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Malladi, Ram; Ahmed, Ikhlaaq; McIlroy, Graham; Dignan, Fiona L.; Protheroe, Rachel; Jackson, Aimee; Moss, Paul; Nunnick, Jane; Siddique, Shamyla; Bishop, Rebecca; Elhaneid, Mohamed; Hodgkinson, Andrea; Craddock, Charles

DOI:

[10.1038/s41409-021-01439-y](https://doi.org/10.1038/s41409-021-01439-y)

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Document Version

Peer reviewed version

Citation for published version (Harvard):

Malladi, R, Ahmed, I, McIlroy, G, Dignan, FL, Protheroe, R, Jackson, A, Moss, P, Nunnick, J, Siddique, S, Bishop, R, Elhaneid, M, Hodgkinson, A & Craddock, C 2021, 'Azacitidine for the treatment of steroid-refractory chronic graft-versus-host disease: the results of the phase II AZTEC clinical trial', *Bone Marrow Transplantation*, vol. 56, no. 12, pp. 2948-2955. <https://doi.org/10.1038/s41409-021-01439-y>

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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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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.

38 **Abstract**

39

40 Chronic graft-versus-host disease (cGvHD) is a major cause of non-relapse morbidity and
41 mortality following allogeneic stem cell transplant. Over half of patients with moderate or
42 severe cGvHD fail to respond adequately to first-line treatment with systemic steroids, and
43 although a range of second-line options have been employed, a lack of prospective evidence
44 means there is no standard of care. The AZTEC trial is a prospective, single-arm, phase II study
45 investigating the safety and activity of azacitidine for the treatment of cGvHD in patients who
46 are resistant to, or intolerant of, systemic steroid therapy. The co-primary outcomes were
47 treatment tolerability, and activity measured as objective response according to modified
48 National Institutes of Health criteria. Fourteen patients were recruited to the first stage of the
49 trial, of whom seven completed the planned six cycles of azacitidine 36mg/m² days 1 to 5 per
50 28-day cycle. Azacitidine was tolerated by 13/14 patients, and 7/14 showed an objective
51 response. Clinical responses were mirrored by improvements in patient-reported cGvHD
52 symptoms and quality of life. AZTEC demonstrates that azacitidine is a safe and promising
53 option for the treatment of cGvHD, and continued evaluation in the second stage of this phase
54 II efficacy study is supported.

55 **Introduction**

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

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

71 Recent advances in understanding of its pathobiology have led to a number of targeted agents
72 being applied to the treatment of cGvHD.¹¹ For example, ibrutinib can produce clinically
73 meaningful responses in steroid-refractory cGvHD, focusing on symptoms most likely to show
74 a rapid response.¹² Early results have also shown ruxolitinb to be effective in this setting.¹³ A
75 further example is azacitidine, a DNA methyltransferase inhibitor licensed for the treatment of
76 AML and high-risk MDS. In trials of azacitidine that sought to reduce the risk of disease relapse
77 post-SCT, low rates of cGvHD were also observed.^{14, 15} Evidence from mouse models,
78 recapitulated in patients, shows azacitidine has an immunomodulatory effect through
79 regulatory T-cells, providing a mechanism that suppresses GvHD.¹⁶ This protection from GvHD
80 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.

100

101 **Methods**

102 **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

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

113 Further inclusion criteria were: unable to receive ECP (for clinical or logistic reasons or patient
114 preference); life expectancy of at least 3 months; performance status 0-2. Exclusion criteria
115 comprised: ocular GvHD only; pulmonary GvHD; active treatment for cGvHD within 14 days of
116 study entry (steroids and calcineurin inhibitors permitted); ECP within six months of study
117 entry; uncontrolled infection requiring treatment at study entry; HIV, HBV or HCV
118 seropositivity; neutrophil count $<1 \times 10^9/\text{L}$ (G-CSF support permitted); platelet count $<30 \times 10^9/\text{L}$;
119 breastfeeding, or risk of pregnancy.

