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Azacitidine for the treatment of steroid-refractory chronic graft-versus-host disease: The results of the Phase II AZTEC clinical trial

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Abstract

Chronic graft-versus-host disease (cGvHD) is a major cause of non-relapse morbidity and mortality following allogeneic stem cell transplant. Over half of patients with moderate or severe cGvHD fail to respond adequately to first-line treatment with systemic steroids, and although a range of second-line options have been employed, a lack of prospective evidence means there is no standard of care. The AZTEC trial is a prospective, single-arm, phase II study investigating the safety and activity of azacitidine for the treatment of cGvHD in patients who are resistant to, or intolerant of, systemic steroid therapy. The co-primary outcomes were treatment tolerability, and activity measured as objective response according to modified National Institutes of Health criteria. Fourteen patients were recruited to the first stage of the trial, of whom seven completed the planned six cycles of azacitidine 36mg/m^2 days 1 to 5 per 28-day cycle. Azacitidine was tolerated by 13/14 patients, and 7/14 showed an objective response. Clinical responses were mirrored by improvements in patient-reported cGvHD symptoms and quality of life. AZTEC demonstrates that azacitidine is a safe and promising option for the treatment of cGvHD, and continued evaluation in the second stage of this phase II efficacy study is supported.

Introduction

Allogeneic haematopoietic stem cell transplantation (SCT) is a highly effective curative treatment for patients with high risk haematological malignancies. Chronic graft-versus-host disease (cGvHD) is a major cause of non-relapse morbidity and mortality[1, 2] affecting up to half of transplant recipients,[3, 4] leading to a significant reduction in the quality of life (QoL) of transplant survivors.[5]

The standard first-line treatment for severe cGvHD includes high dose corticosteroid therapy, with the addition of a calcineurin inhibitor as a steroid-sparing agent.[6] Up to half of patients are expected to respond to first-line therapy, whereas many require second-line therapy for steroid-refractory cGvHD.[7, 8] There are currently multiple agents that could be selected for second-line use, however the lack of prospective evidence means there is currently no standard of care for the treatment of steroid-refractory cGvHD. Extracorporeal photopheresis (ECP) is approved for steroid-refractory cGvHD.[9, 10] However, ECP requires a lengthy treatment schedule, many patients require indwelling venous access, it is expensive, and is not widely available. For patients with cGvHD failing to respond to first-line treatment, and those who are unable to tolerate steroids, there is currently no standard of care.

Recent advances in understanding of its pathobiology have led to a number of targeted agents being applied to the treatment of cGvHD.[11] For example, ibrutinib can produce clinically meaningful responses in steroid-refractory cGvHD, focusing on symptoms most likely to show a rapid response.[12] Early results have also shown ruxolitinb to be effective in this setting.[13] A further example is azacitidine, a DNA methyltransferase inhibitor licensed for the treatment of AML and high-risk MDS. In trials of azacitidine that sought to reduce the risk of disease relapse post-SCT, low rates of cGvHD were also observed.[14, 15] Evidence from mouse models, recapitulated in patients, shows azacitidine has an immunomodulatory effect through regulatory T-cells, providing a mechanism that suppresses GvHD.[16] This protection from GvHD is not at the expense of the graft-versus-leukaemia effect,[17, 18] which conversely may be

enhanced through induction of cytotoxic T-cell responses against tumour associated antigens, including Wilms Tumour 1.[19] Azacitidine's efficacy as a treatment for cGvHD has not previously been tested in a prospective clinical trial.

The diagnosis and staging of cGvHD was standardised in 2005 and updated in 2014 with the publication of the National Institutes of Health (NIH) Consensus Criteria, which set out organ-specific and global scales to more accurately describe the extent, severity and functional impact of cGvHD.[20, 21] Consequently, clinical trials in cGvHD now have a detailed framework for assessing and objectively describing response to treatment.[22] Crucially, patient-reported symptoms, global severity ratings and global impression of change form a key part of the assessment. The patient-reported and cGvHD-specific Lee Symptom Scale is also a core measure of cGvHD impact and response to treatment,[23] with further non-cGvHD-specific measures of quality of life strongly encouraged by the NIH consensus recommendations.[22]

For patients with cGvHD who are resistant to or intolerant of systemic steroids, novel treatments options with objective and prospectively-collected evidence of effectiveness are needed. The AZTEC trial is a two-stage, single-arm, open-label phase II study of the safety and efficacy of azacitidine in this patient group (ISRCTN15649711, EudraCT 2014-005659-19). We present here the results of the planned interim analysis after stage one of the trial, at which point the independent trial steering committee recommended early stopping, as continuing was felt unlikely to bring significant additional information.

Methods

Participants

Adults with moderate or severe cGvHD at any time after allogeneic SCT, as defined by the NIH consensus criteria,[21] who failed therapy with steroids, were eligible for the trial. Failure of steroids was defined as either: progression of cGvHD on 1mg/kg/day prednisolone over two

weeks; stable cGvHD on ≥0.5mg/kg/day over four weeks; inability to taper prednisolone dose below 0.5mg/kg/day without recurrence of cGvHD; inability to tolerate first line therapy (for example, steroid-induced myopathy). Patients with a prior history of moderate and severe cGvHD, but graded lower at the time trial screening due to an inability to taper steroids, were eligible for trial registration. Patients with progressive, recurrent or delayed-onset acute GvHD of the skin (including overlap syndrome) were also eligible, according to validated consensus criteria.[24]

Further inclusion criteria were: unable to receive ECP (for clinical or logistic reasons or patient preference); life expectancy of at least 3 months; performance status 0-2. Exclusion criteria comprised: ocular GvHD only; pulmonary GvHD; active treatment for cGvHD within 14 days of study entry (steroids and calcineurin inhibitors permitted); ECP within six months of study entry; uncontrolled infection requiring treatment at study entry; HIV, HBV or HCV seropositivity; neutrophil count $<1x10^9$/L (G-CSF support permitted); platelet count $<30\ x10^9$/L; breastfeeding, or risk of pregnancy.

