



ARTICLE

Evaluation of infliximab as second-line treatment of acute graft versus host disease -validating response on day 7 and 28 as predictors of survival

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Abstract

Several immunosuppressive drugs have been proposed for second-line treatment of steroid-refractory acute graft versus host disease (aGvHD) after allogeneic hematopoietic stem cell transplantation. However, the studies on these drugs are small, retrospective, uncontrolled and use different endpoints. Therefore, it remains unknown which treatment is superior. We retrospectively evaluated 68 consecutive patients treated with infliximab for aGvHD. We adhered to recently proposed guidelines for aGvHD trials and thus evaluated response on day 7 and 28. Furthermore, we assessed the composite endpoint 6 months freedom from treatment failure (6MFTF). The majority of patients had grade III-IV aGvHD. We found that 41 patients (60%) responded on day 7 and 31 patients (46%) on day 28. Twenty-four patients (35%) achieved 6MFTF. The main reasons for failure within 6 months were death ($n = 31$) or additional immunosuppression ($n = 16$). By six and 24 months, 44 and 34% of the patients were alive respectively. Patients with response to infliximab on day 7 and 28 had significantly higher overall survival (OS) probability than non-responders. We show that response on day 7 and 28 identifies high and low risk groups. Patients who fail to respond should be identified early and offered alternative therapy.

Introduction

Acute Graft versus Host Disease (aGvHD) is a common and severe complication to hematopoietic stem cell transplantation (HSCT). There is consensus that first-line treatment is 1–2 mg/kg glucocorticoid; however for steroid-refractory patients, it remains unknown which treatment option is superior [1].

Several second-line therapies have been proposed; most of which add further immunosuppression and thus increase risk of infection and relapse. Most studies show some effect

of the studied second-line agent, but the prognosis remains poor with only approximately 50% of patients surviving 6 months [1].

Comparison of studies is complicated by the small number of studied patients, lack of control groups, inconsistent treatment schedules and different criteria for response assessment [2]. Recent guidelines for response assessment in aGvHD studies recommend evaluating treatment response at fixed timepoints [3, 4]. Furthermore, a composite endpoint, 6 months freedom from treatment failure (6MFTF), is increasingly used [5, 6].

In our center, we have used the tumor necrosis factor α (TNF- α) inhibitor infliximab as second-or further-line treatment for aGvHD since the year 2000. TNF- α is significantly increased in patients with aGvHD [7], and inhibition of TNF- α may potentially control aGvHD [8]. Inhibition of TNF- α has been suggested in all phases of aGvHD treatment; as prevention [9, 10], as part of primary aGvHD treatment [11–15], and most commonly as treatment for steroid-refractory or steroid-dependent aGvHD [16–25].

In this retrospective evaluation, we adhere to the recently proposed guidelines for response assessment. We intend to supplement earlier evidence of the effect of infliximab as

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second-line-treatment and to validate if response on day 7 and 28 after second-or-further line treatment is predictive of survival as proposed in above referred guidelines. To our knowledge, this is the largest study on patients with mainly severe steroid-resistant aGvHD treated with infliximab. Hopefully this will further elucidate the role of infliximab in the treatment of aGvHD.

Patients and methods

A single investigator (MN) reviewed the records of all patients treated with infliximab between 01-01-2000 and 17-06-2014. All patients gave informed consent for use of data and the study was conducted according to the directions of the regional Ethics Committee. Myeloablative conditioning regimens were total body irradiation (TBI) 12 Gy with cyclophosphamide 120 mg/kg or etoposide phosphate 1800 mg/m² or busulphane 12.8 mg/kg plus cyclophosphamide 120 mg/kg. Non-myeloablative regimens were TBI 200 cGy (or in a few cases 300 or 400 cGy) combined with fludarabine 90 mg/m² or fludarabine 90 mg/m² without TBI. Graft versus host disease prophylaxis was cyclosporine A (CsA) with short-course methotrexate in myeloablative HSCT and tacrolimus (TAC) or CsA and mycophenolate mofetil (MMF) in non-myeloablative HSCT. Donors were 10/10 or 9/10 human leucocyte antigen (HLA) identical siblings or HLA-matched unrelated donors; except for two with single antigen mismatch.

