Regular article

Targeting Hedgehog Signaling with Glasdegib in Patients with Refractory Sclerotic Chronic Graft vs. Host Disease: A Report of Two Phase I/II Trials

Running head: Glasdegib in patients with sclerotic cGVHD

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Translational relevance statement

Prior studies have revealed Hedgehog (HH) signaling activation in dermal fibroblasts as a key biological hallmark of sclerotic chronic graft-versus-host disease (scGVHD). The results from these two phase I/II trials provide proof of concept of the actionability of the HH signaling pathway in this clinical setting. Treatment with glasdegib, a selective Smoothened inhibitor, was associated with clinically meaningful and sustained improvements in a range of standardized response measures in patients with heavily refractory scGVHD. Tolerability was constrained by the frequent emergence of on-target toxicities, but prolonged treatment was feasible in some patients. Taken together, our data support the use of glasdegib as novel targeted therapeutic option for scGVHD patients not responding to established therapies. Notably, our analyses failed to identify fibroblast-independent immunomodulatory effects upon HH signaling inhibition.

Abstract

Purpose:

Sclerotic chronic graft vs. host disease (scGVHD) is characterized by progressive skin fibrosis and frequent refractoriness to available therapies. Aberrant activation of Hedgehog signaling in dermal fibroblasts has been implicated in scGVHD. Here, we report the results of two phase I/II studies (NCT03415867, GETH-TC; NCT04111497, FHD) that evaluated glasdegib, a SMO antagonist, as a novel therapeutic agent in refractory scGVHD.

Patients and Methods:

Adult patients with active scGVHD after ≥1 (FHD) or ≥2 (GETH-TC) lines of therapy were enrolled. Primary endpoints were dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) in the GETH-TC trial, and safety and tolerability measures in the FHD trial. Glasdegib was administered once daily in 28-day cycles. Responses were scored per 2014 NIH cGVHD criteria. Correlative studies were performed to evaluate the role of fibroblast-independent immune mechanisms on clinical activity.

Results:

Twenty (GETH-TC) and 15 (FHD) patients were recruited. Treatment-emergent grade (G) ≥2 adverse events (AEs) in the GETH-TC trial included muscle cramps (85%), alopecia (50%) and dysgeusia (35%). Two patients experienced a DLT (G3 muscle cramps), and the MTD was established at 50 mg. G3 muscle cramps were the most frequently reported AE (33%) in the FHD trial. At 12-months, the skin/joint scGVHD overall response rate was 65% (all partial responses) in the GETH-TC trial and 47% (6 partial responses, 1 complete response) in the FHD cohort. No immune correlates of response were identified.

Conclusions:

Glasdegib demonstrated promising responses in patients with refractory scGVHD, but tolerability was limited by muscle cramping.

Introduction

Chronic graft-versus-host disease (cGVHD), a late complication after allogeneic hematopoietic stem cell transplantation (alloSCT), is a pleomorphic condition in which immune-mediated clinical manifestations in multiple organs usually coexist (1–3). Sclerotic cGVHD (scGVHD), a distinctive phenotypic variant that affects up to 20% of patients requiring systemic treatment for cGVHD, is characterized by progressive skin fibrosis and frequent refractoriness to available therapies(4–6). In advanced stages, scGVHD can lead to chronic skin ulceration, joint contractures and pulmonary restriction, often resulting in major disability, prolonged requirements of immunosuppressive therapy, and severely impaired quality of life. Yet, despite an expansion of the cGVHD armamentarium in recent years(7–10), the development of effective therapeutic options for steroid-refractory scGVHD remains a largely unmet need(11,12).

Although our mechanistic understanding of cGVHD-induced fibrosis is still far from complete, prior studies have revealed Hedgehog (HH) signaling activation in dermal fibroblasts as a key hallmark in the pathogenesis of scGVHD. HH signaling, a developmental morphogen pathway tightly regulated in adult tissues, promotes fibrosis

147 in scGVHD through the differentiation of resting fibroblasts into metabolically active,
148 collagen-releasing myofibroblasts(13,14). Notably, blockade of HH signaling has been
149 shown to prevent the onset of sclerosis and to halt the progression of established
150 sclerotic features in murine models of scGVHD(14). In addition to its direct role in the
151 modulation of fibroblast activity, HH signaling is also known to be involved in a
152 number of immune regulatory circuits relevant to cGVHD biology. For instance,
153 overactivation of the HH pathway in thymocytes reduces the intensity of the TCR
154 signal, thereby interfering with TCR-mediated positive selection and allowing the
155 escape of autoreactive cells from clonal deletion(15). Likewise, SHH modulates
156 lymphocyte activation and cytokine production in peripheral CD4+ T lymphocytes,
157 cytotoxic T lymphocyte function, proliferation in germinal center B cells, and M2
158 polarization in macrophages(16–19). Whether aberrant HH activation in immune cell
159 subpopulations might therefore contribute to scGVHD onset and maintenance, and
160 account to certain extent for the clinical activity of SMOi in this setting, remains
161 unexplored.

162 HH signaling is amenable to therapeutic intervention through the inhibition of
163 smoothened (SMO), a transducer protein with a central role in this pathway. Upon
164 binding of HH ligands to the patched homolog-1 (PTCH1) membrane receptor, SMO is
165 released to direct the downstream activation of the HH signaling transcriptional
166 program through the stabilization of GLI family zinc finger transcription factors(20–
167 22). Prominent HH signaling dysregulation across cancer types(23) has propelled the
168 development of SMO inhibitors (SMOi), with approved agents in clinical use for the
169 treatment of basal-cell carcinoma and acute myeloid leukemia(24–26).

170 Here, we report the results of two independent phase I/II clinical trials that evaluated
171 glasdegib, an orally bioavailable, selective, small-molecule SMO antagonist(27,28), as a

novel therapeutic agent in patients with refractory scGVHD. Our clinical data was complemented by correlative studies conducted with the aim of determining if fibroblast-independent immune modulation might contribute to glasdegib activity in this patient population.

Methods

Study design

Two independent, single-arm, open-label, phase I/II investigator-initiated studies [NCT03415867, Spanish Hematopoietic Transplantation and Cellular Therapy Cooperative Group (GETH-TC); NCT04111497, Fred Hutchinson Cancer Center/Huntsman Cancer Institute/Duke University Medical Center (FHD)] were conducted at 5 centers in Spain and 3 centers in the United States.

Primary endpoints were dose-limiting toxicity (DLT) and maximum tolerated dose (MTD) in the GETH-TC trial, and safety and tolerability measures [i.e. type and severity of adverse events (AEs), time on treatment, and reasons for discontinuation] in the FHD trial. Secondary endpoints included best overall response rate (ORR) in cGVHD and sclerotic manifestations, patient-reported outcomes, and immunological correlates of response. Data cut-off was at 12 months from the time of glasdegib initiation.

Glasdegib was administered orally once daily (OD) in continuous 28-day cycles. The GETH-TC trial followed a dose-finding strategy, whereas the FHD trial employed a fixed-dose design. In the GETH-TC study, the starting treatment dose of glasdegib was 50 mg (Level 1), with subsequent dose escalation/de-escalation stages planned at 25 mg (Level -1), 100 mg (Level 2), 150 mg (Level 3), and 200 mg (Level 4) per a standard 3+3 escalation design. DLTs were defined as any treatment-related grade (G) ≥3 toxicity that was uncontrolled despite optimal medical management, excluding G ≥3 electrolyte abnormalities and ALT/AST elevations that returned to G ≤1 within 7 days. The DLT-evaluable period spanned the first two cycles of treatment. An independent data monitoring committee (DMC) was established to evaluate the emerging safety profile before each dose escalation stage. Intra-patient dose re-escalation after a toxicity-related dose reduction was not permitted. Patients in the FHD study received glasdegib at an initial dose of 50 mg. Dose reduction or interruption was indicated in the event of treatment-related toxicity G ≥3 or intolerable treatment-related toxicity of any grade, at least until the AE resolved to G ≤2 or stabilized to an acceptable degree. Taper and eventual withdrawal of concurrent immunosuppressive treatment was allowed in the event of a clinical response that was maintained after cycle 2 in the GETH-TC trial, or at any time in the FHD trial. Target sample size was 20-24 patients in the GETH-TC trial and 20 patients in the FHD trial. AEs were graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE), version 4.0 (GETH-TC) or 5.0 (FHD).

The study protocols were approved by the institutional review boards of the participating centers. All patients provided written informed consent, and the studies were conducted in accordance with the principles of the Declaration of Helsinki. Both trials were registered at ClinicalTrials.gov.

