Reduced-Intensity Allogeneic Hematopoietic Stem Cell Transplantation in Metastatic Colorectal Cancer as a Novel Adoptive Cell Therapy Approach. The European Group for Blood and Marrow Transplantation Experience

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Reduced-intensity conditioning (RIC) regimens for allogeneic hematopoietic stem cell transplantation (HCT) allowed the existence of an allogeneic cell-mediated antitumor effect in metastatic colorectal cancer (mCRC) to be explored. We report on 39 patients with progressing mCRC treated with different RIC regimens in a multicenter clinical trial of the European Bone Marrow Transplantation Group. Disease status at transplant was progressive disease (PD) in 31 patients (80%), stable disease (SD) in 6 (15%), and partial response (PR) in 2 (5%). All patients engrafted (median donor T cell chimerism of 90% at day +60). Transplant-related morbidities were limited. Grades II-IV acute graft-versus-host disease (aGVHD) occurred in 14 patients (35%) and chronic GVHD (cGVHD) in 9 patients (23%). Transplant-related mortality occurred in 4 patients (10%). The best tumor responses were: 1 complete response (CR) (2%), 7 PR (18 %), and 10 SD (26%), giving an overall disease control in 18 of 39 patients (46%). Allogeneic HCT after RIC is feasible; the collected results compared favorably in terms of tumor response with those observed using conventional approaches beyond second-line therapies. The study of an allogeneic cell based therapy in less advanced patients is warranted.

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INTRODUCTION

Metastatic Colorectal Cancer (mCRC) is an incurable disease [1]. For patients inoperable at diagnosis or relapsing after surgery, the best available treatment is represented by oxaliplatin or by irinotecan in combination with fluorouracil and folinic acid [2]. These treatments, used as first-line procedures, give a response rate of 55% to 60%, with a median time to progression (TTP) of 7 to 10 months and a median survival of 18 to 22 months. Once resistance to these agents has developed, second-line chemotherapy offers a low overall response rate (4% to 15%), with a median TTP of 3 to 7 months and a median survival of 9 to 12 months [3]. Molecular targeted therapies have recently been introduced in the treatment of mCRC. Their association with chemotherapy further improved remission rates and survival but, in resistant disease, their impact remains limited and no long-lasting remissions have been reported [4-8].

Therefore, new therapeutic strategies are required for the management of mCRC.

Although first-generation clinical trials of adoptive or vaccine therapy reported only limited success, based on growing knowledge on the immune system and T cell biology, there is a renewed interest to explore immunotherapy as a novel therapeutic strategy in CRC [9-11]. In the context of adoptive cell-based therapy, allogeneic hematopoietic stem cell transplantation (HCT) represents a promising approach that may help in overcoming some of the limitations of the previous experiences [12].

The introduction of allogeneic HCT was founded on the principles of maximal tumor cytoreduction and adequate immunosuppression to permit engraftment of HLA-identical donor stem cells. Evidence has accumulated over the last 2 decades that the donor stem cells may exert not only a repopulating role but also, through the lymphocytes, a graft-versus-tumor effect (GVT) [13]. Thus, many efforts have been made to transform allogeneic HCT from a chemotherapy- to an immunotherapy-based approach. Allogeneic HCT after reduced-intensity conditioning (RIC) accomplished this new concept [14-17]. The lower toxicity of the procedure allowed not only to proceed with success with allogeneic transplantation as an up-front treatment in selected malignancies but also to explore the existence of alloreactivity in metastatic solid tumors [18-22]. These diseases are often diagnosed in elderly patients and their growth kinetic is sometimes slow, allowing the development of a GVT effect. In metastatic CRC, early clinical experiences demonstrated the feasibility of the approach, giving immunologic evidence of a graft-versus-CRC effect [23,24].

To better understand the potential role of RIC and allogeneic HCT in the treatment of mCRC and to better select candidates for second-generation studies, we collected and analyzed the clinical data of patients with resistant/refractory mCRC transplanted and reported to the European Group for Blood and Marrow Transplantation (EBMT).