Acute GVHD diagnosis

Both classical aGvHD, occurring within 100 days after HSCT, and persistent or late-onset aGvHD were included. Gastro-intestinal GvHD was diagnosed by histological examination of a sigmoidum biopsy. Skin GVHD was diagnosed by presence of a typical maculopapular rash. Liver GVHD was diagnosed by increase in serum bilirubin without other possible causes. Grading of aGvHD was assessed by standard criteria [26].

Insufficient response to standard high-dose (1–2 mg/kg) glucocorticoid treatment was defined as aggressive progression of de novo aGvHD grade within 3 days, no response after 1 week of therapy or inability to taper glucocorticoid dose after an initial response. If glucocorticoids had been tapered below 0.5 mg/kg before reoccurrence of aGvHD, the day of increased glucocorticoid dose was considered the start of the current aGvHD episode.

Response assessment

Baseline for aGvHD grading score was the day infliximab was started. Overall response of aGvHD was assessed on day

7 and 28 after start of infliximab treatment. Complete response (CR) was defined as resolution of all indicators of aGvHD, very good partial response (VGPR) was an approximation of CR as described by Martin et al. [4]. Partial response (PR) was improvement of one or more grades in any affected organ without progression or de novo aGvHD in other organs. If progression was noted in any organ or additional treatment was administered, patients were classified as having progressive disease (PD). Change in immunosuppression due to side effects was not considered PD. Both substitution and addition of immunosuppression were at the discretion of the treating physician. Stable disease (SD) was unaltered grading in all affected organs.

On day 7, any response (CR, VGPR or PR) was considered significant treatment effect. On day 28 only CR and VGPR were considered significant treatment effect. We also evaluated 6MFTF where failure was defined as death, relapse of malignancy or need for additional systemic immunosuppression within 6 months [5, 6].

Treatment with infliximab

Infliximab was used off label for steroid-refractory or steroid-dependent aGvHD as allowed by the national health authorities. No patient was excluded from start of infliximab treatment due to poor performance status, infection or financial issues. Standard dosage of infliximab was 10 mg/kg once weekly, but if the patient had PD, an intensified schedule with two doses weekly could be used. Unless severe infections or PD occurred, infliximab was continued until the patient had CR/VGPR.

Infections

All patients received posaconazole or liposomal Amphotericin B, acyclovir or valaciclovir, and co-trimoxazole or dapsone. If neutrophil count was below $0.5 \times 10^9/L$, ceftazidime or meropenem was administered. When body temperature exceeded 38 °C, blood, urine and sputum cultures were taken followed by preemptive antibiotic administration. Quantitative PCR analysis for CMV in blood was performed weekly, and if positive, valganciclovir, ganciclovir or foscarnet replaced acyclovir/valaciclovir. Verified bacterial, fungal or viral infections were recorded for 6 months after start of infliximab. Occurrence of severe or life threatening/fatal infections was registered according to BMT CTN Data Coordinating center's definition of infection severity (https://web.emmes.com/study/bmt2/public/Definition/Definitions_of_Inf_Severity.pdf).

Statistics

Association of baseline variables to day 7 and 28 response and 6MFTF was analyzed using the χ^2 test or Fishers exact