Patient eligibility

Adult (≥ 18 years) patients with active scGVHD per 2014 NIH Consensus Criteria(29) after ≥1 (FHD) or ≥2 (GETH-TC) prior lines of systemic therapy were recruited into the studies. Patients with baseline QTc interval prolongation (i.e. QTc >470 or 480 milliseconds in the GETH-TC and FHD trials, respectively) were excluded. Patients with a history of clinically significant muscle cramping (G2-3, or G1 that occurred at least weekly) were excluded from the FHD study after protocol amendment. Concurrent use of known strong CYP3A4/5 inducers was not allowed. Patients could maintain ongoing (begun prior to study enrollment) treatment for cGVHD, but addition of new lines of systemic immunosuppresive therapy while on study treatment was prohibited.

cGVHD response assessment

Chronic GVHD organ-specific response assessments were performed per 2014 NIH Response Criteria Working Group Report recommendations(30). Changes in sclerosis were serially measured using the NIH skin and/or joint tightening semiquantitative (0-10 points) scale. Additionally, a modified scGVHD partial response (PR) was defined as skin and/or joints score improvement (≥1 point for body surface area, skin features or joints/fascia scores; ≥2 points for skin/joint tightening or P-ROM scores) in the absence of concomitant worsening in any other of those scores or worsening ≥2 points in the global severity score. Response assessments were obtained at the end of cycles 1, 3, 6, 9 and 12 in the GETH-TC trial, and monthly in the FHD trial.

Patient-reported outcomes

In the GETH-TC trial, patient-reported cGVHD global severity and skin tightness scores were obtained through the NIH self-report questionnaire. The modified Lee

cGVHD Symptom Scale (mLSS)(31) was used in the FHD trial. Patient-reported outcomes were obtained at the end of cycles 1, 3, 6, 9 and 12 in both studies.

Immune correlative studies

All studies were performed on peripheral blood samples that were serially obtained on day 1 of cycles 1, 2 and 4 from patients in the GETH-TC trial.

The monoclonal antibodies used are listed in the **Supplementary Tables 1-3**.

Flow cytometry and immunophenotypic analysis

Antibody-stained samples were acquired in a FACSCanto II or a FACSLyric flow cytometer [Becton Dickinson (BD)] using the FACSuite software (BD). Data analysis was performed using the Infinicyt software 2.0 (Cytognos).

T cell receptor (TCR) repertoire diversity

The IOTest® Beta Mark Kit (Beckman Coulter) was employed for the quantitative, flow cytometric-based analysis of the TCR Vβ repertoire according to the manufacturer's instructions. TCR Vβ repertoire diversity was quantified using a Gini-like diversity index(32).

T-cell co-stimulatory and co-inhibitory molecules expression assays

Expression of T-cell co-stimulatory and co-inhibitory molecules (4-IBB, PD-1, OX40, CTLA-4 and TNFRSF18) in the CD4 and CD8-positive compartments was measured by flow cytometry under resting conditions and after 4-hour incubation with ionomycin (0.91 μg/mL) and phorbol myristate acetate (PMA) (20 μg/mL). Brefeldin A (10 μg/mL) was added in both conditions.

Phosphoflow analysis of T-cell activation signalling pathways

Phosphoflow analysis of ERK (pT202/204), p38 (pT180/y182), STAT3 (pY705), and STAT5 (pY694) was performed using the Phosflow T Cell Activation Kit (BD). Briefly, after staining for extracellular markers (CD3, CD4, and CD8) for 30 minutes, samples were activated (or not) with PMA 40 nM (pERK and p38 analyses), IL-2 50 ng/mL (pSTAT5 analysis) or IL-6 20 ng/mL (pSTAT3 analysis) for 15 minutes at 37 ºC. Peripheral mononuclear cells were then fixed, washed and permeabilized with Phosflow Perm Buffer III (BD). Lastly, cells were stained for phosphoproteins for 1 hour, and washed before acquisition in the cytometer. Median fluorescence intensity (MFI) was determined for each marker.

Statistical analyses

Safety data were summarized using descriptive statistics. Chronic GVHD response rates were estimated with 80% binomial confidence intervals (CIs) using the Clopper-Pearson exact method. For immune correlative studies, the Friedman test was employed to evaluate differences between serial measurements, and the Wilcoxon rank sum test was used for post-hoc pairwise comparisons. Bonferroni corrections were applied to account for multiple testing. Statistical analyses were performed using R (http://www.r-project.org).

Data availability

The data generated in this study are available upon request from the corresponding author.

Results

Patient demographics and baseline characteristics

Thirty six patients are included in this analysis, 21 from GETH-TC (enrolled January 2018-October 2019) and 15 patients from FHD (enrolled January 2020-January 2022). A non-DLT-evaluable patient in the GETH-TC trial was replaced per protocol after study exit due to a G3 treatment-unrelated acute cerebrovascular ischemic event early after enrollment in Cohort 1, resulting in his exclusion from the primary analysis. Median age was 47 (range 27-73) and 64 (33-74) years in the GETH-TC and FHD studies, respectively. Median time from the onset of cGVHD to study entry was 31 (7-106) and 30 (6-101) months. Chronic GVHD involvement in ≥4 organs was present in 80% and 60% of patients, and the baseline median NIH skin/joint tightening score was 8 (6-9) and 7 (2-9) points. Enrolled patients had received a median of 4 (2-10) and 3 (1-16) lines of therapy. Of note, 10 (50%) and 9 (60%) patients had undergone prior treatment with ruxolitinib. Patient and disease characteristics are detailed in **Table 1**.

Dose escalation and safety analysis

In the GETH-TC trial, three patients were recruited into Cohort 1 (Level 1, glasdegib 50 mg), and 3 patients into Cohort 2 (Level 2, glasdegib 100 mg). Two patients in Cohort 2 experienced G3 treatment-related muscle cramps that qualified as DLTs. Further dose escalation was halted following DMC evaluation of these events, and the MTD was established at 50 mg daily. Fourteen additional patients received treatment at the MTD. In the FHD study, a pre-specified trial stopping rule was triggered by the emergence of muscle cramps, and the protocol was modified to exclude patients with G≥2 cramps or G1 cramps at least once per week. Despite these changes, the stopping rule was met again resulting in recruitment closure. A total of 6 patients in the FHD study stopped glasdegib due to muscle cramping.

A summary of treatment-emergent AEs (TEAEs) is reported in **Table 2**. Frequent G≥2 TEAEs in the GETH-TC trial included muscle cramps (85%), alopecia (50%) and dysgeusia (35%). Two (10%) patients developed G3 treatment-related creatine kinase (CK) elevations that resolved after treatment interruption, not requiring study discontinuation. Similarly, muscle cramps were the most frequently reported TEAE in the FHD trial, with G3 events occurring in 5 (33%) patients. No clinically significant QTcF prolongation events were reported in either trial.

In the GETH-TC trial, 8 patients prematurely stopped glasdegib before completion of the first 12 cycles of therapy. Reasons for study discontinuation included muscle cramps (n=2), muscle cramps and diarrhea (n=1), biochemical relapse of multiple myeloma (n=1), thrombocytopenia in the absence of alternative explanatory cause (n=1), transaminitis and repeated infections (n=1), myocardial infarction (n=1), and withdrawal of consent (n=1). Glasdegib dose reductions were undertaken in 10 patients

as a result of the ocurrence of muscle cramps (n=5), CPK elevation and muscle cramps (n=2), diarrhea (n=1), nausea and vomiting (n=1), and repeated infections (n=1). Thirteen patients were active in the study at the end of cycle 12, and 10 patients remained on treatment beyond the data cut-off. In the FHD trial, muscle cramping (n=4), myalgia (n=2) and dysgeusia (n=2) led to dose reductions in 5 patients. Reasons for study discontinuation included muscle cramping or myopathy (n=8), lack of efficacy (n=2), disease relapse (n=1), and cGVHD progression (n=1). Three patients remained on treatment for longer than 12 months. Median time until discontinuation of glasdegib was 6.7 months (range 1.6-12.0) in the GETH-TC trial and 2.7 months (0.7-8.3) in the FHD trial. No patients died on study treatment.

