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# Effect of emicizumab prophylaxis on bone and joint health markers in people with haemophilia A without factor VIII inhibitors in the HAVEN 3 study

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## Abstract

**Introduction:** Emicizumab prophylaxis significantly reduces bleeding events; however, the associated impact on bone/joint health is unknown.

**Aim:** To explore the effect of emicizumab prophylaxis on bone/joint health in people with haemophilia A (PwHA) without FVIII inhibitors enrolled in HAVEN 3 (NCT02847637).

**Methods:** Haemophilia joint health scores (HJHS; v2.1) were evaluated at baseline and Weeks 49 and 97 in PwHA receiving emicizumab ($n = 134$), and at baseline and Weeks 49, 73 and 97 in PwHA who switched to emicizumab after 24 weeks of no prophylaxis ($n = 17$). Bone and joint biomarkers were measured in 117 PwHA at baseline and at Weeks 13, 25, 49 and 73.

**Results:** HJHS was lower for PwHA who were previously on FVIII prophylaxis, aged <40 years or had no target joints at baseline compared with PwHA who were receiving no prophylaxis, aged ≥40 years or with target joints. Clinically significant mean (95% confidence interval) improvements from baseline of −2.13 (−3.96, −.29) in HJHS joint-specific domains were observed at Week 49 in PwHA with at least one target joint at study entry ($n = 71$); these changes were maintained through Week 97. Improvements in HJHS from baseline were also observed for PwHA aged 12–39 years. Biomarkers of bone resorption/formation, cartilage degradation/synthesis, and inflammation did not change significantly during emicizumab prophylaxis.

**Conclusions:** Clinically relevant improvements in HJHS were observed in younger PwHA and those with target joints after 48 weeks of emicizumab in HAVEN 3. Biomarkers of bone/joint health did not show significant changes during 72 weeks of emicizumab prophylaxis.

KEYWORDS
biomarkers, bone, emicizumab, factor VIII, haemophilia A, joints, prophylaxis

## 1 | INTRODUCTION

Haemophilic arthropathy can lead to a considerable reduction in quality of life (QoL) in people with haemophilia A (PwHA).[1,2] Haemophilia A (HA) is associated with decreased bone mineral density (BMD),[3–5] which can further reduce QoL.[6] The mechanism leading to reduced BMD is unclear, although it is likely multifactorial. Deficiencies in coagulation factors and reduced mobility due to haemophilic arthropathy and target joints have been suggested to contribute to reduced BMD.[7]

Factor (F)VIII prophylaxis has long been the standard of care for PwHA without FVIII inhibitors.[8] However, despite improvements in trough factor level and improved bleed control,[9] subclinical joint bleeding can still lead to debilitating joint damage.[10,11]

Emicizumab is a bispecific monoclonal antibody that bridges FX and activated FIX to restore the function of missing activated FVIII, resulting in downstream thrombin generation and fibrin clot formation.[12] The randomised phase III study HAVEN 3, part of the HAVEN clinical development programme, demonstrated the efficacy and favourable safety profile of emicizumab prophylaxis administered subcutaneously once weekly (QW) or every 2 weeks (Q2W) in PwHA aged ≥12 years without FVIII inhibitors. Emicizumab prophylaxis led to a significant reduction in bleed rate compared with no prophylaxis ($p < .001$) and with previous FVIII prophylaxis ($p < .001$; intraindividual comparison).[13] Emicizumab significantly reduced the risk of treated joint bleeds compared with previous episodic FVIII (96% and 97% reductions with QW and Q2W prophylaxis, respectively; both $p < .001$).[13]

This exploratory analysis aims to describe the bone and joint health of PwHA without FVIII inhibitors who received emicizumab prophylaxis during the HAVEN 3 study, and to explore the potential impact of reduced FVIII exposure on bone and joint health.[13] To quantify potential changes, the Haemophilia Joint Health Score (HJHS) and biomarkers for cartilage degradation, cartilage turnover, cartilage synthesis/repair and inflammation were used to examine joint health, and biomarkers for bone formation and resorption, and a receptor-ligand system involved in bone regulation were used to investigate bone health.