Table 1 Patient characteristics

Characteristics	N	%
Age, mean (range)	50.2	(25–73)
Sex		
Male	44	65
Female	24	35
Diagnosis		
Acute myeloid leukemia	18	26
MDS	16	24
Acute lymphoid leukemia	8	12
Chronic lymphocytic leukemia	8	12
Chronic myeloid leukemia	4	6
Hodgkins lymphoma	3	4
Non-Hodgkins lymphoma	6	9
Multiple myeloma	3	4
Severe aplastic anemia	1	1
Prolymphocyte leukemia	1	1
Remission status at HCT		
In remission	47	69
Not in remission	21	31
Conditioning		
Myeloablative	22	32
Non-myeloablative	46	68
Donorrelation		
Related	34	50
Unrelated	34	50
Cell source		
Peripheral blood	54	79
Bone marrow	14	21
Recipient/donor sex		
Female/male	11	20
Female/female	9	16
Male/female	9	16
Male/male	26	47
GVHD prophylaxis (at time of aGVHD diagnosis)		
CsA	21	31
TCR	11	16
CsA + MMF	15	22
TCR + MMF	17	25
MMF	4	6
Overall GVHD grade		
Grade II	17	25
Grade III	33	49
Grade IV	18	26
Organ involvement of aGVHD		
Gastrointestinal only	30	44
Gastrointestinal + liver	11	16
Gastrointestinal + skin	10	15
Gastrointestinal + liver + skin	1	2

Table 1 (continued)

Characteristics	N	%
Skin only	15	22
Liver only	1	2
Days from HCT/DLI to aGVHD onset. Median (range)	48	(14–337)
Days from steroid to infliximab. Median (range)	6	(1–125)
Number of infliximab doses at day 28. Median (range)	3	(1–9)
Total number of infliximab doses. Median (range)	4	(1–13)

test for categorical variables and Mann–Whitney's test for continuous variables. Overall survival (OS) probability was estimated by the Kaplan–Meier method with comparison done by the log rank test. Survival probability was estimated with start of infliximab therapy as day 0. When using day 7 and day 28 as landmarks for impact on survival, only patients alive at that time point were included in the analysis. Statistical tests were two-tailed and a p -value < 0.05 was considered statistically significant. The SPSS[®] software version 22 was used for all statistical analysis.

Results

Between 01-01-2000 and 17-06-2014, a total of 938 patients received an allogeneic HSCT at our center and of these, 315 (35.8%) developed grade II–IV aGVHD. Sixty-eight patients (7.2%) received infliximab as second-or-further-line treatment of aGVHD. Patient and treatment characteristics of these patients are shown in Table 1. Three patients developed aGVHD after donor lymphocyte infusion, six patients after a second transplant.

Treatment of aGVHD

At initial diagnosis of aGVHD, 66 patients received 2 mg/kg methylprednisolone (MP) or prednisolone. Two patients started on lower doses but were increased to 2 mg/kg due to progression in aGVHD. When infliximab treatment was initiated, nine patients received less than 1 mg/kg glucocorticoid due to uncontrolled diabetes ($n = 2$), active CMV infection ($n = 1$) or tapering of prolonged steroid treatment ($n = 6$). Tapering of glucocorticoids had been attempted in 32 (47%) patients before aGVHD progression necessitated increase to 2 mg/kg again and thereafter infliximab.

At start of infliximab treatment, concomitant immunosuppressive medication was: CsA ($n = 21$), TAC ($n = 11$), TAC + MMF ($n = 17$), CsA + MMF ($n = 15$), MMF ($n = 3$), and MMF plus sirolimus ($n = 1$). Four patients received other aGVHD therapies prior to or at the same time