Trial design and sample size

AZTEC is a single-arm, non-blinded, phase II study of azacitidine, following the Bryant and Day two-stage design[25] to jointly evaluate tolerability and efficacy, as defined by the co-primary outcomes, with the aim of determining whether the intervention should be recommended for further evaluation. Based on clinical judgement, a tolerability rate of 85% or more was defined as the acceptable level to warrant further investigation, whereas 70% or less would be undesirable. An overall response rate of 40% was deemed the minimum clinically acceptable level, whilst 20% or less would be undesirable. See below for the definitions of tolerability and treatment response. With the probability of obtaining false positive results for tolerability set at 20% and efficacy at 15%, and the probability of false negative results set at 20% for both, a total sample size of 32 patients was required. At least 25 patients tolerating treatment and 9 or more with a response were required to conclude the treatment deserved further

investigation. At the planned interim analysis, 14 evaluable patients were required, with at least 10 tolerating treatment and at least three showing a response, before the proceeding to the second stage of the trial. The results of the pre-specified interim analysis are presented here. The AZTEC trial was approved by UK Research Ethics Committee (reference 15/EM/044), and institutional review boards at participating sites; all patients gave written informed consent in accordance with the Declaration of Helsinki to enter the trial.

Treatment

Azacitidine was administered at 36mg/m^2 on days 1 to 5 of a 28-day cycle, by either intravenous or subcutaneous route. This dose has previously been well-tolerated by patients post-SCT.[19] A dose delay of up to three days was permitted for logistical reasons. Treatment was paused or the dose reduced to 24mg/m^2 in the event of a transient grade 3-4 adverse event, however recurrent or persistent toxicity required discontinuation as per the trial's tolerability co-primary outcome. A minimum of six cycles of azacitidine were planned, with patients able to complete a further four cycles if clinical benefit was observed.

Concomitant treatment with steroids is expected, except where toxicity has been proven. A prednisolone-equivalent dose of up to 1mg/kg/day for skin cGvHD or 2mg/kg/day for other organ involvement was permitted at trial entry. Investigators were required to taper the dose of steroids following the first two cycles of azacitidine. Concomitant treatment with calcineurin inhibitors, ciclosporin or tacrolimus, was strongly recommended. Other immunosuppressive therapies were not permitted.

Outcome measures

The NIH consensus criteria provide a detailed and standardised framework for assessing cGvHD severity and response to treatment.[22] In brief, a complete response (CR) to treatment requires resolution of all manifestations of cGvHD at all involved organ sites; partial response

(PR) requires improvement in at least one organ site and no progression elsewhere; no response (NR) does not meet the criteria for CR or PR; and mixed response (MR) describes a subset of non-responders with resolution or improvement in at least one organ site but with progression elsewhere. The criteria were modified for the purpose of this study in order to ensure that patients achieving a PR or CR could not be receiving a prednisolone-equivalent dose of 0.125mg/kg/day or greater. This study did not apply response criteria to ocular cGvHD.

The co-primary outcomes were: best overall response rate (CR or PR) of cGvHD within six months of trial entry, as defined by modified NIH criteria; and tolerability of azacitidine, defined as the absence of clinically relevant and drug-related grade 3+ adverse event resulting in stopping treatment early within six months. The secondary outcomes were: best overall response between trial entry and six months after the end of trial treatment; best organ-level response, determined by improvements in individual organ system involved in cGvHD, according to modified NIH criteria; proportion of patients with a mixed response; duration of response, defined as time from CR or PR until NR or initiation of a new treatment for cGvHD; reduction in steroid use; QoL. All cGvHD assessments were performed by treating clinicians using NIH the *pro forma*, with primary outcome attribution subject to central review by the chief investigator.

Patient-reported measures of cGvHD were collected using the NIH patient self-report form, capturing global ratings of severity and global impression of change compared with the preceding month.[22] Health-related QoL was measured using the functional assessment of cancer therapy – bone marrow transplantation (FACT-BMT) questionnaire,[26] and the cGvHD-specific Lee Symptom Scale.[23] For all patient-reported secondary outcome measures, the total number of patients is too small to draw definitive conclusions. Where used, graphical representations and statistical tests are applied solely to help summarise the data.

Statistical analysis

The number and proportion of patients for each response category for the co-primary and secondary outcomes are reported as a proportion of the total number of patients recruited with 95% confidence intervals. The number and proportion of patients who tolerate treatment within 6 cycles of trial treatment are also presented in the same manner. Average duration of response is calculated using the Kaplan-Meier method. Percentage change from baseline in steroid dosage at the end of six cycles of trial treatment and six months post end of trial treatment is presented along with the 95% confidence interval. QoL outcomes were analysed using multi-level mixed effects models, where repeated measurements from baseline through to six months post treatment were analysed as random effects and response status for the primary outcome (responder vs. non-responder) was analysed as a fixed effect. Stata v16.0 was used for the analysis.