## 2 | METHODS

### 2.1 | Study design and participants

The study design and eligibility criteria for HAVEN 3 (NCT02847637) were published previously.[13] Participants receiving previous episodic FVIII were randomised (2:2:1) to treatment arms A–C (Table 1). For participants receiving emicizumab, a loading dose of 3.0 mg/kg QW was administered for 4 weeks followed by a maintenance dose of either 1.5 mg/kg QW (Arm A, $n = 36$) or 3.0 mg/kg Q2W (Arm B, $n = 35$). Participants in Arm C ($n = 18$) received no prophylaxis; after 24 weeks, they could switch to emicizumab 3.0 mg/kg Q2W. Participants previously receiving FVIII prophylaxis were assigned to Arm D ($n = 63$) and received a loading dose of emicizumab 3.0 mg/kg QW for 4 weeks followed by a maintenance dose of 1.5 mg/kg QW.[13] Data cut-offs for the exploratory analyses of HAVEN 3 bone and joint health data were May 2020 for haemophilia joint health score (HJHS) and October 2018 for biomarkers.

The study was conducted in compliance with the International Conference on Harmonisation Guidelines for Good Clinical Practice and the principles of the Declaration of Helsinki. The trial protocol was approved by the relevant independent review boards/ethics committees at participating sites and carried out in accordance with applicable regulations. All participants provided informed consent.

### 2.2 | Procedures

Joint health was evaluated using the HJHS v2.1, an internationally validated tool developed for the assessment of joint health in people with haemophilia.[14] HJHS v2.1 consists of eight item scores (swelling, duration of swelling, muscle atrophy, crepitus on motion, flexion loss, extension loss, joint pain and strength) for each joint and a global gait score. Scores range from 0 to 20 per joint and the global gait score ranges from 0 to 4, resulting in a HJHS total score of 0–124. HJHS was measured at Week 1 (baseline) and Weeks 49 and 97 (Arms A, B and D); and at baseline, Weeks 49, 73 and 97 (Arm C participants who switched to emicizumab prophylaxis after Week 24). For Arms A, B and D, Weeks 49 and 97 correspond to 48 or 96 weeks of emicizumab prophylaxis, respectively, and for Arm C, Weeks 49, 73 and 97 correspond to 24, 48 or 72 weeks of emicizumab, respectively.

Target joints were defined as major joints (e.g., hip, elbow, wrist, shoulder, knee and ankle) in which three or more bleeding events occurred in the same joint over the 24-week period before enrolment in HAVEN 3.

For bone and joint biomarker analysis, blood samples were collected on Day 1 prior to treatment (baseline), and after 12, 24, 48 and 72 weeks of emicizumab prophylaxis (Arms A, B and D) or at Weeks 1, 25, 37, 49, 73 and 97 (Arm C participants switching to emicizumab). Blood samples were collected per protocol before noon after at least 8 h of fasting and prior to emicizumab administration. Interleukin (IL)-1$\beta$, IL-6, tumour necrosis factor alpha (TNF$\alpha$), *C*-terminal telopeptide of type I collagen (CTX-I), and *N*-terminal propeptide of type I procollagen (P1NP) were evaluated in plasma. Cartilage oligomeric matrix protein (COMP), CTX-II, aggrecan chondroitin

