	PB	MUD	Skin, joint	2	Severe	No	280
12	CLL	F	66	PB	MUD	No	1	Mild	Yes	294
13	RAEB-1	M	66	PB	Related	Skin	2	Moderate	Yes	406
14	MM	M	65	PB	MUD	Skin	1	Mild	Excluded	203
15	AML	M	52	PB	Related	Lung, mouth	2	Severe	No	426
16	AML	M	63	PB	MUD	Skin	1	Mild	Yes	193
17	AML	F	50	PB	Related	Liver, mouth	2	Moderate	No	218
18	MM	M	60	PB	MUD	Gut, skin	2	Severe	Excluded	141
19	BPDCN	M	64	PB	MUD	Mouth	2	Moderate	No	280
20	MM	F	62	PB	MUD	Mouth	2	Mild	No	275
21	ALL	F	64	PB	MUD	Skin	2	Mild	Yes	141
22	AML	F	63	BM	MUD	Mouth	2	Moderate	No	211
23	ALL	M	25	PB	Related	Skin, mouth	2	Moderate	No	362
24	MDS	M	63	BM	MUD	Mouth	1	Mild	No	241
25	AML	F	61	PB	MUD	Skin	2	Mild	Yes	98
26	MDS	M	64	PB	Related	Sierositis	2	Moderate	No	146
27	MDS	M	47	PB	Related	Mouth, skin	2	Moderate	No	181
28	NHL	F	53	PB	MUD	No	1	Mild	Yes	97
29	ALL	F	37	PB	Related	Skin, lung	3	Severe	Excluded	90

M indicates male; RAEB, refractory anemia with excess blasts; F, female; CML, chronic myeloid leukemia; MI, myelofibrosis idiopathic; CLL, chronic lymphocytic leukemia; MM, multiple myeloma; BPDCN, blastic plasmacytoid dendritic cell neoplasm.

General Outcome of Ocular GVHD Compared with Extraocular GVHD

Of the 26 patients diagnosed with ocular cGVHD, 6 (20%) relapsed after allo-HSCT and 4 (15%) died of disease progression. Twenty-two patients (76%) are currently alive at follow up.

To understand the outcome of our cohort of patients, we compared OS and NRM curves based on the presence of ocular involvement (29 patients) versus patients with extraocular cGVHD (limited, 13 patients and extensive, 30 patients) or without evidence of this complication (55 patients). In OS analysis, 11 patients were censored because of early death before day +100. We found that 5-year OS of patients with ocular cGVHD was 65%, which is comparable to that of patients with limited cGVHD (75%). This was significantly better than OS of patients with extensive nonocular cGVHD or without cGVHD, which were 30% and 59%, respectively ($P = .013$) (Figure 3). Interestingly, the 9 patients who were classified as having extensive cGVHD only for ocular symptoms had a 3-year OS of 100%. Figure 1S shows similar results, with slightly lower OS rate for oGVHD at 60% ($P = .024$).

Patients with extensive ocular cGVHD had a significantly better cumulative incidence of NRM (19.6%, 7.7%, 34.7%, and 3.6% at 3 years for patients without cGVHD, with limited cGVHD, with extensive nonocular cGVHD, and with

Table 3
Subjective and Objective Results of Patients treated with Platelet-Derived Eye Drops

	Baseline	30 Days			180 Days		
	Score	Score	Difference with Baseline	*P* Value	Score	Difference with Baseline	*P* Value
Symptoms							
OSDI score (range)	69 (27-93)	51 (81-15)	−18 (−3 to +12)	.074	21 (72-12)	−48 (−72 to +3)	.004
NIH score (range)	2 (1-3)	1 (0-3)	−1 (−1 to 0)	.0001	1 (0-2)	−1 (−2 to 0)	.0001
Test							
Schirmer test (mm/5 min)	7 (1-25)	13 (6-26)	+6 (−10 to +22)	.091	10 (3-30)	+3 (−13 to +27)	.126
TBUT test (range), sec	4 (0-8)	7 (3-15)	+3 (+1 to +9)	.005	9 (4-15)	+5 (−4 to +11)	.011
BCVA test (range), decimals	9 (4-10)	10 (8-10)	+1 (0 to +4)	.02	10 (9-10)	+1 (0 to +6)	.034
Damage							
Flurescein score	1 (0-3)	0 (0-2)	−1 (−1 to 0)	.003	0 (0-1)	−1 (−2 to 0)	.003
Lissamine score	1 (0-3)	0 (0-2)	−1 (−2 to 0)	.004	0 (0-1)	−1 (−2 to 0)	.005