120 **Trial design and sample size**

121 AZTEC is a single-arm, non-blinded, phase II study of azacitidine, following the Bryant and Day
122 two-stage design²⁵ to jointly evaluate tolerability and efficacy, as defined by the co-primary
123 outcomes, with the aim of determining whether the intervention should be recommended for
124 further evaluation. Based on clinical judgement, a tolerability rate of 85% or more was defined
125 as the acceptable level to warrant further investigation, whereas 70% or less would be
126 undesirable. An overall response rate of 40% was deemed the minimum clinically acceptable
127 level, whilst 20% or less would be undesirable. See below for the definitions of tolerability and
128 treatment response. With the probability of obtaining false positive results for tolerability set
129 at 20% and efficacy at 15%, and the probability of false negative results set at 20% for both, a
130 total sample size of 32 patients was required. At least 25 patients tolerating treatment and 9
131 or more with a response were required to conclude the treatment deserved further

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

138

139 **Treatment**

140 Azacitidine was administered at 36mg/m² on days 1 to 5 of a 28-day cycle, by either
141 intravenous or subcutaneous route. This dose has previously been well-tolerated by patients
142 post-SCT.¹⁹ A dose delay of up to three days was permitted for logistical reasons. Treatment
143 was paused or the dose reduced to 24mg/m² in the event of a transient grade 3-4 adverse
144 event, however recurrent or persistent toxicity required discontinuation as per the trial's
145 tolerability co-primary outcome. A minimum of six cycles of azacitidine were planned, with
146 patients able to complete a further four cycles if clinical benefit was observed.

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

153 **Outcome measures**

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

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

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

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

181 **Statistical analysis**

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

193

194 **Results**

195 **Patients**

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

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

212 **Treatment tolerability**

213 A total of 55 adverse events (AEs) were experienced by 10 patients, including 32 grade 3-4 AEs
214 observed in 10 patients. Grade 3-4 AEs are shown in Table 3, the largest proportion of which
215 were haematological (38%). The most common non-haematological AE was infection in three
216 patients (21%). Eight serious AEs were experienced by seven patients, including three that
217 were related to treatment observed in three patients – episodes of fever, sepsis, and
218 dyspnoea. One patient death due to sepsis and multi-organ failure was judged to be related to
219 azacitidine treatment. The two further deaths were due to relapse of underlying lymphoma.
220 13/14 (93%) patients (95% confidence interval (CI) 66% to 100%) met the tolerability co-
221 primary outcome, exceeding the pre-specified threshold of 10 patients.

222 **Activity**

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

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

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

243 **Reduction in steroid use**

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

250 **Patient-reported outcomes**

251 Self-reported cGvHD symptoms are integral to the NIH cGvHD activity assessment, and are
252 essential for ensuring clinical improvements are meaningful for patients. Global severity is
253 measured on both 3-point (mild, moderate or severe) and 11-point (scored 0 to 10) scales,
254 with an additional 7-point scale measuring month-on-month changes in symptoms (very much
255 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

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

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

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

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

315

316 **Acknowledgements**

317 The AZTEC trial was funded by Bloodwise (now Blood Cancer UK). Azacitidine was provided
318 free of charge by Celgene.

319 The trial was supported by the facilities funded through Birmingham Science City Translational
320 Medicine Clinical Research Infrastructure and Trials Platform, an Advantage West Midlands
321 (AWM) funded project which forms part of the Science City University of Warwick and
322 University of Birmingham Research Alliance.

323 **Competing interests**

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

328 **Authorship**

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

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482 **Table and Figure legends**

483 **Table 1**

484 Baseline characteristics of patients recruited to AZTEC.

485 **Table 2**

486 Baseline cGvHD severity.

487 **Table 3**

488 All grade 3 to 4 adverse events.

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

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

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

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

503

504

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

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

511

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%)

¹Steroid dose given as prednisolone equivalent

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 <i>plus</i> lichenoid 0-3 <i>plus</i> 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) ³	
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.	