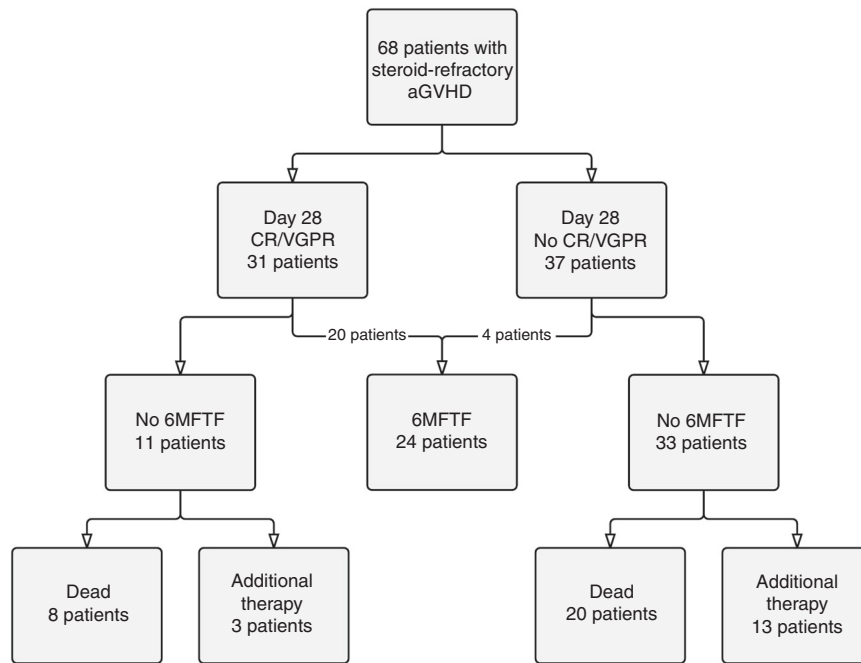


Fig. 1 Flowchart of the course of the patients according to response on day 28

as starting infliximab; antithymocyte globulin (ATG) ($n = 2$), extracorporeal photopheresis ($n = 1$) or ultraviolet phototherapy (UVA1) ($n = 1$).

Median of three infliximab doses (range 1–9) was administered within 28 days. Schedules for administration of infliximab were: standard weekly ($n = 24$), twice weekly ($n = 31$), twice weekly for a total of two doses ($n = 7$) or only one dose ($n = 6$). Infliximab was stopped before day 28 ($n = 53$) for the following reasons: improvement in aGvHD ($n = 29$), infection ($n = 6$), progression with need for additional treatment ($n = 8$) and death ($n = 10$).

Additional therapy before day 28 was MMF ($n = 4$), MMF + 1 g MP ($n = 1$), daclizumab + ATG ($n = 1$), rituximab ($n = 1$) or sirolimus ($n = 1$). Additions after day 28 were UVA1 ($n = 3$), MMF ($n = 2$), sirolimus ($n = 1$), daclizumab ($n = 1$), 1 g MP + sirolimus ($n = 1$). Eleven patients continued infliximab after addition of therapy, whereas infliximab had stopped prior to addition in five patients. Eight patients changed immunosuppression due to side effects, usually thrombotic microangiopathy or impaired kidney function.

Glucocorticoid dose was reduced by 50% in 45 patients after a median of 3 weeks (range 1–8). At 24 weeks, 30 evaluable patients received no glucocorticoid ($n = 13$), ≤ 10 mg daily ($n = 9$), and > 10 mg daily ($n = 8$). Overall, glucocorticoids were discontinued in 18 patients with 13 of those eventually free from all immunosuppressive medication.

Response

On day 7, CR, VGPR or PR was obtained in 41 patients (60%), and on day 28, CR or VGPR was obtained in 31 (46%) patients. When including PR in the day 28 response, we found a response rate of 66%. Twenty-four patients (35%) achieved 6MFTF, where the majority had CR/VGPR on day 28 ($n = 20$, 83%). Reasons for treatment failure within 6 months were death ($n = 31$) or additional immunosuppression ($n = 16$). See flow chart in Fig. 1. Of the eight patients with additional treatment before day 28, one had CR on day 28, five had PD and two had died.

The only variable significantly predictive of responses on day 7, day 28 and 6MFTF was grade of aGvHD at start of infliximab treatment (day 7 $p = 0.001$, day 28 $p = 0.005$ and 6MFTF $p = 0.002$). Analysis of other possible baseline predictors of response is shown in supplemental Table 1. For patients with gastrointestinal involvement, there was a significantly positive association with CR/VGPR on day 28 ($p = 0.014$), but not with other endpoints.