OSDI indicates ocular surface disease index; TBUT, tear break-up test; BCVA, best corrected visual acuity.
OSDI score: minimum = 0 maximum = 100; NIH score: minimum = 0 maximum = 4; Schirmer test: normal values > 15 mm/5 min; TBUT test: normal values > 10 seconds; BCVA: minimum 0/10; maximum 10/10; fluorescein/lissamine score: Oxford scheme (0 = absence of conjuctival damage; 5 = maximum conjuctival damage). Values are expressed as medians.

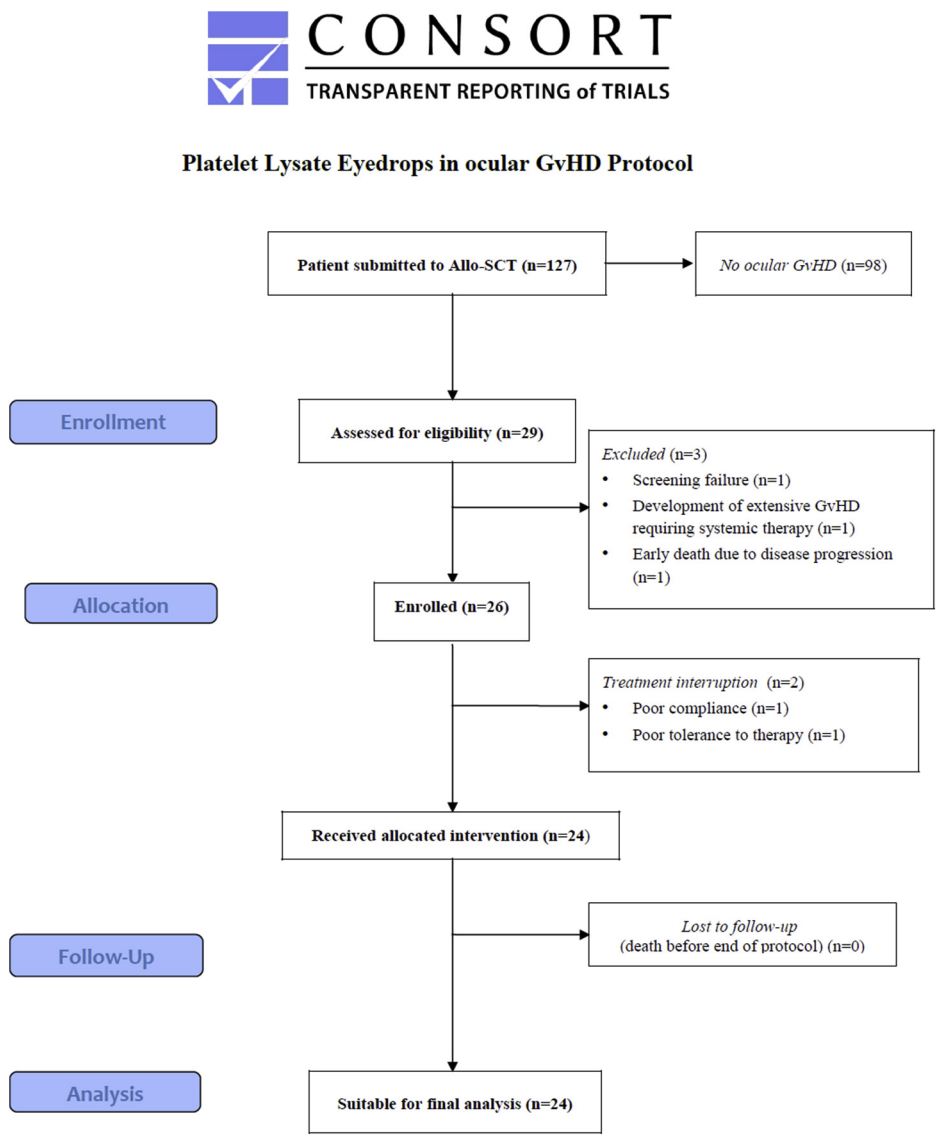

Figure 1. CONSORT flow diagram of study protocol.

extensive ocular cGVHD, respectively [$P = .016$]) (Figure 4). Conversely, no statistically significant difference was reported for the cumulative incidence of relapse when stratified by the same risk factor (31.6%, 33.6%, 21.5%, and 22.1%, respectively, $P = .406$).