Results

Patients

Between October 2016 and July 2019, 14 patients with moderate or severe cGvHD from three UK sites entered the AZTEC trial. Median age was 58 (range 32 to 67), and although the trial was open to patients of both sexes, all were men. Two patients had received donor lymphocyte infusions prior to trial entry, indicated in Supplementary Table S1. Patient characteristics are described in Table 1. Baseline cGvHD severity including patient-reported measures are shown in Table 2 and Supplementary Table S1. All patients were on steroid treatment at trial entry, despite which eight patients continued to experience moderate or severe cGvHD according to the NIH global severity score.[20] Six patients with absent or mild symptoms at trial entry were unable to taper their systemic steroid dose. Skin and mouth cGvHD were the most commonly reported symptoms, shown in more detail in Supplementary Table S2. Lung scores in 3 patients were entirely attributable to infective, non-GvHD causes. A

total of 73 cycles of azacitidine were delivered, median 4.5 (range 1 to 10) cycles per patient, seven patients completed the planned course of six or more cycles. The reasons for stopping treatment early were disease relapse (two patients), death due to sepsis (one patient); or by patient and clinician choice due to perceived lack of efficacy (two patients), recovery of cGvHD (one patient), or the availability of treatment with ECP (one patient).

Treatment tolerability

A total of 55 adverse events (AEs) were experienced by 10 patients, including 32 grade 3-4 AEs observed in 10 patients. Grade 3-4 AEs are shown in Table 3, the largest proportion of which were haematological (38%). The most common non-haematological AE was infection in three patients (21%). Eight serious AEs were experienced by seven patients, including three that were related to treatment observed in three patients – episodes of fever, sepsis, and dyspnoea. One patient death due to sepsis and multi-organ failure was judged to be related to azacitidine treatment. The two further deaths were due to relapse of underlying lymphoma. 13/14 (93%) patients (95% confidence interval (CI) 66% to 100%) met the tolerability co-primary outcome, exceeding the pre-specified threshold of 10 patients.

Activity

The co-primary outcome of overall response within six months of starting treatment was observed in 7/14 (50%) patients (95% CI 23% to 77%), including one CR and six PRs. This exceeds the pre-specified, clinically relevant threshold of three patients requiring a response. The remaining seven patients showed NR. Four of the responding patients completed at least six cycles of azacitidine, compared with three patients without a disease response. Given that AZTEC met the tolerability and efficacy co-primary endpoints of the planned interim analysis, the trial would be appropriate to advance to its second stage. There was no correlation between treatment response and baseline cGvHD global severity score.

The secondary efficacy outcome of overall response between trial entry and six months after end of trial treatment was observed in 8/14 (57%) patients (95% CI 29% to 82%), comprising five CR and three PR; six patients showed NR. Only one patient with an improved response received additional therapy (ECP) after stopping trial treatment; two further patients maintained a PR having received a ECP outside of the trial. An organ-level response within six months of the end of trial was observed eight patients, including four CR and four PR; four patients showed NR, whilst two showed organ progression. Skin and mouth cGvHD were the most common manifestations: sequential, individual-level treatment responses are shown for all evaluated timepoints for skin (11 patients) and mouth (7 patients) symptoms (Figure 1).

The median time to response was 5.0 months (95% CI 3.2 months to not estimable). The median duration of response was 4.7 months (95% CI 1.0 to not estimable), following cGvHD relapses or new treatments started in four of the eight responding patients.

Reduction in steroid use

The average concomitant steroid dose reduced with azacitidine treatment. Six of the seven patients who completed six or more cycles reduced their steroid dose during treatment. The mean steroid dose was reduced by 72% (95% CI 33% to 100%) after six cycles compared with baseline. Ten of the 11 patients who completed six months of follow-up after finishing AZTEC treatment reduced their steroid dose, the mean reduction was by 78% (95% CI 56% to 95%) compared with baseline.

Patient-reported outcomes

Self-reported cGvHD symptoms are integral to the NIH cGvHD activity assessment, and are essential for ensuring clinical improvements are meaningful for patients. Global severity is measured on both 3-point (mild, moderate or severe) and 11-point (scored 0 to 10) scales, with an additional 7-point scale measuring month-on-month changes in symptoms (very much better to very much worse). The sequential distributions of self-reported global severity ratings

256 throughout azacitidine treatment, amongst patients demonstrating an objective clinical
257 response, are shown in Figure 2. Changes in both of the self-reported global severity ratings
258 are consistent with improvements in cGvHD symptoms. Similarly, patients were more likely to
259 report a month-on-month improvement and less likely to describe a worsening in symptoms.

260 Patient-reported QoL was measured using the FACT-BMT instrument, with higher scores
261 indicating better quality of life. Individual-level improvements in FACT-BMT score during
262 treatment, amongst patients demonstrating a clinical response, are shown in Figure 3.
263 Multilevel modelling of the FACT-BMT total score confirms a monthly improvement in quality
264 of life (time coefficient 0.94, 95% CI 0.27 to 1.61, p=0.006). No significant change was seen in
265 the summary scores of the cGvHD-specific Lee symptom scale, where lower values indicate
266 less bothersome symptoms (time coefficient -0.32, 95% CI -0.80 to 0.17, p=0.199).