PATIENTS AND METHODS

We reviewed the data of 39 patients with mCRC who were treated with RIC and allogeneic HCT between 1999 and 2004 at 9 EBMT centers accordingly to a multicenter trial (http://www.ebmt.org/ClinicalTrials/Trials.aspx then select STWP-02) that allowed 5 different RICs. Some of these patients have been already described in single institution reports [24,25]. Patients were required to have a disease that could be evaluated radiographically and to have an HLA-identical donor or a matched unrelated donor (URD) typed by allele level polymerase chain reaction (PCR) single-stranded polymorphism, and who was at least A, B, and DRβ1 compatible with the recipient. At each center, all patients and

donors had to sign a written informed consent, and the protocol was approved by the Local Ethical Committees. Patients were treated according to different regimens for reduced-intensity HCT (Table 2). The conditioning regimens were total-body irradiation (TBI) 2 Gy/Fludarabine (25 mg/mg days −3, −2, and −1), Cyclophosphamyde (30 mg/kg/day days −4 and −3)/Fludarabine (30 mg/mEq days −4 and −3), Thiotepa (5 mg/kg day −5)/Cyclophosphamyde (30 mg/kg/day days −4 and −3)/Fludarabine (30 mg/mEq days −4 and −3), Busulfan (4 mg/kg days −8 and −7)/Fludarabine (30 mg/mEq days −8, −7, −6, −5, −4, and −3) in 22, 15, 1, and 1 patients, respectively.

To prevent graft rejection and graft-versus-host disease (GVHD), post transplant immunosuppression consisted of the combination of cyclosporine (CSA) and mycophenolate mofetil (MMF) in TBI-based regimens and of the combination of CSA and methotrexate (MTX) short course in all the others; in 5 patients (4 unrelated, 1 related) as GVHD prophylaxis antithymocyte globulin (ATG) was also added at a dose of 1.5 mg/kg for 4 days before transplant.

In TBI regimens, CSA was started on day −3 and given at a dose of 6 mg/kg (oral) or 1.5 mg/kg (intravenously) every 12 hours. CSA levels were targeted at the upper therapeutic ranges (500 ng/L as defined by the fluorescence polarization method by Abbott TDX, Abbott Park, IL) in the first 28 days, maintained in normal ranges until day +56, and then tapered at 25% per week to be discontinued on day +90. MMF was started at a dose of 15 mg/kg (oral) every 12 hours on day 0 and stopped without tapering on day +27.

In the other regimens CSA (target blood levels, 150-300 ng/mL) and short-course methotrexate (MTX; 10 mg/msq day 1; 8 mg/msq days 3 and 6) were used; CSA was started on day −3, maintained in normal ranges until day +56, and then tapered at 25% per week to be discontinued on day +90. Tapering schedules were modified if GVHD developed and according to the disease status. All patients but 1 who was a 1 antigen mismatch were grafted with HLA identical sibling donors matched for classes I and II (A, B, C, DRβ1) with high-resolution molecular typing [26]. Patients transplanted using URD had 6 of 6 HLA loci in common. Donors were given 16 µg/kg daily of granulocyte-colony stimulating factor (G-CSF) subcutaneously for 4 to 5 days, after which peripheral blood stem cells were collected. After transplant, all patients received prophylaxis against bacterial, viral, fungal, and *Pneumocystis carinii* infection according to previously published protocols [27-30].

Chimerism, Treatment-Related Toxicities, GVHD, and Donor Lymphocyte Infusions

The degree of donor chimerism was assessed at days +30, +60, +90, +180, and +360 after

transplantation on circulating CD3$^+$ lymphocytes and CD 13$^+$ or CD 33$^+$ myeloid cells, as well as in some centers on bone marrow cells accordingly to previously published protocols. Mixed chimerism was defined as the presence of 1% to 95% donor CD3$^+$ cells, whereas complete chimerism was defined as >95% donor CD3$^+$ cells [31]. Treatment-related toxicities were graded according to the Common Toxicity Criteria of the National Cancer Institute 2.0 (http://ctep. cancer.gov/reporting/ctc-3.htlm). The severity of GVHD was graded according to the modified Seattle criteria [32]. Acute GVHD was treated with CSA if it occurred after discontinuation. If the patient was still on CSA, methylprednisolone i.v. or oral prednisone 1.0-2.0 mg/kg/day was started. Patients who had progressive disease after they had discontinued immunosuppression in the absence of severe GVHD (ie, grade III-IV) were eligible for a donor leukocyte infusion (DLI). T lymphocytes (CD3$^+$) were administered in escalating doses starting with the 1×10^6 CD3$^+$ cells/kg, followed by 1×10^7 CD3$^+$ cells/kg 30 days later and $5-10 \times 10^7$ CD3$^+$ cells/kg 30 days later if no response or GVHD occurs. In 2 patients, DLI was depleted of CD8$^+$ cells, and in another case, the patient received infusions of CD3$^+$/CD 56$^+$ cells according to a single institution protocol. The preocedures of depletion and selection were performed by immunomagnetic labeling of cells followed by separation of the positive and negative fraction using an automated system (CliniMACS).