Survival

At six and 24 months, 44 and 34% of the patients were alive, respectively. The median observation time was 116 months (range 30.5–205). Patients with response on day 7 and 28 had significantly higher OS probability than non-responders with an OS rate after two years of 49 and 58%, respectively. Overall survival and OS according to

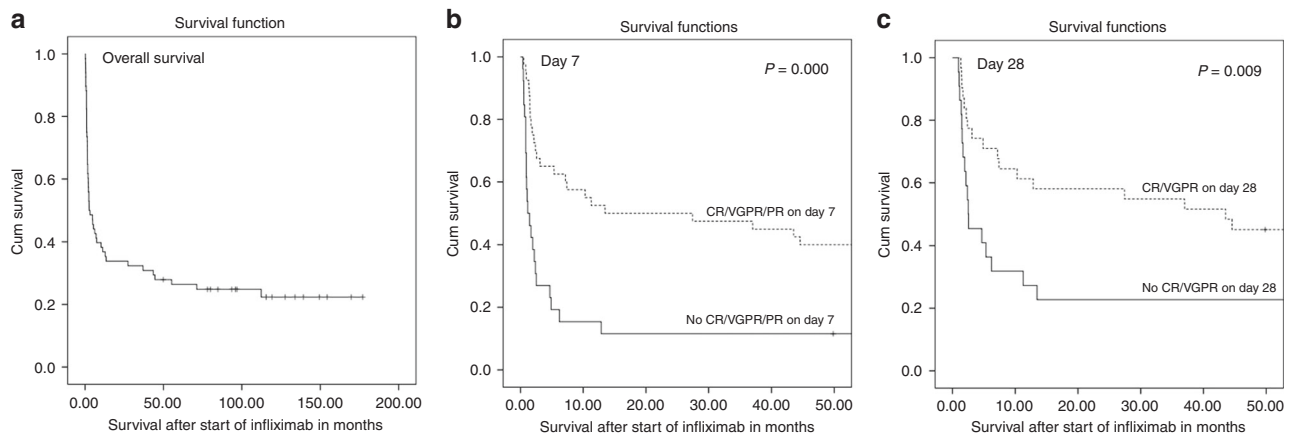


Fig. 2 Overall survival (a) and overall survival according to response status on day 7 (b) and day 28 (c)

Table 2 Causes of death

	N (%)
Organ failure caused by GVHD	20 (38.5)
Infections	17 (32.7)
Invasive fungal (N = 8)	
Viral (N = 4)	
Bacterial (N = 3)	
Unspecified (N = 2)	
Relapse	5 (9.6)
Multi-organ-failure	4 (7.7)
Other	6 (11.5)
Cerebral hemorrhage (N = 3)	
Cancer pharynx (N = 1)	
Myocardial infarction (N = 1)	
Hepatic failure of unknown cause (N = 1)	

response are shown in Fig. 2. Patients with no more than PR on day 28 had OS comparable to patients with PD on day 28 (data not shown). In univariate analysis, higher grade of aGvHD, unrelated donor, and no response on day 7 and 28 were associated with poor survival. See supplemental Table 2. Causes of death are shown in Table 2.

Infections

Infections were observed in 61 (90%) of the patients. Bacterial infections were seen in 45 (66%), viral infections in 36 (53%) and fungal infections in 35 (52%) patients. The microbiological agents are listed in Table 3. There were 90 infectious episodes classified as severe (mean = 1.3, range = 0–7) and 32 life-threatening or fatal (mean = 0.47, range = 0–2) in 53 patients. Number of severe or fatal infections pr. patient month were median 0.5 (range 0–5.36).

Chronic GVHD

Chronic GVHD occurred in 22 of the 34 patients who survived at least 3 months. Only four of the 16 patients still alive at the time of analysis were free from cGvHD. Median time to diagnosis of cGvHD from administration of infliximab was 30 weeks (range 16–108).