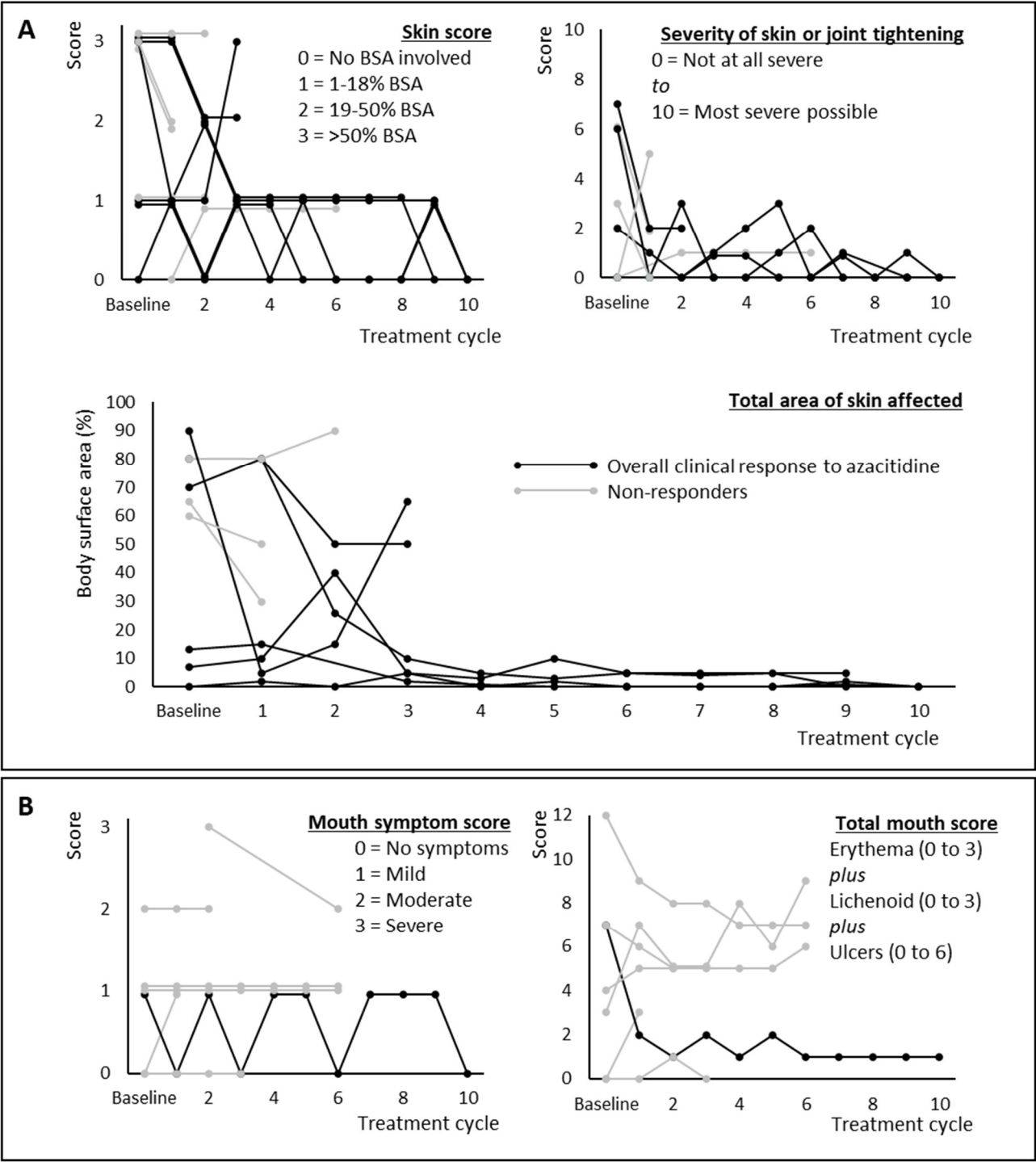
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Table 3