Efficacy

Skin/joint scGVHD

At the 12-month data cut-off, 9 patients (45%) in the GETH-TC trial had achieved a skin body surface area and/or sclerotic features PR, and 9 patients (45%) had obtained a joints/fascia response (6 PR, 3 complete responses [CR]). Corresponding findings in the FHD trial were 5 patients (33%; 3 PR, 2 CR) and 3 patients (20%; 2 PR, 1 CR). An improvement ≥2 points in the total P-ROM score was observed in 11 patients (55%) in the GETH-TC study and 5 patients (38%; 2 patients with normal baseline P-ROM were excluded from the denominator) in the FHD study (**Table 3**). Among these, median total P-ROM score maximum change was 4 (range 2-8) and 2 (2-5) points (**Figure 1A-B**). Additionally, an improvement ≥2 points in the 0-10 skin and/or joints tightening severity scale was reported in 13 (65%) and 4 patients (31%; not applicable to 2 patients with baseline sclerotic features score <3) in the GETH-TC and FHD trials, respectively (**Table 3**). Among responders, median improvement in skin and/or joints tightening

severity scores was 3 (range 2-6) and 2.5 (2-5) points (**Figure 1C-D**). Overall, 13 patients (65%) obtained a skin/joint scGVHD PR in the GETH-TC trial, with a median time of 2.1 (range 0.9-6.0) and 8.4 (2.1-12.0) months to first and best response. Likewise, 7 (47%) patients had a skin/joint scGVHD response (6 PR, 1 CR) in the FHD cohort, with a median time of 0.9 (range 0.9-2.8) and 2.8 (1.7-6.0) months to first and best response (**Table 3 and Figure 2A-B**). At the 12-month data cut-off, median time of skin/joint scGVHD response on study was 9.0 months (range 0.0-11.0) in the GETH-TC trial and 1.9 months (range 0.0-11.0) in the FHD trial (**Figure 2A-B**). Median time on study for responder patients was 12.0 (2.1-12.0) months in the GETH-TC trial vs. 3.6 (0.7-12) months in the FHD trial. Similarly, median time on study for non-responding patients was 5.6 (1.6-12.0) months in the GETH-TC trial vs. 2.7 (1.0-12.0) months in the FHD trial. Of note, there were patients who achieved a response after prior treatment with ruxolitinib (GETH-TC n=6, FHD n=4), and ibrutinib (GETH-TC n=1).

Other target cGVHD organs

Clinical responses were also seen across other cGVHD target organs. Mouth (11/16, 69%; 5/9, 56%), eye (5/16, 31%; 3/10, 30%), lungs (2/7, 29%; 3/7, 43%), esophagus (6/6,100%; 1/1, 100%), upper gastrointestinal tract (1/3, 33%; 2/2, 100%), and lower gastrointestinal tract (2/2, 100%; 3/3, 100%) responses were reported in the GETH-TC and FHD trials, respectively. Organ-specific response rates are detailed in **Table 3**.

Corticosteroid sparing

Sixteen (80%) patients in the GETH-TC trial were receiving treatment with corticosteroids (CS) at the time of study entry. Among these, thirteen patients (81%; all skin/joint scGVHD responders and 2 non-responders) achieved a CS dose reduction.

379 The median CS dose decreased from 0.22 mg/kg per day (range 0.02-0.49) (prednisone
380 equivalent) at baseline to 0.11 mg/kg per day (0.01-0.39; median change -52%).

381 In the FHD trial, 7 (47%) patients were on CS at baseline, of whom 6 (86%; all
382 skin/joint scGVHD responders and 3 non-responders) reduced their CS dose. Here, the
383 median CS dose decreased from 0.18 mg/kg per day (range 0.11-0.34) to 0.14 mg/kg
384 per day (0.09-0.45; median change -23%) (**Table 3**).

385 **Patient-reported outcomes**

386 In the GETH-TC trial, self-reported cGVHD global severity and skin tightness scores
387 were serially available (≥75% of scheduled visits) in 13 patients, 8 of whom were
388 responders per NIH physician-assessed response criteria. Seven (54%; 6 skin/joint
389 scGVHD responders) and 5 (38%; 4 skin/joint scGVHD responders) patients reported a
390 ≥2-point improvement lasting for ≥2 consecutive visits in cGVHD global severity and
391 skin tightness scores, respectively.

392 In the FHD trial, the median mLSS summary score at baseline was 21.2 points (range
393 2.8-26.1), and median change during follow-up was -3.1 points (range -18.3-11.4). Only
394 1 non-responder patient reported a clinically significant improvement ≥7 points. Two
395 patients had a ≥7-point mLSS worsening (1 responder and 1 non-responder).

396 **Analyses of fibroblast-independent immunomodulatory effects of glasdegib**

397 The baseline distributions of key immune cell subpopulations in patients and in healthy
398 controls are shown in **Supplementary Table 4**. TCR Vβ repertoire in scGVHD patients
399 was often characterized by clonotypic dominance resulting in decreased diversity
400 quantified by a Gini-like index (**Figure 3A**). Yet, treatment with glasdegib was not
401 associated with changes in TCR Vβ repertoire diversity over time (**Figure 3B**).
402 Similarly, no differences from baseline were detected in the distribution of circulating

T-cell subpopulations (i.e. naive, central memory, effector, or peripheral memory $CD4^+$ and $CD8^+$ lymphocytes), or in the prevalence of regulatory-enriched T cells and monocyte subsets (**Figure 3C-E**). Treatment with glasdegib also failed to show an impact on the expression of co-stimulatory and co-inhibitory molecules (4-IBB, PD-1, OX40, CTLA-4 and TNFRSF18) in the $CD4^+$ and $CD8^+$ compartments (**Supplementary Figure 1**). No effect of glasdegib on downstream T-cell activation as measured by the phosphoflow analysis of ERK, p38, STAT3, and STAT5 was detected (**Supplementary Figure 2**). Finally, no changes were seen in the distribution of total, naive, unswitched memory, and switched memory B-cells, nor in the expression of BAFF (**Supplemementary Figure 3**). Overall, no differential patterns in the immune profiles between glasdegib responders and non-responder patients were observed. As 11 out of 13 skin/joint scGVHD responders in the GETH trial achieved their first response within 3 months of treatment with glasdegib, it was not possible to make any meaningful inferences regarding potential differences in the immunophenotypic repertoires between early responders and late responders in our analyses.

Discussion

These two prospective studies represent the largest reported cohort of patients with scGVHD treated with a SMOi. With response rates of 65% and 47% in skin/joint scGVHD after treatment with glasdegib, our findings provide proof of concept of the clinical actionability of the HH signaling pathway in this hard-to-treat patient population. Likewise, our data highlight the distinct challenges derived from the use of SMOi in this setting, and more generally, the difficulties associated with the performance of clinical trials in patients with scGVHD.

Notably, both of our studies encountered similar obstacles related to the emergence of DLT-defining muscle cramps, which precluded dose escalation and resulted in study closure in the GETH-TC and FHD trials, respectively. Muscle cramps, a well-characterized class-effect of HH signaling inhibitors, are thought to result from the non-canonical, SMO-mediated, paradoxical activation of muscle glucose uptake and Ca^{2+} influx(33). Contrasting with the very high incidence of muscle cramps in our studies

(including G3 events in one third of the patients), muscle cramps of any grade were experienced by 23% of patients in the experimental arm of the randomized trial of glasdegib (100 mg daily) plus low-dose cytarabine in acute myeloid leukemia or high-risk myelodysplastic syndrome, with G3 events reported in only 5%(26). Thus, our data suggest that the triggering threshold for SMOi-induced muscle AEs may be unusually low in patients with scGVHD. Of note, the high baseline prevalence of muscle cramps and myalgias among cGVHD patients may interfere with determining causality and could worsen this muscle toxicity(34). However, the exclusion from the FHD trial of patients with a significant history of muscle cramping did not appear to mitigate the risk of intolerable cramping. Other on-target SMOi-related AEs such as dysgeusia and alopecia, although not constituting DLTs, were common and could be expected to impose a substantial burden on quality of life for patients undergoing long-term treatment with glasdegib. Both pharmacological interventions and non-continuous dosing schedules have been proposed for the management of SMOi-associated muscle cramping when treating basal cell carcinoma(35). Among pharmacological interventions, there is a rationale for the use of calcium channel antagonists based on the pathophysiology of muscle cramps. In a pilot study, treatment with amlodipine was associated with a decrease in the frequency of muscle cramps, but no changes in their intensity or duration were detected(36). Other drugs have also been proposed for the control of muscle cramps, although the level of evidence regarding their efficacy is limited. These include antimuscarinic agents, anticholinergics, or baclofen(35). Intermittent treatment regimens could represent another strategy to optimize SMOi tolerability. In this regard, the results of a randomized phase 2 study evaluating two intermittent treatment regimens with vismodegib suggest that therapeutic rest periods could reduce the incidence of limiting toxicities in patients on long-term therapy(37).

These symptom palliation strategies were not assessed in our studies, but may have improved the tolerability and net clinical benefit of glasdegib in the scGVHD population.