Endpoints and Assessment of Response

The following endpoints were assessed: achievement of a status of mixed chimerism that was defined as between 1% and 95% peripheral blood (PB) donor CD3$^+$ cells, incidence of acute GVHD (aGVHD) and chronic GVHD (cGHVD), treatment-related mortality (TRM) and toxicities, tumor response, overall survival (OS), and, in responding patients, time to treatment failure.

Tumor response was scored according to the international RECIST criteria [33]. Tumor size was assessed by a spiral computed tomography (CT) of the brain, chest, and abdomen at days +30, +60, +90, +180, and +365, or when clinically indicated. To be considered responsive, a patient had to fulfill criteria of tumor-size changes that define complete response (CR), partial response (PR) or stable disease (SD) compared to base-line CT measurement.

Statistical Analysis

Proportions were compared between groups with Fisher's exact test. To estimate the association between some potential predictors of success and the response after transplant a logistic regression model was used. OS was estimated by the Kaplan-Meier method from the date of transplant until the date of death (because of any cause). The Log-rank test was used to compare the survival of subgroups of patients, stratified according to some prognostic factors. Adjusted hazard ratios (HR) and a corresponding 95% confidence interval (CI) for OS were estimated with the Cox proportional hazards model. The cumulative incidence of GVHD during the first 100 days after transplant was estimated with the Gooley method, taking into account mortality from any cause as a competitive risk [34]. Analyses were conducted by SAS 8.2 (SAS Institute, Cary, NC) and by R 2.1.0, package "cmprsk."

RESULTS

Patients Characteristics

The characteristics of the 39 patients are given in Table 1. The pretransplant status was PD in 31 patients (80%), SD in 6 (15%), and PR in 2 (5%) cases. Thirty-eight patients (97%) had been previously treated: 23 (58%) only with chemotherapy regimens containing 5-fluorouracil (5FU), oxaliplatin, and irinotecan, 15 (38%) with surgery and/or chemotherapy. Among previously treated patients, 13 (33%) patients were treated with 1 line, 24 (62%) with 2 or more lines of therapy (Table 1). In regard to the 2 patients who were nor treated before transplant, 1 was not considered eligible to chemotherapy by an oncologist because of a severe vascular disease, and the other patient was treated only with hepatic surgery and then referred to the transplant center by a local oncologist.

Engraftment, Chimerism, and Transplant-Related Toxicity

In 1 case, bone marrow represented the only source of the graft; otherwise, patients were reinfused with donor PB stem cells. The patients received a median of 7.65×10^6 (2.5-55) CD34$^+$ cells/kg and a median of 3.86×10^8 (0.11-33.7) CD3$^+$ cells/kg for a total of 7.9×10^8 (2.4-17.6) mononucleated cells/kg. After transplant, all patients had a hematologic recovery with a median absolute neutrophil count nadir (ANC) of 640 (0-15,050)/μL. The median platelet nadir was 91,000 (4000-191,000) / μL (Table 2).

Median chimerism on CD3$^+$ cells at days +30, +60, +90, and +180 was 70% (range: 20-90), 90% (30-100), 90% (7-100), 99% (0-100), respectively. All patients surviving more than 365 days (11 [28%]) had complete chimerism. An inversion of donor chimerism was observed in 2 patients at days +124 and +220. The first patient was successfully retransplanted from the same donor, whereas the second was treated with 2 courses of DLI but the chimerism level did not improve and the patient later died for progressing

Table 1. Patient Characteristics

Characteristics	No.	%
No. of patients	39	
Sex		
Male	25	64
Female	14	36
Age at transplant, years		
Median	55	
Range	3-76	
Time from		
diagnosis to transplant, months		
Median	16	
Range	4-47	
Status pretransplant		
PR	2	5
SD	6	15
PD	31	80
No lines CHT pretransplant		
Median	2	
Range	0-4	
Most frequently		
used chemotherapy agents		
Oxaliplatin	24	61
5-Fluoro-uracil	37	94
Irinotecan	21	53

CHT indicates chemotherapy; PR, partial response; SD, stable disease; PD, progressive disease.
High Tumor Burden was defined by the presence of at least one of the following conditions: (1) >5 liver metastasis with the largest more than 5 cm in diameter or a single metastasis more than 10 cm in diameter; (2) lung metastasis >5 cm in diameter; (3) lymphoadenopathy >5 cm in diameter; (4) peritoneal carcinosis.

disease. New onsets of alopecia, diarrhea, and veno-occlusive diseases were not observed. Only 2 patients developed grade 3 nonhematologic toxicities (liver and renal, respectively), whereas no grade 4 toxicity was registered. Cytomegalovirus (CMV) reactivation