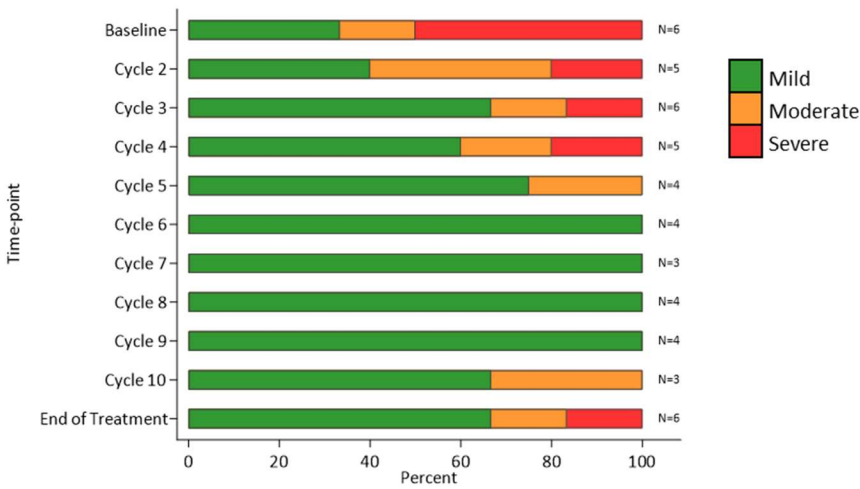
Adverse event	Events (Patients)	
	Grade 3	Grade 4
Haematological		
Neutropenia	3 (2)	2 (2)
Thrombocytopenia	4 (2)	1 (1)
Leukopenia	1 (1)	1 (1)
Infective		
Sepsis		1 (1)
Bladder infection	1 (1)	
Lung infection	1 (1)	
Sinusitis	1 (1)	
Metabolic		
Hypokalaemia	3 (2)	
Hypocalcaemia	1 (1)	
Hyperglycaemia	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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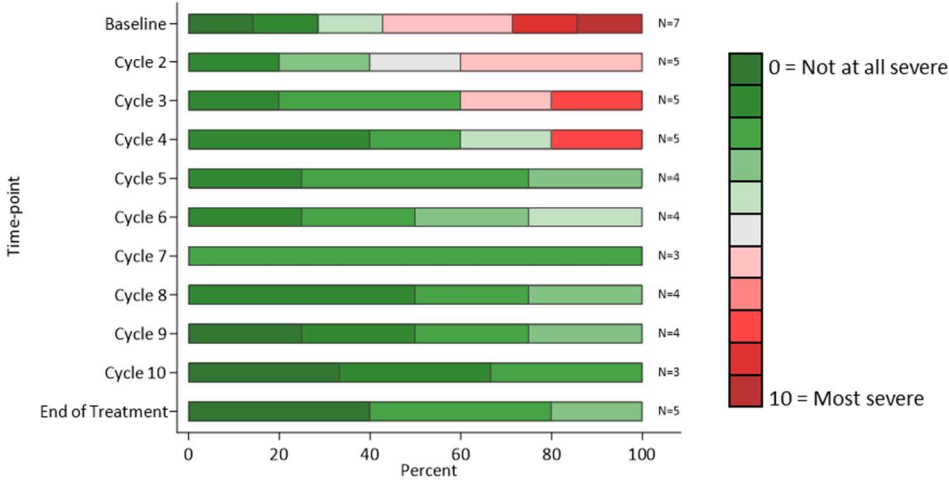
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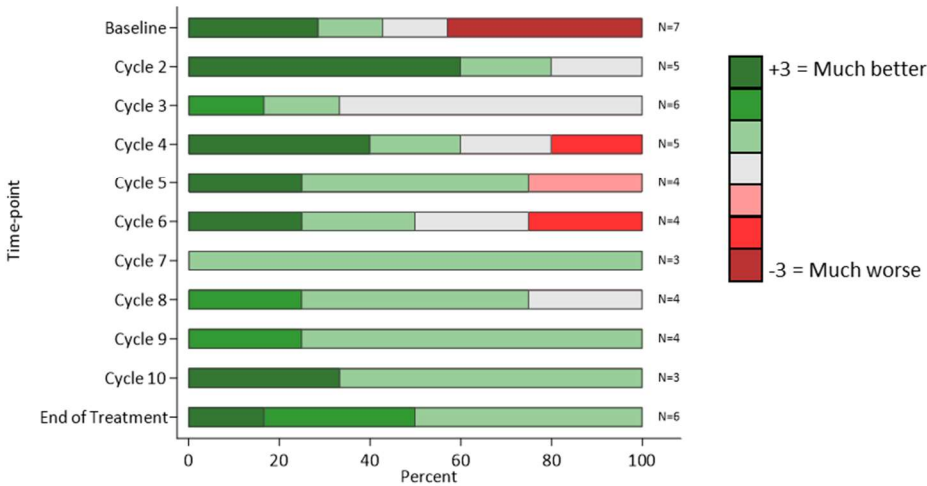
A: Overall, do you think that your chronic graft versus host disease is mild, moderate or severe?