Discussion

In this study, we show that infliximab has effect in steroid-refractory or steroid-dependent aGvHD as it has also been shown previously. Patriarca et al. [20] reported on 32 patients with mainly severe aGvHD and found 19% of patients had CR and 40% PR on day 7. Couriel et al. [21] evaluated 21 patients with primarily grade II steroid-refractory aGvHD and found response in 67% on day 7. These day 7 responses are comparable to our day 7 CR/VGPR/PR rate of 60% in patients with mainly severe aGvHD.

Pidala et al. [23] reported that only 15% of the patients treated with infliximab achieved CR at median 6 days (range 2–26). They concluded that infliximab may be of limited value in grade III-IV aGvHD. This is contradicted by our findings where the majority of patients also have severe aGvHD. The reasons for the poor results reported by Pidala et al. are not clear, but may be due to less intensive treatment or early response assessment. The PR rates are not stated in the study.

As shown by Martin et al. [1] results on second-line treatments are variable, but with most agents demonstrating some activity. Overall CR/PR rate for 29 studies was 58% but this should be interpreted with caution because the timing and definition of response varies, and furthermore there are differences in patient populations. Of the included studies, five reported response on day 28 with CR

Table 3 Overview of the microbiological agents found in the patients during treatment with infliximab

Blood	N	Sputum/BAL*fluid	N	Feces/intestine	N	Urine	N
Gram-negative		Gram-negative		Gram-negative		Gram-negative	
<i>Eschericia coli</i>	8	<i>Pseudomonas aeruginosa</i>	5	<i>Pseudomonas aeruginosa</i>	1	<i>E.coli</i>	2
<i>Pseudomonas aeruginosa</i>	4	<i>Moraxhella catharalis</i>	3	<i>Aeromonas hydrophila</i>	1	<i>Klebsiella pneumoniae</i>	1
<i>Klebsiella pneumoniae</i>	3	<i>Eschericia coli</i>	1	Gram-positive		<i>Citrobacter freundii</i>	1
<i>Listeria monocytogenes</i>	2	<i>Branhamella catharalis</i>	1	<i>Clostridium difficile</i>	19	Gram-positive	
Gram-positive		<i>Haemophilus influenzae</i>	1	Yeast		<i>Enterococcus faecium</i>	3
<i>Enterococcus faecalis</i>	6	<i>Legionella pneumophila</i>	3	<i>Candida albicans</i>	6	<i>Enterococcus faecalis</i>	1
<i>Streptococcus pneumoniae</i>	2	<i>Pseudomonas putida</i>	1	<i>Candida parapsilosis</i>	3	Yeast	
<i>Micrococcus luteus</i>	1	Gram-positive		<i>Saccharamyces cerevisiae</i>	4	<i>Candida albicans</i>	1
<i>Staphylococcus epidermidis</i>	7	<i>Staphylococcus aureus</i>	4	<i>Candida glabrata</i>	2	<i>Candida glabrata</i>	3
<i>Staphylococcus aureus</i>	2	<i>Streptococcus pneumoniae</i>	2	<i>Candida krusei</i>	1	<i>Candida krusei</i>	1
<i>Staphylococcus haemolyticus</i>	1	<i>Enterococcus faecium</i>	3	Unclassified yeast	4	<i>Candida parapsilosis</i>	2
<i>Koagulase-negative coccus</i>	1	Gram-positive coccus	1	Virus		Unclassified yeast	1
<i>Non-hemolytic streptococcus</i>	1	Moulds		<i>Cytomegalovirus</i>	10	Virus	
Yeast		<i>Aspergillus fumigatus</i>	11	<i>Rotavirus</i>	1	<i>Polyomavirus</i>	5
<i>Candida albicans</i>	2	<i>Candida albicans</i>	2	<i>Adenovirus</i>	1		
<i>Candida parapsilosis</i>	1	<i>Candida glabrata</i>	2	<i>Norwalkvirus</i>	1		
<i>Candida glabrata</i>	3	Virus					
Virus		<i>Influenza A</i>	2				
<i>Cytomegalovirus</i>	17	<i>Respiratory syncytial virus</i>	2				
<i>Epstein Barr virus</i>	2	<i>Parainfluenza</i>	2				
		<i>Cytomegalovirus</i>	1				
		<i>Corona virus</i>	1				
		<i>Boca virus</i>	1				
		<i>Metapneumovirus</i>	1				
CNS		Skin		Liver			
Gram-negative		Gram-positive		Moulds			
<i>Listeria monocytogenes</i>	1	<i>Staphylococcus aureus</i>	1	<i>Aspergillus fumigatus</i>	1		
Moulds		Yeast					
<i>Aspergillus fumigatus</i>	2	<i>Trichosporon mucoides</i>	1				
Virus		Virus					
<i>Cytomegalovirus</i>	1	<i>Varicella zoster virus</i>	1				