**TABLE 1** Demographics and clinical characteristics of PwHA receiving emicizumab[a] in HAVEN 3[13]

| | PwHA in HAVEN 3 (all participants) | | | | | |
|---|---|---|---|---|---|---|
| | Arm A (emicizumab 1.5 mg/kg QW; *n* = 36) | Arm B (emicizumab 3 mg/kg Q2W; *n* = 35) | Arm C (no prophylaxis; *n* = 18) | Arm D (emicizumab 1.5 mg/kg QW; *n* = 63) | Total (*N* = 152) | PwHA in HAVEN 3 with biomarker data (*n* = 117) |
| Mean age (min–max), years | 39.8 (19–77) | 40.4 (20–65) | 37.8 (16–57) | 36.4 (13–68) | 38.3 (13–77) | 38.4 (13–77) |
| Age groups, *n* (%) | | | | | | |
| <18 years | 0 (0) | 0 (0) | 1 (5.6) | 7 (11.1) | 8 (5.3) | 7[b] (6.0) |
| Mean BMI (min–max), kg/m2 | 26.6 (20.0–33.7) | 26.7 (19.0–38.4) | 23.8 (16.8–36.2) | 25.6 (19.2–40.6) | 25.8 (16.8–40.6) | 26.0 (16.8–40.6) |
| Race, *n* (%) | | | | | | |
| White | 24 (66.7) | 20 (57.1) | 11 (61.1) | 47 (74.6) | 102 (67.1) | 80 (68.4) |
| Asian | 6 (16.7) | 10 (28.6) | 4 (22.2) | 12 (19.0) | 32 (21.1) | 22 (18.8) |
| Black/African American | 3 (8.3) | 1 (2.9) | 3 (16.7) | 1 (1.6) | 8 (5.3) | 5 (4.3) |
| Other | 1 (2.8) | 0 (0) | 0 (0) | 0 (0) | 1 (.7) | 1 (.8) |
| Unknown | 2 (5.6) | 4 (11.4) | 0 (0) | 3 (4.8) | 9 (5.9) | 9 (7.7) |
| Prior FVIII,[c] *n* (%) | | | | | | |
| Episodic | 36 (100) | 35 (100) | 18 (100) | 5 (7.9) | 94 (61.8) | 67 (57.3) |
| Prophylaxis | 3 (8.3) | 5 (14.3) | 2 (11.1) | 63 (100) | 73 (48.0) | 50 (42.7) |
| Target joints, *n* (%) | | | | | | |
| None | 2 (5.6) | 8 (22.9) | 3 (16.7) | 37 (58.7) | 50 (32.9) | 38 (32.5) |
| ≥1 | 34 (94.4) | 27 (77.1) | 15 (83.3) | 26 (41.3) | 102 (67.1) | 79 (67.5) |
| History of HIV infection,[d] *n* (%) | 5 (13.9) | 9 (25.7) | 5 (27.8) | 8 (12.7) | 27 (17.8) | 31 (26.5) |
| Osteoporosis, *n* (%) | | | | | | |
| Any | 1 (2.8) | 1 (2.9) | 0 (0) | 6 (9.5) | 8 (5.3) | 5[b] (4.3) |
| Treated | 1 (2.8) | 0 (0) | 0 (0) | 5 (7.9) | 6 (3.9) | 4[b] (3.4) |

Abbreviations: BMI, body mass index; FVIII, factor VIII; HIV, human immunodeficiency virus; PwHA, people with haemophilia A; QW, once weekly; Q2W; every 2 weeks.

[a]Participants received a loading dose of emicizumab 3 mg/kg QW for 4 weeks followed by either emicizumab 1.5 mg/kg QW or 3 mg/kg Q2W maintenance doses, according to the HAVEN 3 protocol.

[b]All in Arm D of HAVEN 3 (participants who had received prior FVIII prophylaxis, and received loading doses of emicizumab 3 mg/kg QW for 4 weeks followed by emicizumab 1.5 mg/kg QW maintenance).

[c]Some participants may have received both episodic and prophylactic FVIII treatment.

[d]Participants with HIV infection with CD4 counts >200 cells per $\mu$L who met all other inclusion criteria were eligible for inclusion in HAVEN 3.

sulphate epitope 846 (CS846), osteocalcin (OC), osteoprotegerin (OPG) and soluble receptor activator of nuclear factor-kappaB ligand (sRANKL) were evaluated in serum (for further details, see Table 2 and Supplement).

## 2.3 | Statistical analysis

Participant demographics and characteristics were summarised using descriptive statistics. HJHS total scores (overall or by domains/location) were re-calculated based on the original items and presented along with the HJHS totals recorded in the case report form (CRF). Re-calculated totals were set as missing if some of the items contributing to the total were missing. The re-calculated HJHS totals were considered primary, while the CRF-based scores were considered supportive. HJHS data (baseline and change from baseline) were described using summary statistics.