Table 2. Characteristics of Allogeneic HCT

Characteristics	N°	%
HLA-sibling	35	89
MUD	4	10
Conditioning regimen		
Seattle	22	56
Fludara + CTX	11	28
Thymoglobuline + CTX + Fludara	4	10
Thiotepa+Fludara+CTX	1	3
Bus + Fludara + ATG	1	3
CD 34+cells infused, $10^{\wedge 6}$/g		
Median	7.65	
Range	2.5-55	
CD 3+cells infused, $10^{\wedge 8}$/g		
Median	3.8	
Range	0.11-33	
MNC infused, $10^{\wedge 8}$/kg		
Median	7.9	
Range	2.4-17.65	

MUD indicates matched unrelated donors; Seattle regimen, Fludarabine 30 mg/sm on days −4, −3, −2, +2 Gy of total body irradiation on day 0; Fludara + CTX, Fludarabine 25 mg/sm × 5 days + Cyclophosphamide 60 mg/kg; Thymoglobuline + CTX + Fludara, Thymoglobuline 2.5 mg/kg/day; Fludarabine 25 mg/sm × 5 days + Cyclophosphamyde 60 mg/kg; Thiotepa + Fludara + CTX = Thiotepa 5 mg/kg, Fludarabine 50 mg/sm, Cyclophosphamide 60 mg/kg; Bus + Fludara + ATG, Busulfan 1 mg/kg/day × 2 days; Fludarabine 30 mg/mEq × 6 days mg + antithymocyte globulin 2.5 mg/kg/day × 4 days; MNC, mononuclear cells.

occurred in 21 (54%) patients between days +45 and +150. All cases were successfully treated with preemptive antiviral regimens.

Immunosuppression Taper, GVHD, and DLI

Immunosuppression was discontinued at a median 92 days (range: 9-447) after transplant. Immunosuppression tapering was started earlier than was planned according to protocol in 14 patients (35%) because of disease progression. Acute GVHD grades I-IV occurred in 18 patients (46%) at a median time of 50 (10-92) days after transplant and was grade I in 5 (13%) and grade II-IV in 14 patients (35%).

The gastrointestinal system and the skin were involved in 12 patients (31%), whereas the liver was affected in 5 (13%) patients. Chronic GVHD that developed in 9 patients (23%) was progressive in 5 patients, and de novo in 4. Treating mortality as a competitive risk, the estimated cumulative incidence of aGVHD during the first 100 days after transplant reached a probability of 51.4% (95% CI = 34.4%; 68.4%) (Figure 1).

Sixteen patients (41%) received DLI between days 175 and 288 because of disease progression (14 cases), disease progression with chimerism loss (1 case), and graft rejection (1 case). The median number of DLI in each patient was 2 (1-8). Acute GVHD after DLI developed in 4 of 16 patients (25%). The 3 patients who received CD 8-depleted or $CD3^+/CD 56^+$-selected DLI did not experience any toxicities; the patient who received $CD3^+/CD 56^+$-selected DLI achieved PR, 1 of the 2 patients reinfused with CD 8-depleted had SD, whereas the other 1 progressed.

Clinical Response, Survival, and Causes of Death

Following transplantation, 1 already reported [35] patient (2%), experienced CR, 7 (18%) had PR, 10 (26%) had SD, giving a total of disease control in 46% of the cohort. In the responding patients, the median time to response onset was +90 (30-365) days, and the median time to treatment failure was 150 (60-335) days. Response was achieved either by patients in PD (n = 31) or by patients with disease control (n = 8) at transplant; precisely, compared to pretransplant disease status, 13 of 39 patients showed an improvement of their disease after transplant, 23 were stable, and 3 worsened (Table 3).

In regard to the chimerism status, responses were achieved both in patients with full chimerism (9 of 17, 52%) or in patients with mixed chimerism (8 of 17, 48%); in all but 2 of mixed-chimera patients, tumor responses were preceded by the achievement of a high percentage of donor chimerism (80%-90%). As far as the conditioning regimen used is concerned, responses were observed in 9 of 17 (52%) patients transplanted with a TBI-based regimen and in 8 of 17 (48%) patients
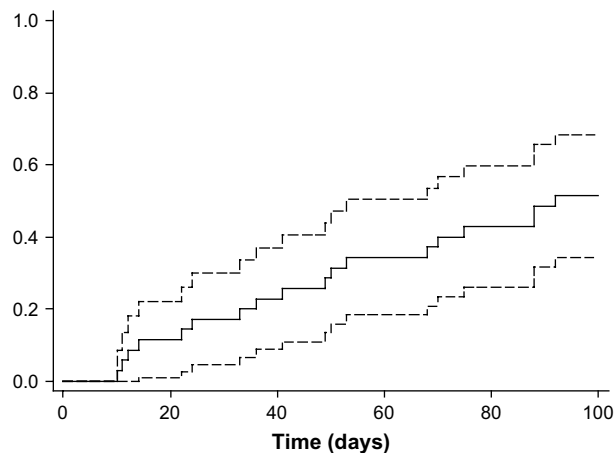
Figure 1. Cumulative incidence of aGVHD. Cumulative incidence (with 95% confidence bounds) of aGVHD during the first 100 days after transplant (estimated with the Gooley method, accounting for mortality as a competing risk).

treated with a Cyclophosphamyde/Fludarabine-based regimen. The median time for onset of response was 90 days (48-209) and 120 days (30-365), respectively.