DISCUSSION

We now report the results of a prospective pilot study using platelet-derived eye drops to treat ocular cGVHD. Our results indicate that PClys eye drops were a safe, feasible, and well-tolerated therapeutic option. They resulted in a

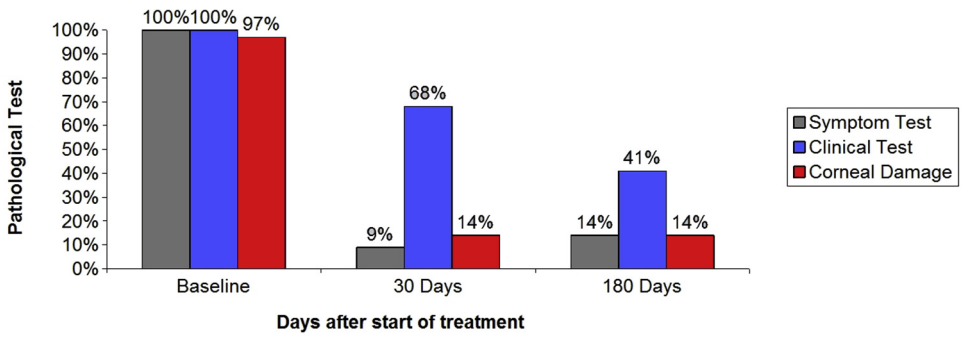

Figure 2. Ophthalmic improvement during study protocol.

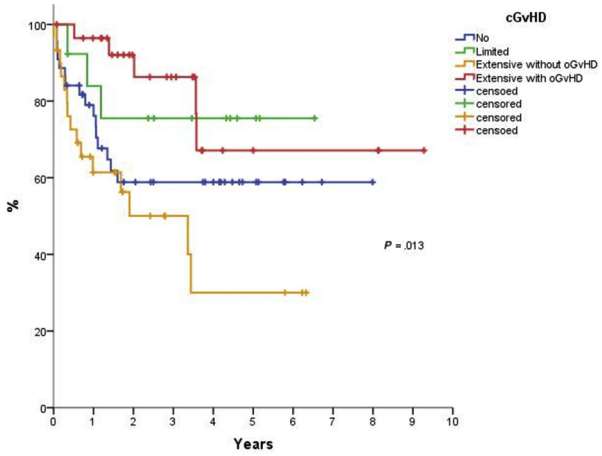

Figure 3. Overall survival stratified for cGVHD. Survival is measured from day 100.

substantial benefit for the majority of our patients. The therapeutic improvement and favorable outcome in those patient in which ocular cGVHD was the most severe symptom of their extensive cGVHD indicate that these patients in particular are ideal candidates for this topical treatment.

cGVHD is a common complication of allo-HSCT for patients surviving more than 100 days. cGVDH has substantial negative implications for quality of life as a result of impaired functional status and often leads to the resumption of immunosuppressive therapy or escalation to more intensive immunosuppressive therapy. For this reason, cGVHD is the leading cause of morbidity and NRM. Ocular symptoms develop in a substantial percentage of patients after allo-HSCT and often represent 1 of the most invalidating manifestation. Indeed, ocular cGVHD often leads to severe ocular symptoms, resulting in decreased quality of life and restriction of daily activities. Consequently, ocular cGVHD is a criterion used to define “extensive cGVHD” according to the Seattle scoring system [23].

Little is known about the influence of ocular cGVHD on survival. For this reason, we split our patients with extensive cGVHD into 2 groups based on ocular involvement. Our results suggest that patients with ocular cGVHD show no increased mortality in comparison to those with limited

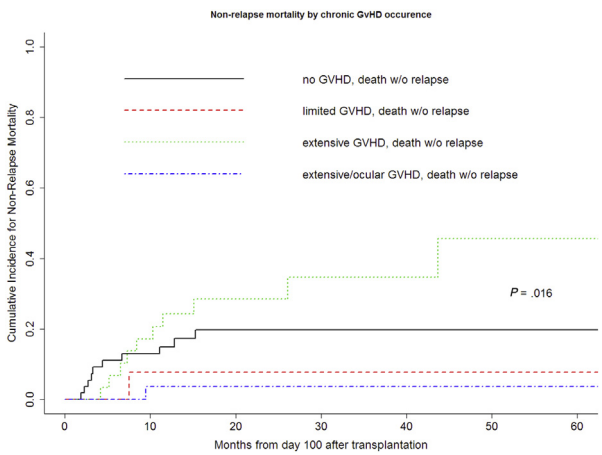

Figure 4. Nonrelapse mortality by occurrence of cGVHD.