267

268 Discussion

269 Chronic GvHD is a major cause of non-relapse morbidity and mortality amongst patients
270 undergoing SCT, many of whom will be cured of their original haematological malignancy.
271 First-line treatment with steroids and calcineurin inhibitors is well-established, and ECP can be
272 offered when practicable.[6] However, for the half of patients with an inadequate response to
273 systemic steroids who require alternative treatments, there is currently no standard of care.[8]

274 The interim data from the AZTEC trial presented here support a role for azacitidine in the
275 treatment of cGvHD in patients who have resistance to or are intolerant of steroids.
276 Azacitidine was generally well-tolerated in this patient group with most adverse events being
277 managed without interrupting treatment. Efficacy was demonstrated in 50% of patients,
278 according to stringent and objective modified NIH criteria, resulting in durable improvements
279 of cGvHD symptoms for most. Improvements in cGvHD allowed a reduction of the steroid dose
280 for 10/11 patients recorded at six months after the trial, protecting them from the significant

risks associated with their long-term use. Importantly, clinically-observed responses to treatment were matched with patient-reported improvements in symptom severity and QoL.

Since opening the AZTEC Trial, a number of additional agents have been investigated as potential second-line options for cGvHD. Ibrutinib was the first US Food and Drug Administration-approved therapy, having demonstrated efficacy against steroid-dependent or -refractory cGvHD in a phase I/II trial.[12] A large retrospective study of ruxolitinib in steroid-refractory GvHD (acute and chronic) showed promising results and led to the prospective REACH trials.[27, 28] Efficacy of ruxolitinib against steroid-refractory acute GvHD was demonstrated, leading to regulatory approval in this setting.[29, 30] The recently presented outcome of REACH3 (NCT03112603), evaluating ruxolitinib against cGvHD, has also shown superiority over best available therapy.[13] Cellular therapies are an emerging area of interest for the treatment and prevention of GvHD, although there is only limited experience in the treatment of steroid-refractory cGvHD.[31-33] And in those settings where it can be delivered, ECP remains an effective and recommended option.[9, 10] In a landscape of new and emerging treatments for cGvHD, this work highlights the potential value of azacitidine in this setting. Well-tolerated by patients undergoing allogeneic SCT, its ability post-transplant to enhance relapse-free survival is currently being investigated in the phase III AMADEUS trial (NCT04173533). Azacitidine could therefore be particularly well-suited to patients with high-risk myeloid malignancies with steroid-resistant cGvHD.

Recruitment to the first stage of the AZTEC Trial took longer than could have been anticipated at the outset. This, at least in part, reflects the increasing number of targeted agents currently under investigation for steroid-refractory cGvHD, many of which have shown promising early results. Whilst the planned interim endpoints for both tolerability and efficacy were met, and progression to the second stage of the trial recommended, the trial steering committee agreed that continuing with AZTEC would be unlikely to provide sufficient additional information to justify continuation of the trial.

Azacitidine is associated with low rates of cGvHD, when used to prevent acute myeloid leukaemia or myelodysplasia relapse post-SCT.[14, 15] In this first prospective trial for the treatment of steroid-refractory cGvHD, we have demonstrated that azacitidine is well-tolerated and can produce objective clinical responses. As the underlying pathobiology of cGvHD becomes better-understood, targeted agents are likely to play an increasing role in its treatment. With a range of patient-, malignancy- and transplant-related factors all likely to contribute to cGvHD, azacitidine remains an option for a subset of patients not responsive to first-line steroids and immunosuppression.

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Competing interests

Ram Malladi has received travel assistance from Celgene. Fiona Dignan has received travel assistance from Gilead and Jazz; speaker's fees from Mallinckrodt, Jazz, Pfizer and Janssen; advisory board for Kiadis, Jazz. Charles Craddock has received research funding from Celgene. All other authors report no competing interests.

Authorship

RM conceived the work; RM, IA, AJ, PM, SS and CC designed the trial; RM, FD, RP, CC and JN acquired the patient data; RM, IA, GM, AJ, RB, ME, and AH analysed and interpreted the results; RM and GM drafted the manuscript; all authors revised the manuscript; all authors approved the final version of the manuscript; all authors are accountable for all aspects of the work, and ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Table and Figure legends

Table 1

Baseline characteristics of patients recruited to AZTEC.

Table 2

Baseline cGvHD severity.

Table 3

All grade 3 to 4 adverse events.

Figure 1. Sequential, individual treatment responses to skin and mouth cGvHD

Serial change in the most common cGvHD symptoms during azacitidine treatment: (A) skin (n=11), and (B) mouth (n=7). Each line in the charts represents one patient. Only patients with symptoms are shown, patients with no symptoms at any point are not shown. Patients with an overall clinical response within six months of starting treatment according to the primary outcome are shown, in contrast to patients without an overall response (non-responders). BSA, body surface area.

Figure 2. Patient-reported ratings of cGvHD severity and change over time

Patient self-reported (A, B) global ratings of symptoms and (C) global impression of change, collected using the NIH cGvHD activity assessment tool. Ratings from patients with an objective clinical response within 6 months of treatment are shown. The global rating questions that patients were asked to respond to are shown. The distributions of responses are indicated for each question, collected over the course of treatment. The key to the right of each bar chart indicates the range of responses available for each question.

Figure 3. Patient-reported quality of life rating over time

Self-reported quality of life ratings, collected using the FACT-BMT tool. Each line represents one patient, with quality of life measured serially during the study. Total scores are shown for patients with an objective clinical response within 6 months of treatment, collected over the course of treatment. The FACT-BMT total score is shown, higher scores indicating a better health-related quality of life.