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



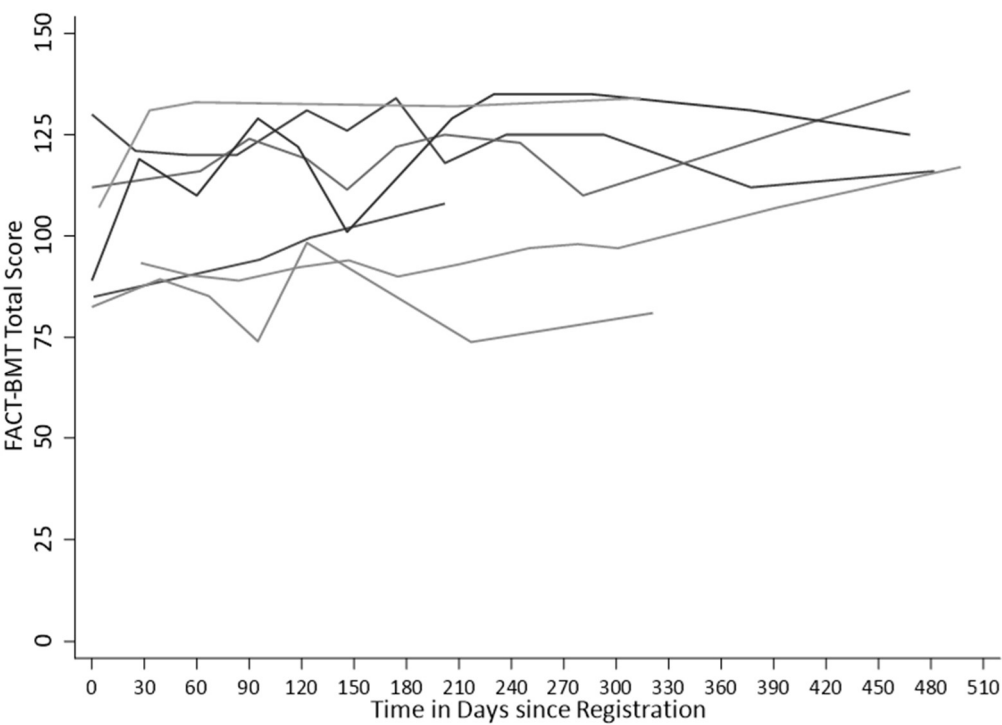
C: Compared to a month ago, overall would you say your cGVHD symptoms are:



525

526

527 **Figure 3**



528

529

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)

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