cGVHD. Moreover, those patients with ocular cGVHD as the sole manifestation of their extensive cGVHD had an OS of 100% at 3 years. Although we have no historical comparison, we believe that this is clearly not an effect of the local treatment applied, as this observation was already reported in previous studies [27,28]. It is possible that the better general outcome of patients with ocular GVHD is not the consequence of a different disease biology but rather an overestimation of the severity of cGVHD in the Seattle classification system compared to the more recent NIH consensus criteria. Indeed, these novel criteria do not consider ocular cGVHD as a parameter of more severe cGVHD. This suggests that the isolated ocular disease, while severe in terms of quality of life and local complications, does not have a major impact on the global burden of cGVHD-related autoimmune phenomena. Consequently, these data indicate that ocular cGVHD is an excellent candidate for topical rather than for systemic treatment. To date, only 2 studies have compared outcomes for ocular versus cGVHD. In agreement with our findings, Jacobs et al. recently described a superior 2-year survival among patients with ocular GVHD versus extraocular [27]. Furthermore, Inamoto et al., in a multicenter observational study, reported that an NIH eye score of 2 or 3 was associated with a decreased risk of recurrent malignancy [28].

The treatment of ocular cGVHD is still not standardized; instead, recommended treatments are based on retrospective series and a few small prospective studies. Previous studies of ocular cGVHD have addressed the efficacy of retinoic acid [29], topical calcineurin inhibitors such as cyclosporine .05% and tacrolimus eye drops [30], and systemic treatments. Tacrolimus .03% eye drops are most commonly prescribed. These are more popular than cyclosporine eye drops because of their good tolerability and direct local immunosuppressive effect [31]. That said, the study that came to this conclusion had a sample size that was too small to be able to draw any definitive statement about safety and efficacy.

The benefits of autologous serum (AS) eye drops for the treatment of dry eye syndrome related to cGVHD was reported by Rocha et al. [32]. It was assumed that serum could recapitulate the function of lacrimal fluid. Indeed, AS eye drops are a rich source of GF, in particular epithelial GF, which induces proliferation and limits apoptosis; serum proteins (fibronectin and vitamin A); and cytokines that improve the healing of the corneal epithelium and conjunctiva [33].

PClys eye drops can provide additional advantages compared with AS eye drops. In particular, their increased concentration of platelet GF (platelet-derived GF, fibronectin, epidermal growth factor, and transforming GF β) can stimulate a faster ocular re-epithelialization [34]. Indeed PClys eye drops might exert a more extensive action; the plasma component contains proteins essential for surface lubrication, whereas platelets donate GFs with which to induce the tissue regeneration needed to accelerate healing.

Based on this rationale, we decided to treat patients with ocular cGVHD using a preparation of PClys eye drops in a prospective pilot study. The eye drop formulation was standardized for platelet number [26] and prepared using a controlled (ie, sterile) and certified procedure. Feasibility of use and tolerability were excellent. Only 1 patient experienced a local reaction to their eye drops of sufficient magnitude to interrupt treatment. Notably, we observed no ocular infections. Our results confirm the efficacy of the

experimental treatment, with an improvement of all ocular cGVHD-related symptoms in almost 80% of patients. Most notably, improvement was rapid and persistent, resulting in a considerable improvement in quality of life. At follow-up, only 1 persistent case of ocular cGVHD was seen, suggesting the long-term benefit of using the PClys eye drops. Based on the excellent toxicity profile and rapid response achieved, we are now investigating whether a shortened program might also allow similar results, without increased risk of late relapse. As an alternative, one might consider the development of response-adapted therapeutic regimens, in which treatment is only indicated when symptoms or signs of cGVHD reappear. Combination therapy with tacrolimus ointment, currently being used as a maintenance-treatment for ocular cGVHD, could also be considered [35].

Our study has some limitations; most notably, the small sample size, a lack of multicenter validation, and the absence of a control arm. The possibility of conducting a multicenter randomized study is currently being investigated and a number of trials from other institutions are currently ongoing (https://clinicaltrials.gov/).

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