Of the patients treated with DLI, 4 of 16 (25%) achieved a tumor-response (all cases PR).

In half of the responding patients (9 of 18) tumor-response followed an initial progression; 5 patients (1 PR and 4 SD) experienced response only tapering immunosuppression, whereas 4 patients achieved the response (PR) after DLI.

The results of explorative analyses on the role of some potential prognostic factors of response after transplant are shown in Table 5. Disease control (CR, PR, SD) was achieved in 13 of 22 (59.1%) patients experiencing any form of GVHD (10 with acute and 3 with chronic) and in 5 of 17 (29.4%) of those without GVHD (OR = 2.62, P = .195). In all the patients with aGVHD who experienced a tumor response the onset of GVHD preceded tumor response. A reduced number of previous lines of chemotherapy also seems to be associated with a higher probability of disease control, but all these results are statistically unstable because of small numbers.

After a median overall follow-up of 202 days (range: 6-1020), 6 patients were still alive and 33 had died. Progression was the cause of death in 29 patients (74%). None died of transplant-related complications

Table 3. Disease status at transplant and response

Disease Status at Transplant	Best Response to Transplant				
	CR	PR	SD	PD	Tot
PR	0	1	1	0	2
SD	0	1	3	2	6
PD	1	5	6	19	31
Tot	1	7	10	21	39

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

before day +100; 4 died after day +100. One patient (3%) died of GVHD and 3 (7%) of infections.

A comparison of OS of patients stratified by some potential prognostic factors (number of metastatic sites, number of previous CHT lines, disease status at transplant, and development of GVHD) are plotted in Figure 2. In a Cox proportional hazard model including all these factors, the number of previous chemotherapy lines (1-2 versus 3 or more) was the strongest predictor of survival (HR = 0.49; 95% CI = 0.23-1.05; P = .066). Response after transplant, was not included in this analysis. However, it is of interest that the achievement of response is associated with a better outcome showing a survival advantage for responders (Figures 2a-f).

DISCUSSION

The disease control rate (46%) achieved in this multicenter trial, with an approach of allo-based adoptive cell therapy is noteworthy for resistant mCRC. The absence of any antitumoral activity of the drugs used in the conditioning regimens of transplants, the delayed onset on the responses, and the high percentage of donor-chimerism observed in the responding patients suggest that the responses observed are entirely because of a cellular mechanism.

In light of these data, the present analysis confirms the feasibility of allogeneic HCT using RIC in mCRC and presents clinical evidence for the existence of a graft-versus-CRC effect as a consequence of that procedure, raising the intriguing possibility that along with molecular therapies, an immunological allo-based strategy may also be explored in the cure of mCRC [24,25,35,36].

Despite these results, the interest of the oncologic community to explore this field seems low. The 2 major criticisms substantiating this skepticism are that morbidity related to the procedure is still high, and that there is lack of clear clinical benefits originated by the transplant. In respect to the first criticism, the introduction of reduced-intensity regimens led to a significant reduction of TRM over the last 5 years [37], and a deeper knowledge of immunologic mechanisms that act in allogeneic HCT might soon translate in a further reduction of the TRM [37,38]. In this setting, the Stanford group recently achieved an impressive low incidence of aGVHD and TRM without hampering the GVT effect through a conditioning regimen based on total lymphoid irradiation. In the near future, the introduction of new methods and agents for the prediction, early diagnosis and treatment of GVHD and infections will lead to a further reduction of the toxicity [39-42].