*BAL broncho-alveolar lavage

proportions between 0.08–0.42 and only two reported response on day 7 with CR/PR proportions of 0.83 and 0.67.

None of the suggested therapies have proved superior to others. Therefore, we adhered to the recently proposed guidelines for response assessment in aGVHD [4, 27] to facilitate comparison both within studies on infliximab but also with other therapeutic options in the absence of large randomized, controlled studies.

Patients treated with second-line therapy for aGVHD have dismal prognoses with a 6 month survival estimate of 0.49 [1]. We confirm this finding with high early mortality with only 44% of the patients alive after 6 months.

Response assessment on day 7 and 28 has primarily been tested in first-line aGVHD treatment where especially response on day 28 is shown to correlate strongly with long-term endpoints including survival [27–29]. For second-line treatment, it is important to have an early predictor of treatment success such as response on day 7. With this study, we validate that both having CR, VGPR or PR on day 7 and having either CR or VGPR on day 28 are predictive of survival. We hypothesized that having only PR by day 28 after second-line therapy indicates insufficient treatment effect. This was confirmed as patients with PR on day 28 had OS comparable to patients with PD on day 28. Early identification of patients with a dismal prognosis is

important in aGvHD, because alternative therapy should be initiated as soon as possible.

We found low incidence of relapse, but infections were a substantial problem with 78% of patients having severe and/or fatal infections despite prophylactic antimicrobial medication. Marty et al. [30] previously reported increased incidence of non-Candida invasive fungal infections in patients treated with infliximab for aGvHD, which is in line with our observation of invasive *Aspergillus* infections. Carcía-Cadenas et al. [31] found 1-year incidence of bacterial, viral and fungal infections to be respectively 74, 65 and 14% after second-line treatment of aGvHD with anti-cytokine therapy. Whether patients treated with infliximab have additional, different, or more severe infections compared to other treatments, cannot be concluded from these data. Careful monitoring of the patients, including relevant sampling and clinical microbiological diagnosis as well as early, aggressive antibiotic treatment, is pivotal.

The results from the present study are limited by the retrospective nature, the heterogeneity of patients and their aGvHD-treatment. Before infliximab was initiated, the patients had been affected by aGvHD for variable periods of time and were on variable systemic immunosuppression. Both steroid-refractory and steroid-dependent patients were treated with infliximab and it is possible that treatment schedules and/or duration should be different for these different groups. The optimal treatment schedule for infliximab remains unknown.

Additional immunosuppression was administered to one fourth of the patients, and this could potentially account for responses or influence survival. However, it was the most severely affected patients who received additional therapy and we found that having additional therapy was associated with worse response on day 28 (seven of eight had PD or had died).

Conclusion

We validate, in the presently largest study on infliximab, that response on day 7 and 28 is able to identify high and low risk groups after second-or-further-line treatment. In patients with CR or VGPR on day 28, 58% were alive after two years, which is encouraging considering the majority of patients had grade III-IV aGvHD. Our results are in line with previously published reports on infliximab and other second-line therapies. Further research, preferably prospective and controlled with comparable endpoints, is warranted. Patients who fail to respond should be identified early and offered alternative therapies.

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Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

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