Changes from baseline (Week 1 for Arms A, B and D; Week 25 for Arm C) in HJHS are presented for all timepoints where scores were captured, with results for Arms A, B and D being combined. These complete case analyses (i.e., no imputation for missing data) were complemented with a 'last observation carried forward (LOCF)' imputation at Week 97. Ninety-five percent confidence intervals (CIs) were produced assuming that the mean and standard deviation (SD) for the

**TABLE 2** Bone and joint health biomarker concentrations at baseline in evaluable PwHA participating in HAVEN 3 ($n = 117$)

| Biomarker | Role in bone and joint health | *N* | Mean | SD | Median | Lower quartile | Upper quartile | Min-max | Lab or literature reference range |
|---|---|---|---|---|---|---|---|---|---|
| OC, nmol/L | Bone formation | 116[a] | 4.5 | 3.7 | 3.5 | 2.78 | 4.73 | 1.09–27.81 | 1.3–6.6 |
| P1NP, μg/L | Bone formation | 117 | 93.6 | 137.2 | 64.5 | 46.86 | 87.36 | 18.92–1197.00 | 13.9–85.5 |
| CTX-I, pg/ml | Bone resorption | 116[a] | 549.9 | 416.6 | 455.0 | 300.00 | 650.00 | 80.00–2890.00 | 40–2470 |
| OPG, ng/ml | Osteoblasts | 117 | .11 | .1 | .1 | .08 | .13 | .03–.31 | 2–584 pg/ml[15] |
| sRANKL,[b] ng/ml | Osteoclastogenesis | 117 | 7.6 | 29.0 | 2.0 | 2.00 | 6.30 | 2.00–313.54 | 11.6–36.7[16] |
| COMP, ng/ml | Cartilage turnover | 117 | 1055.4 | 520.9 | 968.8 | 676.4 | 1345.60 | 100.0–2990.1 | 180–1911 |
| CTX-II, ng/ml | Cartilage degradation | 117 | .4 | .1 | .4 | .32 | .50 | .18–.94 | .182–2.537[17,18] |
| CS846, μg/ml | Cartilage synthesis/repair | 117 | 134.9 | 84.4 | 130.8 | 102.90 | 175.30 | 25.00–411.60 | NA |
| IL-6,[b] ng/L | Inflammation | 117 | 1.7 | 2.9 | 1.1 | .48 | 1.88 | .48–27.78 | 3.1–27.2 |
| TNFα, ng/L | Inflammation | 117 | 3.3 | 1.5 | 3.1 | 2.52 | 3.78 | .19–10.32 | 2.4–4.3 |

Laboratory healthy reference range data are validated; literature reference data are used where limited data exist.
Abbreviations: BLQ, below limit of quantification; COMP, cartilage oligomeric matrix protein; CS846, aggrecan chondroitin sulphate epitope 846; CTX-I, C-terminal telopeptide of type I collagen; CTX-II, C-terminal telopeptide of type II collagen; HJHS, haemophilia joint health score; IL, interleukin; max, maximum; min, minimum; OC, osteocalcin; OPG, osteoprotegerin; P1NP, *N*-terminal pro-peptide of type I procollagen; PwHA, people with haemophilia A; SD, standard deviation; sRANKL, soluble receptor activator of nuclear factor-κβ ligand; TNFα, tumour necrosis factor alpha.
[a]Missing observation at baseline for $n = 1$.
[b]Values BLQ are imputed with half of that limit of quantification; 57% of values for sRANKL and 49% of values for IL-6 were BLQ; all IL-1β samples except for one were BLQ and are not shown here.

participant populations were not known and are estimated based on t-distribution calculations.

Raw/LOCF estimates of HJHS are also supplemented by mixed-effect model repeated measure (MMRM) estimates (assuming that missing values are occurring at random) to account for baseline characteristics and the repeated nature of the longitudinal data. The model includes fixed effects for weeks relative to enrolment, presence of target joints at baseline and age at baseline. An unstructured variance-covariance matrix is applied to model the within-patient errors. Interaction terms are used to estimate the mean change from baseline at each week depending on age or the presence of target joints at baseline. A change of ≥4 for total HJHS and of ≥2 for HJHS joint-specific domain is considered clinically relevant.[2]

Correlations within biomarkers and between biomarker levels and HJHS were determined using Pearson correlation coefficients and non-parametric Spearman correlation coefficients.