512 **Table 1**

	Number (%) of patients, except where indicated
	Total n=14
Age, median (range)	58 years (32 to 67)
Male	14 (100%)
Karnofsky performance status, median (range)	80 (60 to 100)
Personal history of diabetes mellitus	4 (29%)
Haematological diagnosis	
Acute myeloid leukaemia	4 (29%)
Acute lymphoblastic leukaemia	4 (29%)
Myelodysplasia	2 (14%)
Non-Hodgkin lymphoma	2 (14%)
Hodgkin lymphoma	1 (7%)
Peripheral T-cell lymphoma	1 (7%)
Donor	
HLA-identical sibling	5 (36%)
HLA-matched unrelated	5 (36%)
9/10 HLA-mismatched unrelated	3 (21%)
Haploidentical relative	1 (7%)
Transplant type and regimen	
Reduced-intensity conditioning	10 (71%)
Myeloablative	4 (29%)
Total body irradiation	2 (14%)
T-cell depletion of reduced-intensity transplants	
Alemtuzumab	6 (60%)
Anti-thymocyte globulin	1 (10%)
None	3 (30%)
Time from transplant, median (range)	386 days (180 to 1346)
Prior donor lymphocyte infusions	2 (14%)
Prior acute GvHD (maximum grade)	
1	1 (7%)
2	8 (57%)
3	2 (14%)
Not stated	1 (7%)
None	2 (14%)
GvHD diagnosis	
Chronic	6 (43%)
Late acute (progressive/recurrent/delayed)	8 (57%)
Trial eligibility	
Inability to taper steroids	10 (71%)
Intolerance to first-line chronic GvHD therapy	3 (21%)
Late acute GvHD failing first-line therapy	5 (36%)
Initial steroid treatment	
Prednisolone (oral)	12 (86%)
Methylprednisolone (intravenous)	1 (7%)
None	1 (7%)
Initial steroid dose[1], median (range)	1.0mg/kg/day (0.3 to 2.0)
Calcineurin inhibitor	
Ciclosporin	8 (57%)
Tacrolimus	2 (14%)
None	4 (29%)
[1]Steroid dose given as prednisolone equivalent	

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Table 2

Number (%) of patients, except where indicated	Total n=14
NIH Global severity score	
None	2 (14%)
Mild	4 (29%)
Moderate	2 (14%)
Severe	6 (43%)
Skin (body surface area)	
0 (none)	3 (21%)
1 (1-18%)	3 (21%)
2 (19-50%)	0
3 (>50%)	6 (43%)
Not stated	2 (14%)
Skin (sclerotic features)	
0 (none)	12 (86%)
2 (superficial sclerosis)	2 (14%)
3 (deep sclerosis, impaired mobility, ulceration)	0
Mouth (symptoms)	
0 (none)	9 (64%)
1 (mild)	3 (21%)
2 (moderate)	1 (7%)
3 (severe)	0
Not stated	1 (7%)
Lichen planus feature	
Present	5 (36%)
Absent	3 (21%)
Not stated	6 (43%)
Mouth (erythema 0-3 *plus* lichenoid 0-3 *plus* ulcers 0-6)	
Median (IQR)	0 (0 to 4)
Eyes (dry eye symptoms)	
0 (none)	10 (71%)
1 (mild)	3 (21%)
2 (moderate)	1 (7%)
3 (severe)	0
Gastrointestinal tract	
0 (no symptoms)	10 (71%)
1 (<5% weight loss)	4 (29%)
2 (5-15% weight loss, moderate diarrhoea)	0
3 (>15% weight loss, severe diarrhoea, oesophageal dilatation)	0
Liver	
0 (normal bilirubin, ALT or ALP <3x ULN)	14 (100%)
1 (normal bilirubin, ALT 3-5x ULN, ALP ≥3x ULN)	0
2 (elevated bilirubin ≤50μmol/L, ALT >5x ULN)	0
3 (elevated bilirubin >50μmol/L)	0
Lungs (symptom score)[1]	
0 (none)	11 (79%)
1 (mild)	3 (21%)
2 (moderate)	0
3 (severe)	0
Lungs (FEV1)[1]	
0 (≥80%)	10 (71%)
1 (60-79%)	1 (7%)
2 (40-59%)	0
3 (≤39%)	0
Not stated	3 (21%)
Joints and fascia (tightness and movement symptoms)	
0 (none)	14 (100%)
1 (mild)	0
2 (moderate)	0
3 (severe)	0
Healthcare provider global rating (symptoms)	
None	1 (7%)
Mild	3 (21%)
Moderate	3 (21%)
Severe	6 (43%)
Not stated	1 (7%)
Healthcare provider severity scale (0-10)	
Median (IQR)	6 (3 to 7)
Blood results, median (IQR)	
Platelet count ($x10^9$/L)	142 (121 to 201)
Eosinophil count ($x10^9$/L)	0 (0 to 0.3)
Bilirubin (μmol/L)	9 (4 to 15)
Patient self-reported global rating (symptoms)	
Mild	3 (21%)
Moderate	3 (21%)
Severe	7 (50%)
Not stated	1 (7%)
Patient self-reported severity scale (0-10)[2]	
Median (IQR)	7 (5 to 9)
Lee symptom scale (total score, 0-100)[2]	
Median (IQR)	26.2 (21.5 to 32.6)
FACT-BMT (total score, 0-148)[2]	
Median (IQR) (n = 13)	99.8 (85 to 109)

[1]Lung scores were entirely attributable to infective (non-cGvHD) causes. [2]On patient-reported scales, higher scores indicate more severe or more bothersome symptoms, except FACT-BMT where higher scores indicate better quality of life.