As far as the second criticism is concerned, the absence of a clear clinical benefit is mostly because of

Table 4. Patient's post transplant outcome

ID	Previous therapies	Status at transplant	Conditioning regimen	Engrafment	Chimerism+60 CD3+	GVHD prophylaxis	GVHD grade	DLI	Survival	Cause of death
1	No	PD	2Gy+FLU	Yes	100	CSA + MMF	II	No	122	Infection
2	Liver surgery	PD	2Gy+FLU	Yes	90	CSA + MMF	II	Yes	194	PD
3	Nordic FLV	PD	2Gy + FLU	Yes	80	CSA + MMF	0	Yes	330	PD
4	Liver surgery, RF-liver	PD	2Gy+FLU	Yes	100	CSA + MMF	Chronic	Yes	528	PD
5	Nordic FLV, CPT- 11, Ra-imm.therapy	SD	2Gy+FLU	Yes	90	CSA + MMF	I/Chronic	No	222	PD
6	Nordic FLV, CPT-11/Oxyplatin	SD	2Gy+FLU	Yes	30	CSA + MMF	III	Yes	113	Infection
7	FOLFOX, liver surgery, RF-liver	PR	CY + FLU	Yes	95	CSA+MTx	0	Yes	1063	Infection
8	Liver surgery, RF-liver	PD	CY+FLU	Yes	90	CSA + MMF	I/Chronic	Yes	620	PD
9	Liver surgery	SD	Thymo + CY +FLU	Yes	100	CSA+MTx	II	Yes	330	PD
10	FOLFIRI	SD	CY+FLU	Yes	95	CSA+MTx	I/Chronic	Yes	447	
11	FOLFIRI	PD	CY+FLU	Yes	80	CSA+MTx	III	Yes	376	
12	FOLFIRI, FOLFOX, Xeloda	PD	Thymo + CY + FLU	Yes	100	CSA+MTx	III	No	157	PD
13	FOLFIRI, oxyplatin, CPT-11/Xeloda	PD	Thymo + CY +FLU	Yes	100	CSA+MTx	0	Yes	140	PD
14	FLV, FOLFIRI	PD	CY + FLU	Yes	80	CSA+MTx	II	Yes	328	
15	FOLFIRI	PD	Thymo + CY +FLU	Yes	100	CSA+MTx	I	No	210	
16	De Gramont, Xeloda	PD	CY+FLU	Yes	80	CSA	0	No	30	PD
17	FOLFIRI, FOLFOX, Xeloda	PD	2Gy+FLU	Yes	80	CSA + MMF	0	No	44	PD
18	FOLFOX	PD	2Gy+FLU	Yes	97	CSA + MMF	Chronic	No	200	PD
19	FOLFOX	PD	2Gy+FLU	Yes	65	CSA + MMF	0	No	143	PD
20	FOLFOX	PD	2Gy+FLU	Yes	59	CSA + MMF	0	No	145	PD
21	FOLFOX, liver surgery, FOLFOX	PD	2Gy+FLU	Yes	74	CSA + MMF	Chronic	No	361	PD
22	5FU/LV, Tomudex, CPT-11	PD	2Gy+FLU	Yes	70	CSA + MMF	II	Yes	379	PD
23	FOLFOX, Liver surgery, FOLFIRI	PD	2Gy+FLU	Yes	50	CSA + MMF	0	No	123	PD
24	FOLFOX, FOLFIRI	SD	2Gy+FLU	Yes	80	CSA + MMF	IV	No	202	GVHD
25	FOLFOX, liver surgery	PD	2Gy+FLU	Yes	55	CSA + MMF	0	Yes	510	
26	FOLFOX, liver surgery	PD	2Gy+FLU	Yes	60	CSA + MMF	Chronic	No	227	PD
27	FOLFOX	PD	2Gy+FLU	Yes	52	CSA + MMF	0	Yes	330	PD
28	FOLFIRI	SD	2Gy+FLU	Yes	90	CSA + MMF	III	No	501	PD
29	Liver surgery, FOLFIRI, FOLFOX	PD	2Gy+FLU	Yes	50	CSA + MMF	0	No	137	PD
30	FOLFOX	PD	2Gy+FLU	Yes	95	CSA + MMF	0	Yes	90	PD
31	FOLFOX, CPT 11, Xeloda	PD	2Gy+FLU	Yes	90	CSA + MMF	0	No	609	PD
32	Liver surgery, FOLFOX, CPT 11, Tomudex	PD	2Gy+FLU	Yes	100	CSA + MMF	III	No	90	PD
33	LV/5FU	PR	BUS+FLU+ATG	Yes	80	CSA	II	No	450	PD
34	Liver surgery, FOLFOX, Intrahepatic CT	PD	FLU+CTX	Yes	50	CSA+MTx	0	No	165	PD
35	Liver surgery, LV/5FU + carboplatin, FOLFOX, FOLFIRI	PD	FLU+CTX	Yes	65	CSA+MTx	0	No	285	PD
36	LV/5FU, FOLFOX, CPT 11	PD	FLU+CTX	Yes	70	CSA + MMF	I/Chronic	Yes	155	PD
37	De Gramont, FOLFOX, FOLFIRI	PD	FLU+CTX	Yes	75	CSA+MTx	0	No	98	PD
38	Liver surgery, FOLFOX	PD	FLU+CTX	Yes	90	CSA+Basiliximab	II/Chronic	No	977	PD
39	Liver surgery, 5-FU, FOLFOX, FOLFIRI	PD	THIOTEPA+FLU+CTX	Yes	100	MMF+MPDN	I	No	143	PD