Notwithstanding these major tolerability concerns, treatment with glasdegib was associated with clinically meaningful and sustained improvements in a range of skin/joint cGVHD standardized measures. Responses were also reported in other target organs in which fibrosis might play an important role, such as the esophagus and the gastrointestinal tract. Accordingly, and despite the frequent need for dose adjustments, some patients opted for extended treatment with glasdegib beyond the first year. Also notable from a efficacy standpoint, CS sparing was feasible in most patients. Although response rates were moderately higher in the GETH-TC trial, differences might be partially attributable to the longer exposure to glasdegib as compared to patients in the FHD study. Moreover, median times to first and best response in the FHD trial were shorter than in the GETH-TC trial. In this respect, it is worth noting that the median times on study for both skin/joint scGVHD responder and non-responder patients were significantly longer in the GETH trial. Additionally, standardized cGVHD assessments were performed on a monthly basis in the FHD trial, whereas these were only scheduled at the end of cycles 1, 3, 6, 9, and 12 in the GETH-TC trial. Therefore, longer follow-up and sparser cGVHD assessments might have led to the capture of more late-responding patients and deeper responses at later time points in the GETH-TC study compared to the FHD trial. These results are encouraging given the heavily pretreated nature of these two patients cohorts and the addition of glasdegib long after the initiation of the fibrotic cascade, once the irreversibility of fibrotic changes could be anticipated to pose a significant barrier to treatment efficacy. Targeting HH signaling earlier in the onset of sclerosis might help optimise the efficacy and/or tolerability of SMOi. In fact, SMO

498 inhibition has been shown most efficacious when used as prophylaxis in preclinical
499 models(14).

500 Overall, our results are consistent with two previous studies that evaluated the use of
501 sonidegib (n=17) and vismodegib (n=6) in cGVHD. In the sonidegib study, a PR rate of
502 47% was reported in skin or sclerotic disease, though responses were not assessed per
503 NIH criteria. The trial was terminated early as a result of worsening quality of life and
504 cumulative toxicity burden not attributed to sonidegib. No DLT related to muscle
505 cramping was reported, but 3 patients experienced G3 myalgias that were not attributed
506 to sonidegib(38). Similarly, preliminary evidence of efficacy was observed in the
507 vismodegib trial in 5 patients who achieved a PR as determined per 2014 NIH response
508 criteria. Treatment-related AEs, including muscle cramps and dysgeusia, were common,
509 and the study was closed due to slow accrual(19).

510 Beyond its direct influence on myofibroblast activity, HH signaling is involved in key
511 immune processes whose dysregulation might contribute to cGVHD pathogenesis,
512 encompassing TCR repertoire selection and deletion of autoreactive cells in the thymus,
513 B-cell homeostasis and M2 macrophage polarization(18,39–42). In this regard, our
514 extensive analyses failed to identify immunomodulatory effects or immune correlates of
515 response upon HH signaling inhibition with glasdegib. In contrast, treatment with
516 vismodegib has been associated with decreases in M2-like macrophages in skin biopsies
517 and circulating pre-germinal center and plasmablast-like B-cells, neither of which was
518 assessed in our trials(19). Additionally, as immune correlative analyses in the GETH
519 study were only performed in samples obtained within the first 3 months of treatment
520 with glasdegib, the possibility that immunomodulatory effects present at later time
521 points were not captured cannot be excluded. Outside the setting of cGVHD, recent
522 research has also implicated HH signaling activation in Th17 polarization in

inflammatory bowel disease, with vismodegib treatment or genetic ablation of Ihh in $CD4^+$ T cells greatly reducing disease severity in mouse models(43). Of note, the Th17 compartment was not evaluated in our correlative studies. Thus, it remains to be fully elucidated whether fibroblast-independent HH-mediated immune mechanisms might underlie some of the clinical activity of glasdegib.

This study has several limitations. First, no correlative studies were performed in skin samples allowing for analyses of dermal fibroblasts activity. Accordingly, pharmacokinetic/pharmacodynamic (PK/PD) data were not available. Therefore, no firm inferences can be made on the impact of the use of glasdegib at a dose of 50 mg daily on the degree of HH pathway modulation. However, marked (>80%) downregulation of GLI1 expression in the skin has been observed following treatment with glasdegib at doses ≥50 mg in phase 1 trials (28)(44). Taken together with the treatment-emergent safety and efficacy outcomes hereby reported, these data would indicate that a relevant biological effect can be achieved at the reduced doses employed in our studies. Second, caution should be exercised when interpreting our results considering the uncontrolled study designs, the partial overlap between SMOi-associated toxicities and cGVHD manifestations, and the inherent difficulties in accurately assessing clinical responses in scGVHD(30). Still, the fact that both independent studies yielded consistent outcomes supports the validity of our findings.

In recent years, the regulatory approvals of ruxolitinib, ibrutinib and belumosudil have expanded the treatment options for cGVHD. While these drugs have demonstrated activity in cutaneous cGVHD, response rates for sclerotic skin and joint disease have not been explicitly reported(8,45,46). Interestingly, clinical responses were seen in our studies in patients with prior exposure to ruxolitinib and ibrutinib. It is thus conceivable that glasdegib and other SMOi could fill a niche for some patients not responding to

tyrosine kinase inhibitors, particularly if better tolerated dosing schedules are determined. Looking further into the future of SMOi in this setting, the development of molecules lacking non-canonical HH signaling activating properties could allow for a more effective and better tolerated therapeutic blockade of this key pro-fibrotic pathway in patients with scGVHD(33).

Author contributions:

S.J.L. and J.A.P.S. conceived the studies and obtained funding. E.R.A., C.J.L., C.M., M.A.B.R., L.L.C., A.T., M.E.H., S.S., S.J.L., J.A.P.S. enrolled patients and collected data. C.J.L., L.O., S.J.L., E.R.A., and J.A.P.S. analyzed and interpreted the data; T.C.V. and C.G.C performed the flow cytometric analyses. E.R.A. wrote the manuscript draft. All authors revised and approved the final version of the manuscript for submission.

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Figure legends

Figure 1. Waterfall plots showing the individual maximum change from baseline in P-ROM score (A, GETH-TC trial; B, FHD trial) and NIH skin and/or joint tightening severity score (C, GETH-TC trial; D, FHD trial). The red line indicates the 2-point improvement threshold applied to the definition of response. Skin and/or joint tightening severity scores were not available for two patients in the FHD trial (baseline sclerotic features score <3).

Figure 2. Swimmer plots showing the timeline of glasdegib treatment, dose reductions and skin/joint scGVHD responses (A, GETH-TC trial; B, FHD trial). One patient in the GETH-TC trial and 1 patient in the FHD trial achieved a response at their last assessment.

Figure 3. TCR Vβ repertoire and immunophenotypic analyses of key immune cell subpopulations. A) Representative clonogram of a scGVHD patient showing clonotypic dominance resulting in decreased TCR Vβ repertoire diversity. B) Flow cytometric quantification of the TCR Vβ repertoire using a Gini-like diversity index. A value of 0 indicates equal distribution of TCR Vβ usage, and a value of 1 represents complete absence of diversity (C1D1: n=12 responders [R], n=7 non-responders [NR]; C2D1: 13 R, 6 NR; C4D1: 12 R, 4 NR). C) Fold change from baseline in the absolute count of circulating regulatory-enriched T cells (C1D1: 12 R, 7 NR; C2D1: 12 R, 7 NR; C4D1: 12 R, 5 NR). D) Fold change from baseline in the absolute count of circulating classical (left panel), intermediate (central panel) and non-classical monocytes (C1D1: 13 R, 7 NR; C2D1: 13 R, 7 NR; C4D1: 12 R, 5 NR). E) Distribution of circulating $CD4^+$ and $CD8^+$ T-cell subsets (C1D1: 13 R, 7 NR; C2D1: 13 R, 7 NR; C4D1: 12 R, 5 NR). Median + individual values (B-D) and mean + SEM (E) are shown. * Omnibus Friedman test. In post-hoc tests, only a statistically significant difference between visits C2D1 and C4D1 was detected (p=0.007).