IQR, inter-quartile range; ALT, alanine aminotransferase; ALP, alkaline phosphatase; ULN, upper limit of normal; FEV1, forced expiratory volume in 1 second, expressed as a percentage of that predicted.

518 **Table 3**

Adverse event	Events (Patients)	
	Grade 3	Grade 4
Haematological		
Neutropenia	3 (2)	2 (2)
Thrombocytopenia	4 (2)	1 (1)
Leukopenia	1 (1)	1 (1)
Infective		
Sepsis		1 (1)
Bladder infection	1 (1)	
Lung infection	1 (1)	
Sinusitis	1 (1)	
Metabolic		
Hypokalaemia	3 (2)	
Hypocalcaemia	1 (1)	
Hyperglycaemia	1 (1)	
Other		
Encephalopathy		1 (1)
Hypertension	2 (2)	
Chronic kidney disease	2 (1)	
Acute kidney injury	1 (1)	
Neuralgia	1 (1)	
Dyspnoea	1 (1)	
Diarrhoea	1 (1)	
Flu-like symptoms	1 (1)	
Retinal vascular disorder	1 (1)	

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Figure 1

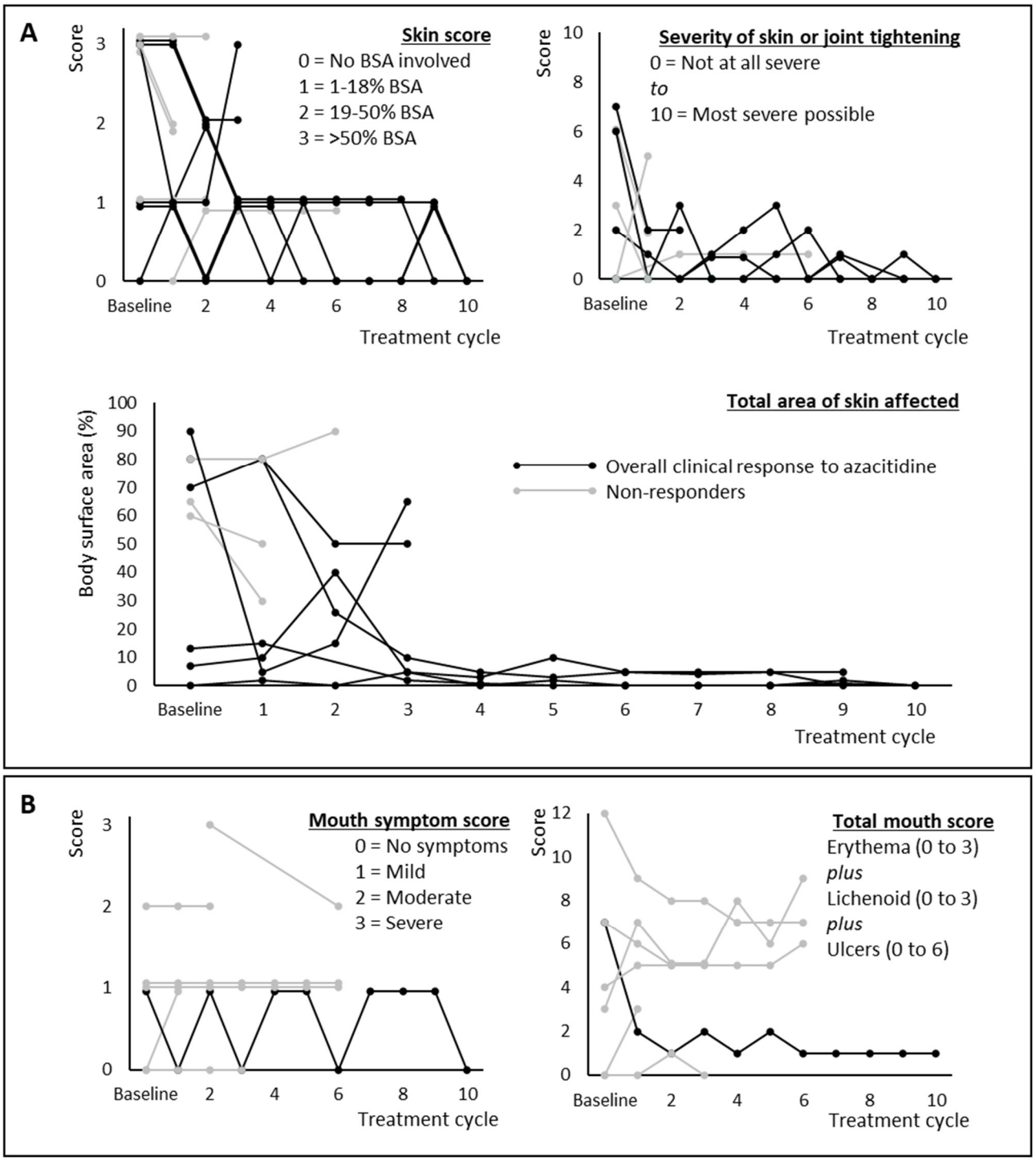
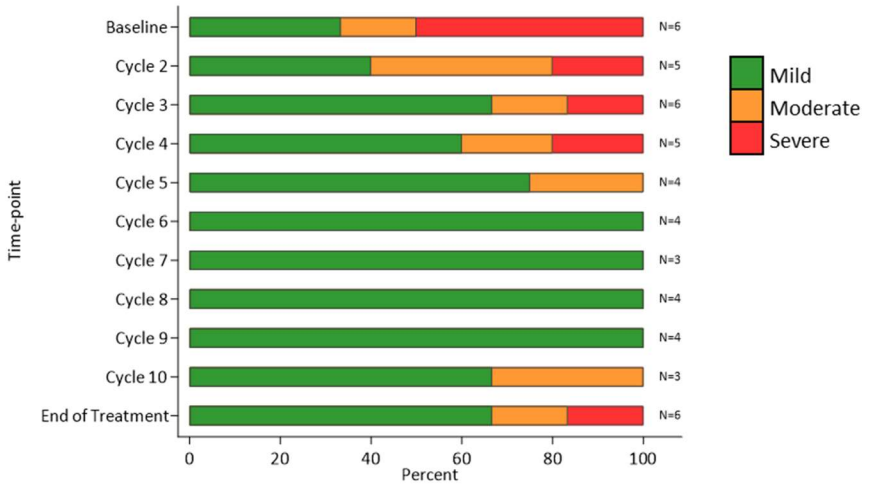
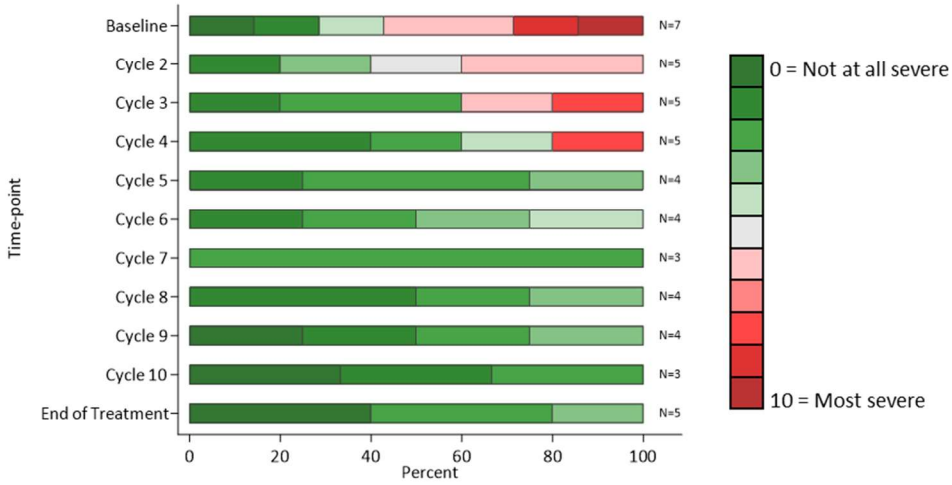