CSA indicates Cyclosporine; MMF, mycophenolate acid.
FOLFIRI = Irinotecan 180 mg/sm; Fluorouracil 400 mg/meEq; Fluorouracil 600 mg/sm; Leucovorin 200 mg/sm; FOLFOX = Oxaliplatin 100 mg/sm; Fluorouracil 400 mg/sm; Fluorouracil 600 mg/sm ; Leucovorin 200 mg/sm; De Gramont = Fluorouracil 400 mg/sm ; Fluorouracil 600 mg/sm; Leucovorin 200 mg/sm; Tomudex = 4 mg/sm; CPT11 = Irinotecan 180 mg/sm.

the difficulty of separating GVT from GVHD. Until recently, our understanding of the mechanism underlying the GVT effect was limited, and the reaction was extremely unpredictable and nonspecific [43]. However, at present, the possibility of generating a specific GVT effect has been demonstrated in different malignancies [44,45]. It has recently been shown that in mCRC-infused donor lymphocytes not only target metastatic sites but also that donor T cells specific to a well-characterized tumor-associated antigen (TAA) are generated in vivo as a consequence of the transplant procedure [25,46].

Table 5. Association between Some Possible Prognostic Factors and Response after Transplant*

Factors	OR	95%CI	P
GVDH	2.62	0.61 - 11.28	.195
Lines of previous chemotherapy (1-2 versus >3)	3.10	0.69 - 14.03	.141
No. of metastatic sites (3-4 versus 1-2)	1.22	0.25 - 5.92	.808
Disease status at transplant (PD versus SD or PR)	0.36	0.05 - 2.64	.318

CI indicates confidence interval; OR, odds ratio; SD, stable disease; PR, partial response.
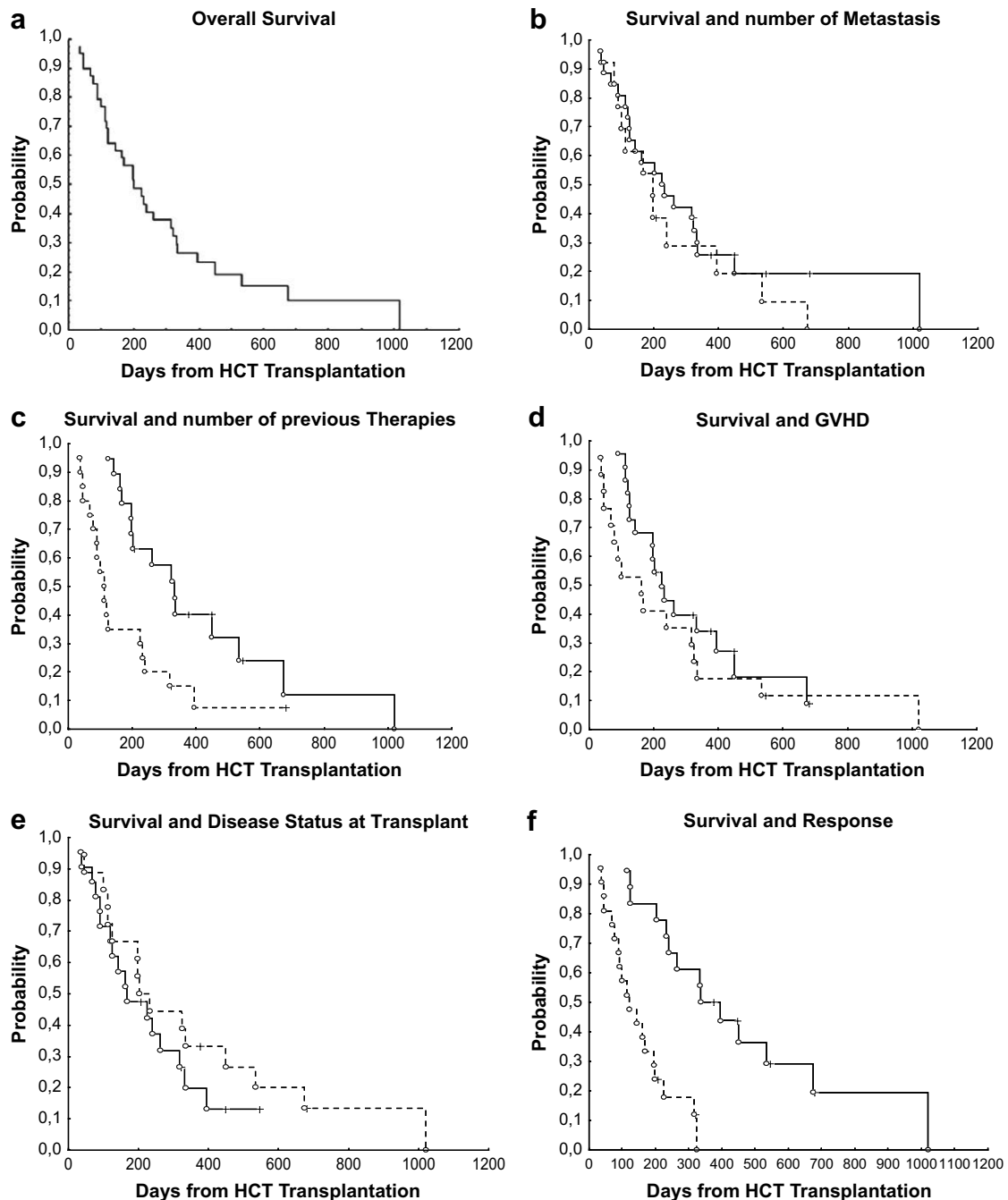*Multiple logistic regression estimates, adjusted for all the variables listed in the table.