767 **TABLE 1. Patient, disease and transplant characteristics.**

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Characteristic	GETH-TC cohort 1 (50 mg) (n=3)	GETH-TC cohort 2 (100 mg) (n=3)	GETH-TC expansion cohort (50 mg) (n=14)	All GETH-TC patients (n=20)	FHD patients (n=15)
Age, median (range), years	50 (42-53)	39 (33-60)	47 (27-73)	47 (27-73)	64 (33-74)
Female sex, n (%)	1 (33)	1 (33)	3 (21)	5 (25)	8 (53)
Myeloablative transplant, n (%)	2 (67)	2 (67)	8 (57)	12 (60)	5 (33)
Donor type, n (%)					
MSD	2 (67)	-	6 (43)	8 (40)	6 (40)
MURD	1 (33)	3 (100)	4 (29)	8 (40)	7 (47)
MMURD	-	-	1 (7)	1 (5)	2 (13)
Haploidentical	-	-	3 (21)	3 (15)	-
Transplant indication, n (%)					
AML	2 (67)	-	6 (43)	8 (40)	5 (33)
ALL	-	2 (67)	2 (14)	4 (20)	3 (20)
CML					1 (7)
Lymphoma	1 (33)	-	2 (14)	3 (15)	3 (20)
MDS					2 (13)
CMPN	-	1 (33)	1 (7)	2 (10)	1 (7)
Other	-	-	3 (21)	3 (15)	-
ECOG score, n (%)					
0-1	2 (67)	2 (67)	11 (79)	15 (75)	-
2	1 (33)	1 (33)	3 (21)	5 (25)	
Time from transplant to cGVHD, median (range), months	6 (3-19)	5 (4-11)	10 (4-59)	9 (3-59)	7 (3-65)
Time from cGVHD to enrolment, median (range), months	71 (63-78)	52 (12-106)	28 (7-65)	31 (7-106)	30 (6-101)

Physician-assessed global severity score, median (range)	8 (8-9)	8 (5-9)	7 (6-9)	8 (5-9)	6 (3-9)
Skin/joint tightening score*, median (range)	8 (7-9)	8 (6-9)	8 (6-9)	8 (6-9)	7 (2-9)
Organ involvement, n (%)					
Mouth	2 (67)	3 (100)	11 (79)	16 (80)	6 (40)
Upper/lower GI tract	1 (33)	1 (33)	4 (29)	6 (30)	4 (27)
Eyes	3 (100)	3 (100)	10 (71)	16 (80)	10 (67)
Lung	1 (33)	1 (33)	6 (43)	8 (40)	5 (33)
Liver	1 (33)	-	2 (14)	3 (15)	0
Skin	3 (100)	3 (100)	14 (100)	20 (100)	15 (100)
Joints/fascia	3 (100)	3 (100)	14 (100)	20 (100)	13 (87)
Organs involved, median (range)	5 (3-6)	5 (4-5)	5 (3-6)	5 (3-6)	4 (1-6)
≥ 4 organs involved, n (%)	2 (67)	3 (100)	11 (79)	16 (80)	9 (60)
Prior treatment lines, median (range)	6 (5-10)	5 (3-10)	3 (2-6)	4 (2-10)	3 (1-16)
Prior treatment regimen, n (%)					
Ruxolitinib	3 (100)	2 (67)	5 (36)	10 (50)	9 (60)
Ibrutinib	-	-	1 (7)	1 (5)	1 (7)
ECP	3 (100)	2 (67)	9 (64)	14 (70)	8 (53)
Rituximab	2 (67)	1 (33)	3 (21)	6 (30)	3 (20)
Imatinib	3 (100)	1 (33)	2 (14)	6 (30)	2 (13)
mTOR inhibitor	1 (33)	1 (33)	6 (43)	8 (40)	7 (47)
CNI	1 (33)	3 (100)	3 (21)	7 (35)	9 (60)
Corticosteroids	3 (100)	3 (100)	14 (100)	20 (100)	13 (87)
Belumosudil	-	-	-	-	1 (7)
MMF	-	-	-	-	5 (33)
Total nodal irradiation	-	-	-	-	2 (13)
Dasatinib	-	-	-	-	2 (13)
Nilotinib	-	-	-	-	1 (7)
MTX	-	-	-	-	2 (13)

Ixazomib	-	-	-	-	1 (7)
Azathioprine	-	-	-	-	1 (7)

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; cGVHD, chronic graft-versus-host disease; CMPN, chronic myeloproliferative neoplasm; CNI, calcineurin inhibitor; ECOG, Eastern Cooperative Oncology Group; ECP, extracorporeal photopheresis; GI, gastrointestinal; MMF, mycophenolate mofetil; MMURD, mismatched unrelated donor; MRD, matched related donor; MTX, methotrexate; MURD, matched unrelated donor; scGVHD, sclerotic chronic graft-versus-host disease.

* Among those patients with skin features score=3 (n=20 in the GETH-TC trial; n=13 in the FHD trial).

783 **TABLE 2. Summary of treatment-emergent adverse events**

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	TEAEs						
	GETH-TC trial				FHD trial		
Event, n (%)	**≥G2**	**G3**	**G4**	**G3-4**	**G3**	**G4**	**G3-4**
Muscle cramps	17 (85)	6 (30)	-	6 (30)	5 (33)	-	5 (33)
Alopecia	10 (50)	-	-	-	-	-	-
Dysgeusia/hypogeusia	7 (35)	-	-	-	-	-	-
ALT/AST elevation	5 (25)	2 (10)	0	2 (10)	-	-	-
Diarrhea	5 (25)	1 (5)	0	1 (5)	-	-	-
Dry eye	5 (25)	1 (5)	-	1 (5)	-	-	-
Asthenia	4 (20)	1 (5)	0	1 (5)	-		
Lymphopenia	4 (20)	1 (5)	0	0	-		
GGT elevation	3 (15)	1 (5)	0	1 (5)	-		
Myalgia/myopathy	3 (15)	2 (10)	-	2 (10)	2 (14)	-	2 (14)
Nausea/vomiting	3 (15)	0	0	0	-	-	-
Neutropenia	3 (15)	1 (5)	0	1 (5)	-	-	-
CK elevation	2 (10)	1 (5)	1 (5)	2 (10)	-	-	-
Thrombocytopenia	2 (10)	1 (5)	1 (5)	2 (10)	-	-	-
Weight loss	2 (10)	0	-	0	-	-	-
Hyponatremia	-	-	-	-	2 (13)	-	2 (13)

Hypokalemia	-	-	-	-	2 (13)	-	2 (13)
Breast wound	-	-	-	-	1 (7)	-	1 (7)

For the GETH-TC study, all ≥ G2 TEAEs in ≥ 10% of patients when at least 1 event was categorized as possibly related to the study drug are included. For the FHD trial, all reported ≥ G3 TEAEs are included. Toxicity grading is based on CTCAE criteria v4.0. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; CK, creatine kinase; TEAE, treatment-emergent adverse event.

TABLE 3. Efficacy summary.

	GETH-TC trial (n=20)			FHD trial (n=15)		
Efficacy endpoint*	**CR**	**PR**	**CR + PR**	**CR**	**PR**	**CR + PR**
Skin/joint scGVHD, n (%, 80% CI)						
Overall response**	0/20	13/20 (65, 48-79)	13/20 (65, 48-79)	1/15 (7, 0.7-24)	6/15 (40, 23-60)	7/15 (47, 28-66)
Body surface area	0/20	9/20 (45, 29-62))	9/20 (45, 29-62)	2/15 (13, 4-32)	1/15 (7, 0.7-24)	3/15 (20, 8-39)
Sclerotic features	0/20	2/20 (10, 3-24))	2/20 (10, 3-24)	1/15 (7, 0.7-24)	2/15 (13, 4-32)	3/15 (20, 8-39)
Joints/fascia	3/20 (15, 6-30)	6/20 (30, 17-47)	9/20 (45, 29-62)	1/15 (7, 0.7-24)	2/15 (13, 4-32)	3/15 (20, 8-39)
P-ROM score†	3/20 (15, 6-30)	9/20 (45, 29-62)	12/20 (60, 43-75)	1/13 (8, 0.8-27)	4/13 (31, 14-52)	5/13 (38, 20-60)
Skin and/or joints tightening severity‡	0/20	13/20 (65, 48-79)	13/20 (65, 48-79)	2/13 (15, 4-36)	2/13 (15, 4-36)	4/13 (31, 14-52)
Other cGVHD target organs, n (%, 80% CI)						
Mouth	10/16 (63, 43-79)	1/16 (6, 0.7-22)	11/16 (69, 50-84)	4/9 (44, 21-70)	1/9 (11, 1-37)	5/9 (56, 30-79)
Liver	0/3	0/3	0/3	-	-	-
Esophagus	4/6 (67, 33-91)	2/6 (33, 9-67)	6/6 (100)	1/1 (100)	0/1	1/1 (100)
Upper GI	1/3 (33, 3-80)	0/3 (33)	1/3 (33, 3-80)	2/2 (100)	0/2	2/2 (100)
Lower GI	2/2 (100)	0/2	2/2 (100)	3/3 (100)	0/3	3/3 (100)
Lung	4/9 (44, 21-70)	0/9	4/9 (44, 21-70)	3/7 (43, 17-72)	0/7	3/7 (43, 17-72)
Eye	4/16 (25, 11-44)	1/16 (6)	5/16 (31,11-44)	3/10 (30, 12-55)	0/10	3/10 (30, 12-55)
CS dose reduction, n (%, 80% CI)						
Overall	13/16 (81, 63-93)			6/7 (86, 55-99)		
Skin/joint scGVHD responder	11/11 (100)			3/3 (100)		
Skin/joint scGVHD no-responder	2/5 (40, 11-75)			3/4 (75, 32-97)		
Median percent change in CS dose from baseline, % (range)						
Overall	-52 (-84, 0)			-23 (-64, 315)		
Skin scGVHD responder	-54 (-84, -15)			-59 (-64, -11)		
Skin scGVHD no-responder	0 (-84, 0)			-13 (-51, 315)		

807

Abbreviations: CI, confidence interval; CR, complete response; CS, corticosteroids; GI, gastrointestinal; PR, partial response; P-ROM, photographic range of motion; scGVHD, sclerotic chronic graft-versus-host-disease;

[*] The best response while on study is shown.