A
Skin score
0 = No BSA involved
1 = 1-18% BSA
2 = 19-50% BSA
3 = >50% BSA
Score
Baseline
Treatment cycle
Severity of skin or joint tightening
0 = Not at all severe
to
10 = Most severe possible
Total area of skin affected
Body surface area (%)
Overall clinical response to azacitidine
Non-responders
B
Mouth symptom score
0 = No symptoms
1 = Mild
2 = Moderate
3 = Severe
Total mouth score
Erythema (0 to 3)
plus
Lichenoid (0 to 3)
plus
Ulcers (0 to 6)

Figure 2

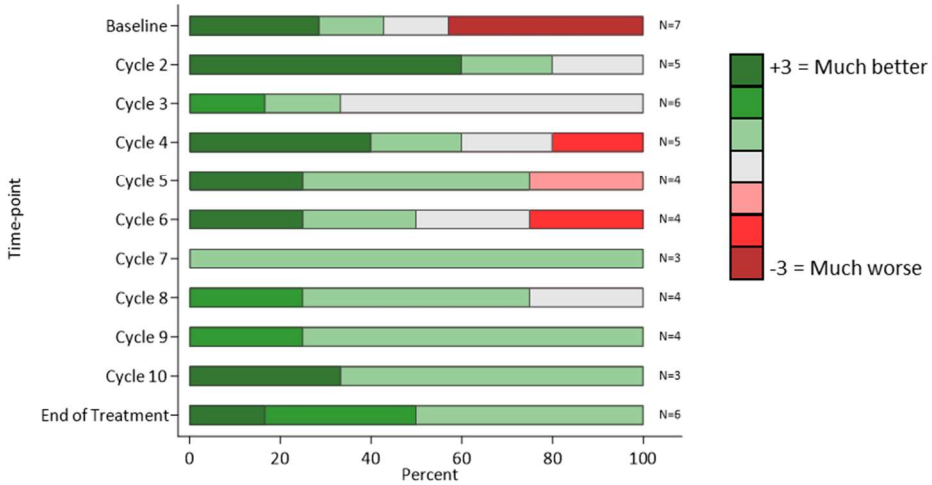
A: Overall, do you think that your chronic graft versus host disease is mild, moderate or severe?