Figure 2. OS of patients with mCRC treated with a reduced-intensity regimen and allogeneic HCT. (a) OS calculated in days from HCT of the 39 patients treated. (b) OS of patients with <3 metastatic sites before HCT (n = 26 solid line) and with >3 metastatic sites (n = 13, dotted line). Log rank test *P* =.39890. (c) OS of patients who were transplanted after 0-1 previous lines of therapies (n = 15, solid line) and who were transplanted after 2 or more previous lines of therapies (n = 24, dotted line). Log rank test *P* =.02953. (d) OS of patients who developed GVHD (n = 22, solid line) and who did not develop aGVHD after HCT (n = 17, dotted line). Log rank test *P* =.02862. (e) OS of patients who were transplanted in a status of disease progression (n = 31, solid line), and those who were transplanted in a disease control status (PR or SD; n = 8, dotted line). Log rank test *P* =.15666. (f) OS of patients who had a response (n = 18, solid line) and who had no response after HCT (n = 21, dotted line). Log rank test *P* = .00018.

Clearly, the result of the present report, similar to those of allogeneic HCT in renal cell carcinoma, breast cancer, and ovarian cancer, must be taken cautiously and do not have to be overemphasized. The small number of patients treated, the still high incidence of GVHD reported, and finally the low rate (20%) of short-lasting responses are objective limitations of the study. However, some of these limitations are not substantial for the following reasons: first, many of the new medical strategies are based on retrospective and small-sized studies if not directly only on case reports [47-50]; second, the incidence of aGVHD, far from being optimal, is similar to that reported in other malignancies in which allogeneic

HCT is routinely employed [51]. Finally, as far as the low reponse rate is concerned, many drugs (gefitinib, erlotinib, bevacizumab, cetuximab, sorafenib) have recently been considered active in different tumors even without the achievement of a high level of tumor regression using traditional Recist criteria [52-55]. If that is true for molecular-targeted therapy, it is even more correct for an immunologic therapy.

Thus, if the data suggest that it would be premature to abandon this field of investigation and second-generation clinical trials of allo-based adoptive cell therapy in less advanced patients should be pursued, what is the next step to be taken [38]?

A first consideration to be made regarding those patients who might be candidates to this approach. Once feasibility has been demonstrated, patients progressing should no longer be referred for this approach. In many hematologic malignancies a disease that is at least stable and has a low tumor burden represents an ideal target to study reduced intensity HCT [56]; the same rules are also valid in solid tumors. In mCRC, patients matching these conditions are probably those who had a partial response or a stable disease after second-line therapy. Second-generation clinical trials of allo-based cell therapy must focus on this population. Together with a selection of patients, every effort to generate safely a specific graft-versus-CRC effect has to be pursued. The introduction of a post-transplant vaccination as well as the infusion of expanded tumor-specific donor lymphocytes represent 2 potential strategies that may specifically enhance the specificity and effectiveness of allogeneic cell therapy. It is also important to remark that in the era of molecular therapies the aim of this new immunologic approach is not to substitute available treatments but to integrate them, to improve the response rates of patients affected by metastatic CRC and, in the future, hopefully achieve long-lasting remissions.

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