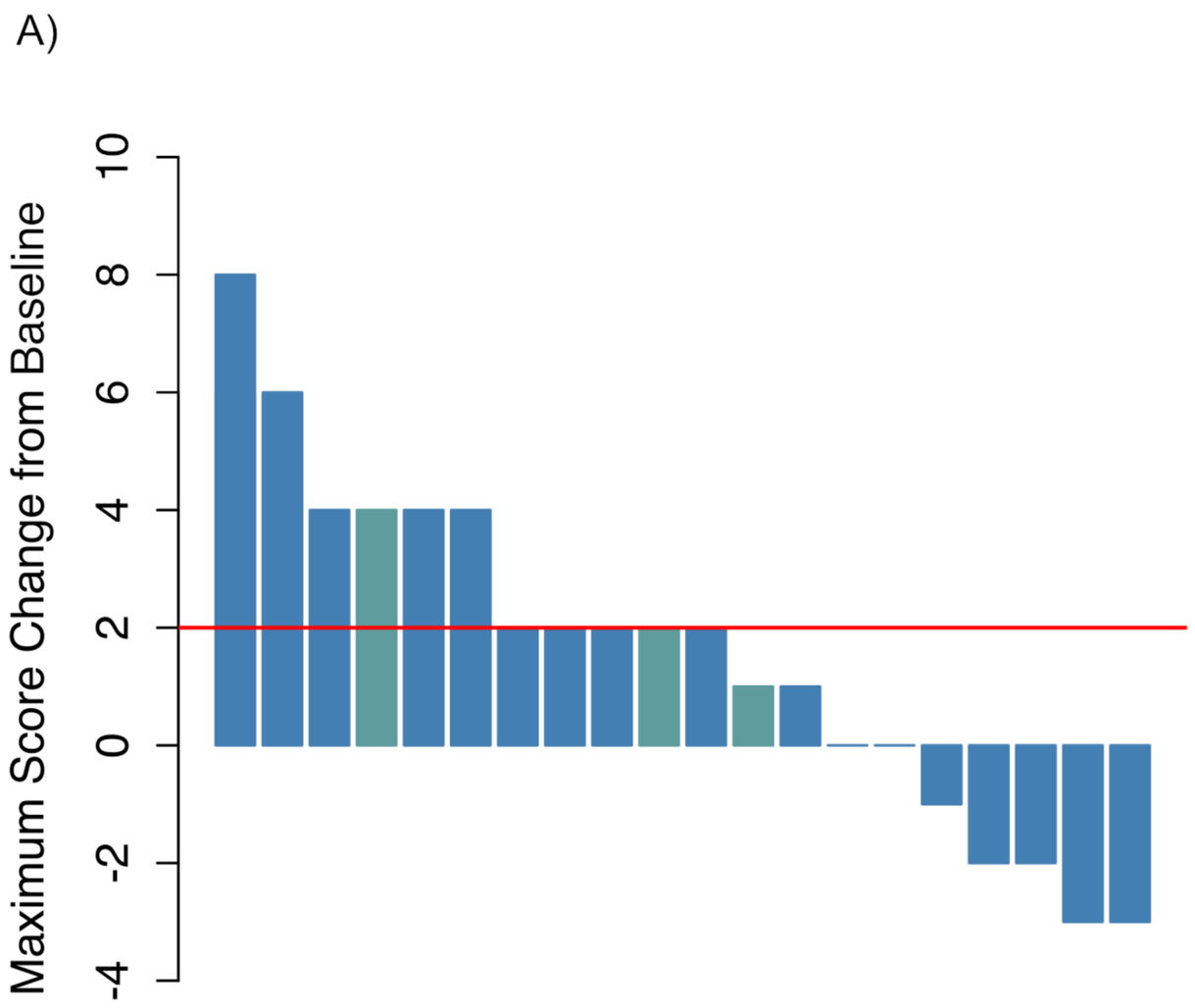
[**] Skin/joint scGVHD response was defined as skin and/or joint score improvement (≥1 point for body surface area, skin features or joint/fascia scores; ≥2 points for skin/joint tightening or P-ROM scores) in the absence of worsening in those scores or an increase ≥2 points in the global severity score.

[†] One patient in the GETH-TC trial with a baseline P-ROM score = 24 points achieved CR (1-point improvement) and was included among the responders. Two patients in the FHD trial had a baseline P-ROM score = 25 points and were excluded from the denominator.

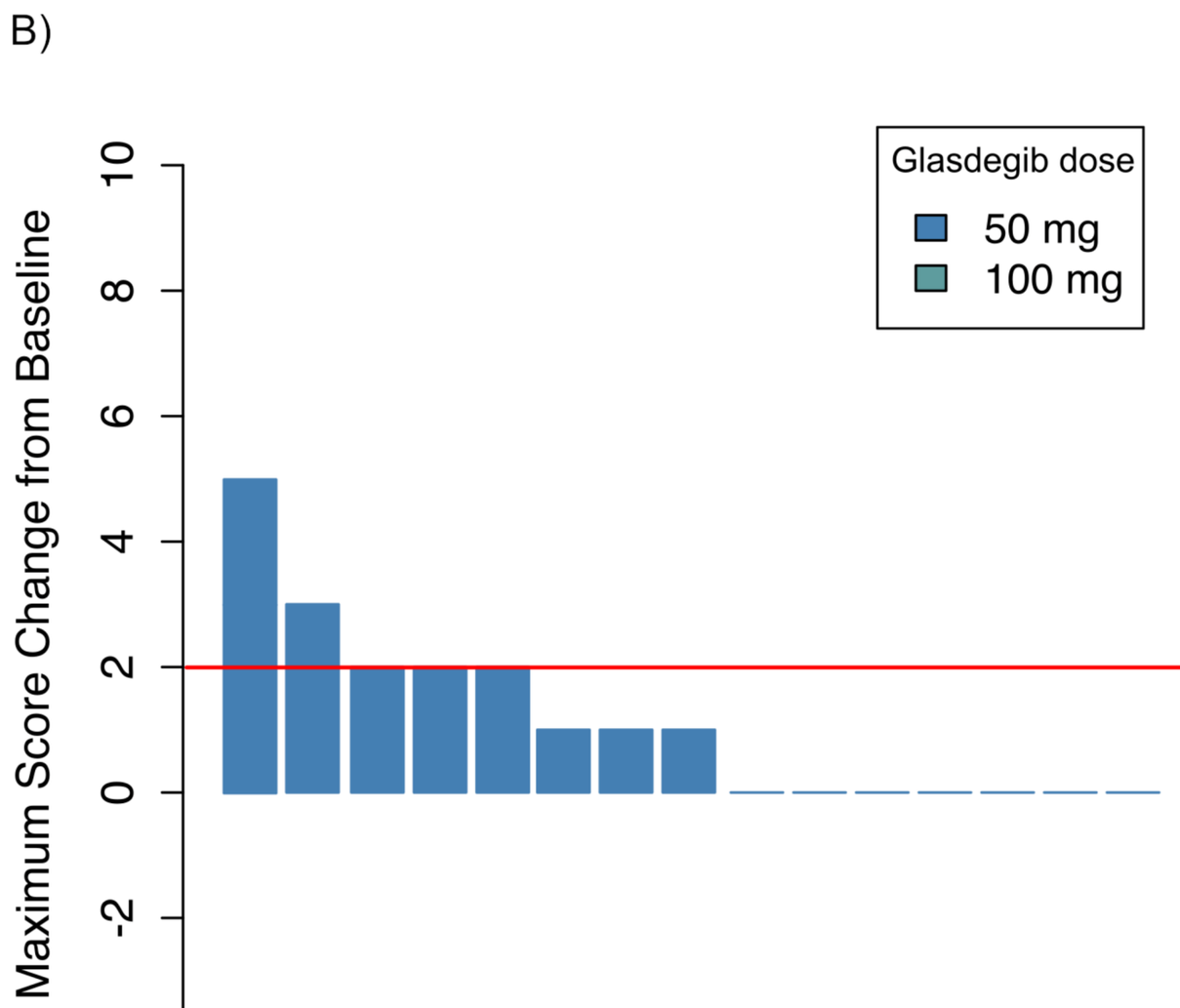
[‡] Two patients in the FHD trial had a baseline sclerotic features score <3 and were excluded from the denominator.