The data for these analyses were generated using SAS software, Version 9.4 of the SAS System for Windows. Copyright © 2021 SAS Institute Inc., Cary, NC, USA.

## 3 | RESULTS

### 3.1 | Study population

HAVEN 3 participants ($N = 152$) had a mean (range) age of 38.3 (13–77) years, while those with evaluable biomarker measurements at baseline ($n = 117$) had a mean age of 38.4 (13–77) years (Table 1). Around half of the participants (48.0%) had previously received prophylactic FVIII treatment for bleeds and 67.1% had at least one target joint at baseline; 5.3% of all participants ($n = 8/152$) and 4.3% of the biomarker group ($n = 5/117$) had a recorded diagnosis of osteoporosis (the majority in Arm D, 75.0%) included in their medical history.

### 3.2 | HJHS at baseline

A cut-off of median HJHS score at baseline among those who had previously received standard-of-care FVIII prophylaxis (which was 11) was used to illustrate the differences in baseline HJHS between subgroups via a categorical analysis.

Regardless of previous treatment, 49% of participants with no target joints had HJHS scores <11 at baseline compared with 29% of those with at least one target joint (Figure 1A). Accordingly, participants with at least one target joint at baseline had a worse HJHS compared with participants with no target joints (25.6 [SD 20.9; $n = 90$] vs. 15.9 [SD 16.8; $n = 43$]).

To explore the effect of age on joint health changes, a rounded cut-off of 40 years was selected based on the age distribution of all 152 HAVEN 3 participants as the median age across the study arms was 38 years. For participants with baseline HJHS available, those aged ≥40 years had higher HJHS, reflecting worse joint health, compared with participants aged 12–39 years (33.4 [SD 22.9; $n = 55$] vs. 14.8 [SD 13.5; $n = 78$]; Figure 1B). HJHS was positively correlated with