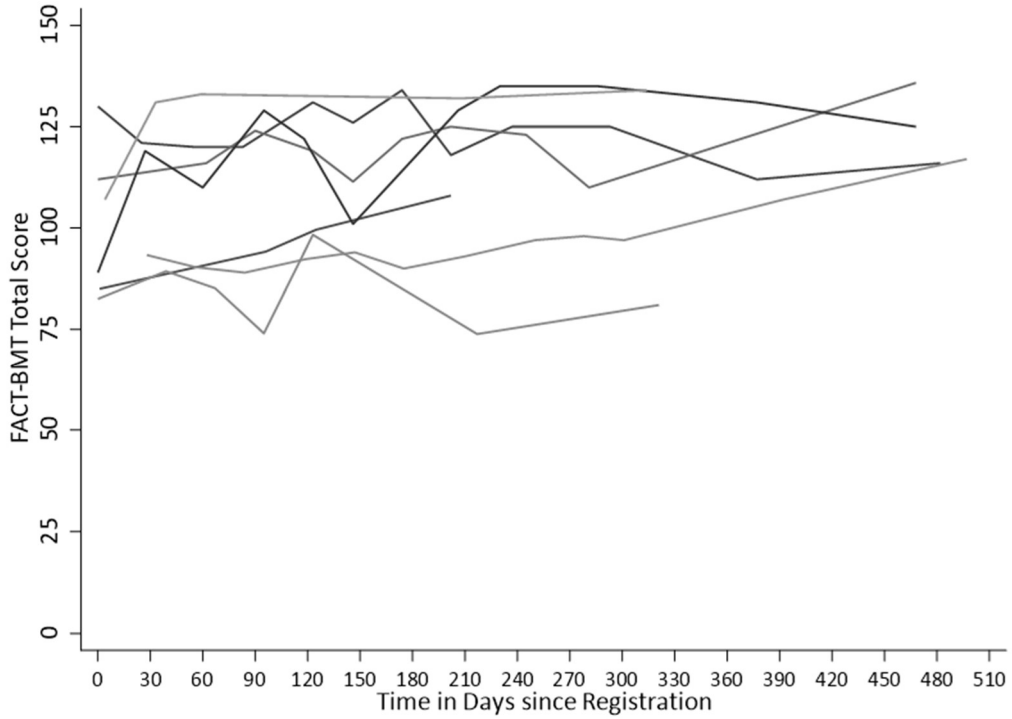
B: Please circle the number indicating how severe your chronic graft versus host disease symptoms are.



C: Compared to a month ago, overall would you say your cGVHD symptoms are:



527 **Figure 3**



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Supplementary

Table S1. Baseline cGvHD organ severity per patient

Patient	Skin (body surface area)	Skin (sclerotic features)	Mouth (symptoms)	Mouth (erythema, lichenoid, ulcers)	Eye	Gastrointestinal tract	Lung (symptoms)	Lung (FEV1)	Liver	Joint and fascia
1	3	0	0	0	1	1	1	0	0	0
2		0		12	0	0	0	0	0	0
3	1	2	1	7	0	1	0	0	0	0
4	0	0	1	4	1	0	0	0	0	0
5		0	0	0	0	1	0	0	0	0
6	3	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0
9	3	0	0	0	1	0	0	0	0	0
10	1	0	0	0	0	0	0	0	0	0
11*	3	2	0	0	0	0	1	1	0	0
12*	0	0	1	3	0	1	0	0	0	0
13	1	0	2	7	2	0	0		0	0
14	3	0	0	0	0	0	0		0	0
15	3	0	0	0	0	0	1		0	0

*Patients received donor lymphocyte infusions before diagnosis of cGvHD. See Table 2 in the main text for details on how each score is defined. Lung scores (symptoms and FEV1 reduction) in all affected patients were entirely attributable to infective (non-cGvHD) causes.

Table S2. Baseline skin and mouth cGvHD

Skin		
Maculopapular rash / erythema	9	(64%)
Lichen planus-like features	1	(7%)
Sclerotic features	1	(7%)
Papulosquamous lesions or ichthyosis	1	(7%)
Keratosis pilaris-like	0	
Skin features score		
0 (no sclerotic features)	12	(86%)
2 (superficial sclerosis)	2	(14%)
3 (deep sclerosis, impaired mobility, ulceration)	0	
Severity of skin tightening (0 to 10 scale), median (IQR), n=10	1	(0 to 6)
Skin score (body surface area)		
0 (none)	3	(21%)
1 (1-18%)	3	(21%)
2 (19-50%)	0	
3 (more than 50%)	6	(43%)
Not stated	2	(14%)
Total body surface area of skin affected, median (IQR)	18%	(0 to 70%)
Patient reported symptoms (0 to 10 scale), median (IQR)		
Skin itching	0	(0 to 6)
Skin tightening	3	(0 to 8)
Mouth		
Mouth symptom score		
0 (none)	9	(64%)
1 (mild)	3	(21%)
2 (moderate)	1	(7%)
3 (severe)	0	
Not stated	1	(7%)
Lichen planus-like features	3	(21%)
Erythema score		
0 (none)	11	(79%)
1 (mild, or moderate <25%)	1	(7%)
2 (moderate ≥25%, or severe <25%)	1	(7%)
3 (severe ≥25%)	1	(7%)
Lichenoid score		
0 (none)	9	(64%)
1 (<25%)	1	(7%)
2 (25-50%)	1	(7%)
3 (>50%)	2	(14%)
Not stated	1	(7%)
Ulceration score		
0 (none)	8	(57%)
3 (≤20%)	4	(29%)
6 (>20%)	1	(7%)
Not stated	1	(7%)
Patient reported mouth sensitivity (0 to 10 scale), median (IQR)	4	(0 to 9)

Number (%) of patients shown, except where indicated.