[‖] Two patients in the GETH-TC trial with altered %FEV1 at baseline were not evaluable.

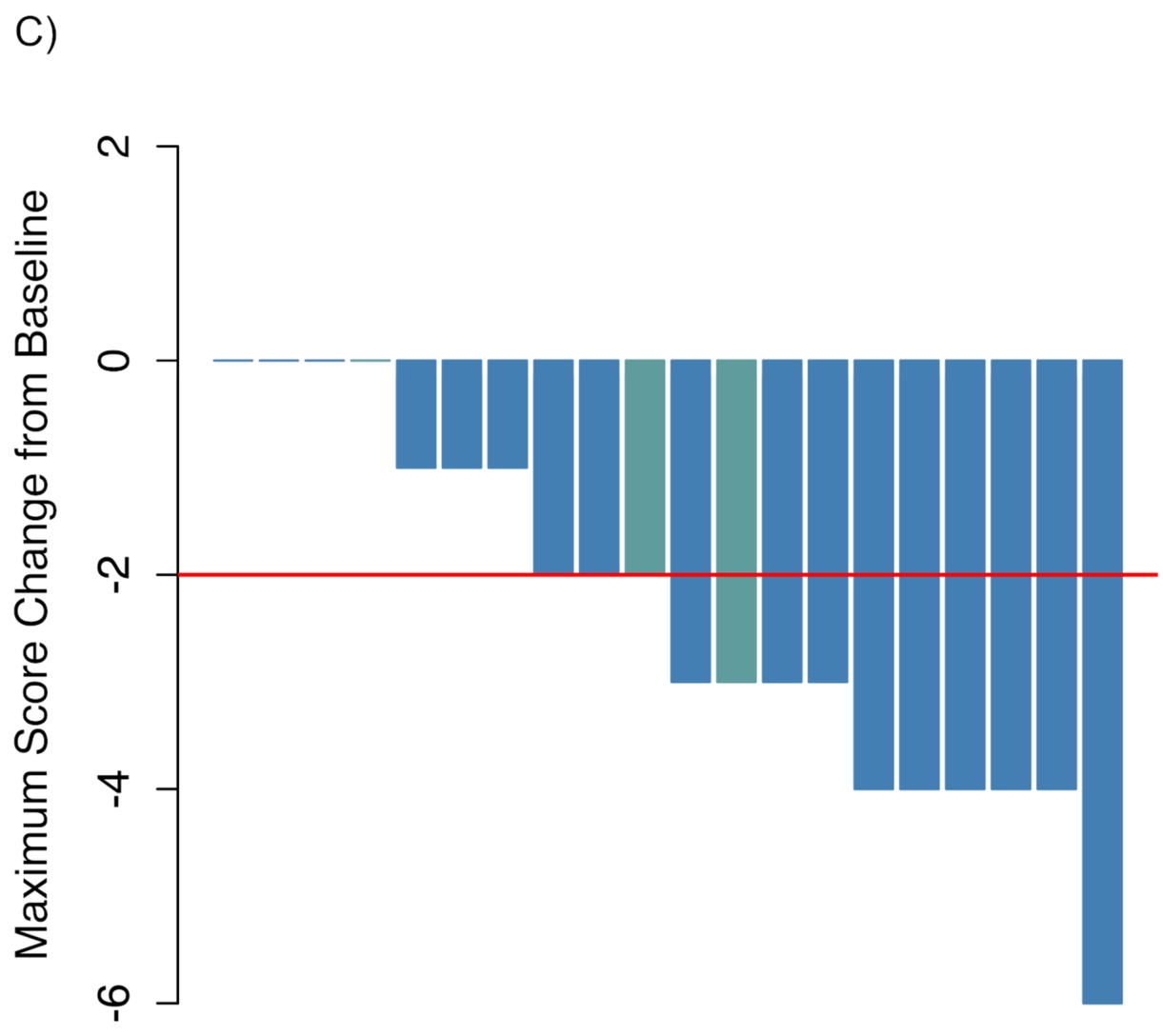
Figure 1



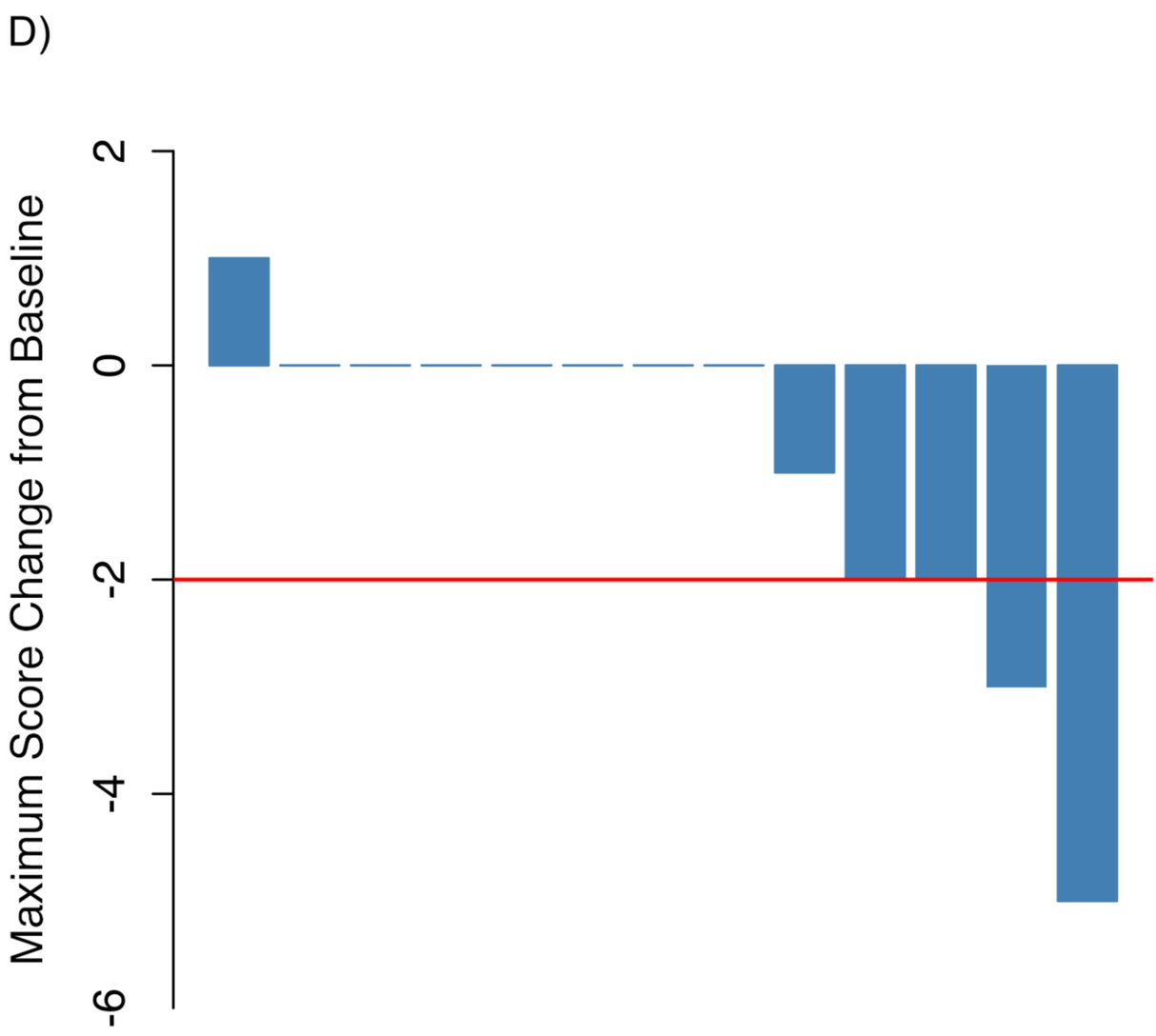
Baseline score	17	15	8	11	15	19	21	22	18	14	23	24	15	23	23	23	16	10	20	21



Baseline score	20	19	19	19	19	23	21	22	19	25	19	24	25	23	24



Baseline score	6	7	9	8	8	8	6	9	9	7	6	8	7	9	6	6	7	9	9	7



Baseline score	5	8	2	9	8	3	7	8	4	2	8	8	5

Figure 2

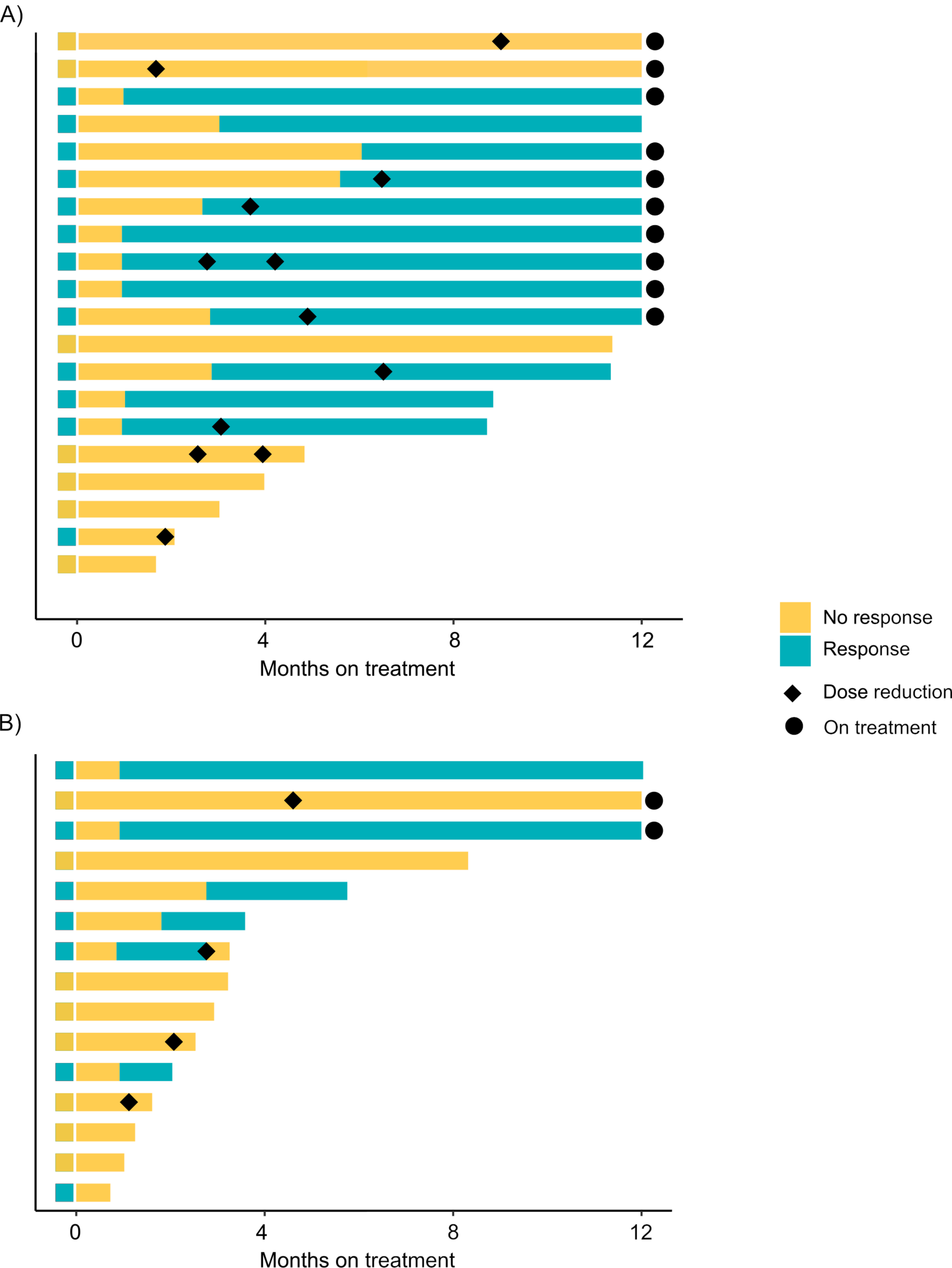
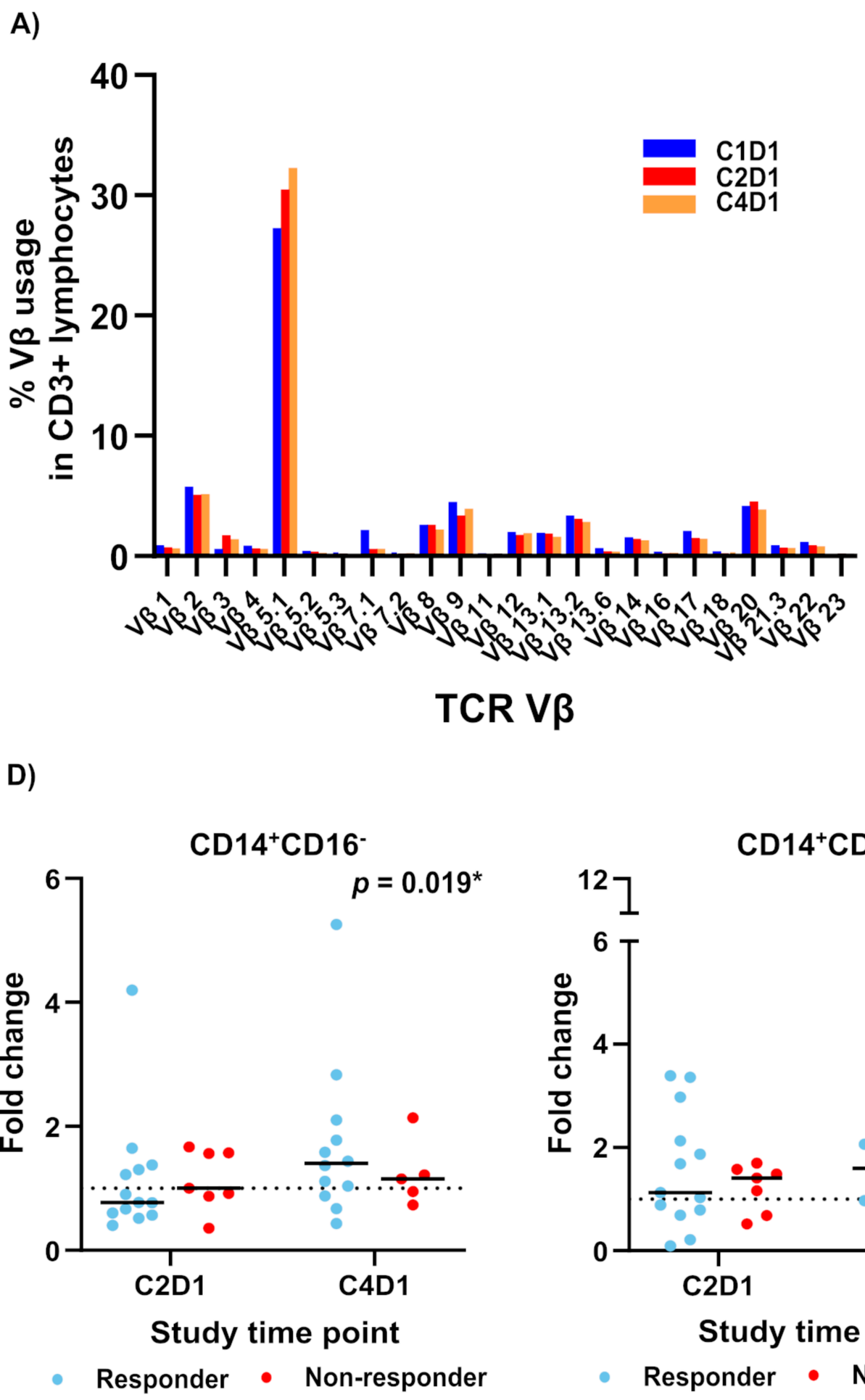

A)
0
4
8
12
Months on treatment
No response
Response
Dose reduction
On treatment
B)
0
4
8
12
Months on treatment

Figure 3


A)
% Vβ usage in CD3+ lymphocytes
C1D1
C2D1
C4D1
TCR Vβ
B)
Gini-TCR index
n.s.
C1D1
C2D1
C4D1
Study time point
Responder
Non-responder
C)
CD4+CD25+CD127-/lo
Fold change
n.s.
C2D1
C4D1
Study time point
Responder
Non-responder
D)
CD14+CD16-
p = 0.019*
CD14+CD16+
n.s.
CD14-CD16+
n.s.
Fold change
C2D1
C4D1
Study time point
Responder
Non-responder
E)
% CD4+ T-cells
% CD8+ T-cells
Peripheral memory (CD45RA-CCR7-)
Effector (CD45RA+CCR7-)
Central memory (CD45RA-CCR7+)
Naive (CD45RA+CCR7+)
C1D1
C2D1
C4D1
Study time point
Responders
Non-responders