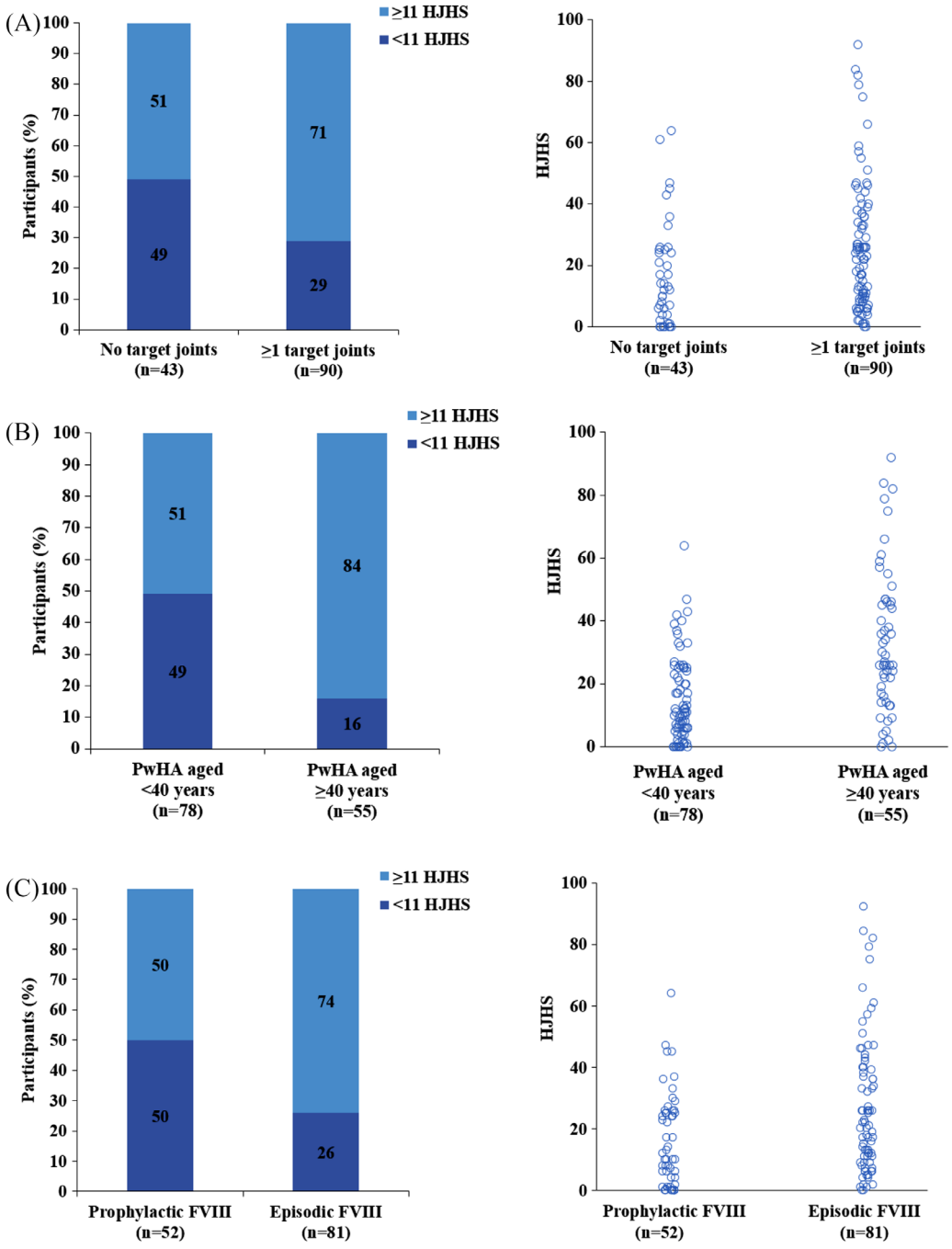



**FIGURE 1** Proportion of HAVEN 3 participants with HJHS scores <11 versus ≥11* at baseline and individual baseline HJHS scores by, target joint status (A), participant age (B) and previous treatment (C). The HJHS 2.1 consists of eight item scores on joint level and a global gait score. Scores range from 0 to 20 per joint and the global gait score ranges from 0 to 4, resulting in a HJHS total score (0–124); a higher score indicates worse joint health. *A cut-off score of 11 was used (median in the FVIII prophylaxis population based on the data collected). FVIII, factor VIII; HJHS, haemophilia joint health score; PwHA, people with haemophilia A

participant age, regardless of previous treatment (on-demand vs prophylaxis; Figure S1) and the number of target joints.

A higher proportion of participants previously taking episodic FVIII had a HJHS score >11 compared with those taking prophylactic FVIII at study entry (74% vs. 50%), indicating worse joint health (Figure 1C).

### 3.3 | HJHS at Weeks 49 and 97 following emicizumab prophylaxis

The mean (95% CI) improvement observed from baseline to Week 49 for total HJHS was −1.86(−3.53, −.20) for all participants. Improvements in HJHS were consistent across different joints. Mean (95% CI)

changes from baseline in HJHS scores at Week 49 were ($n = 107$; left and right combined): elbow, −.55 (−1.24, .14); knee, −.75 (−1.25, −.24); ankle, −.50 (−1.39, .38).

In all participants with at least one target joint at study entry, the mean (95% CI) improvements observed from baseline to Week 49 were −2.28 (−4.15, −.42) and −2.13 (−3.96, −.29) for total HJHS and the HJHS joint-specific domain (excluding gait score), respectively ($n = 71$; Figure 2A). No change in mean total HJHS was observed for participants with no target joints at baseline ($n = 38$). At Week 97, a total of 74/134 participants enrolled in Arms A, B and D had available HJHS data. Overall, changes from baseline in total HJHS and the HJHS joint-specific domain were maintained through Week 97 for PwHA with target joints (Figure 2A).

Similarly, in PwHA aged 12–39 years ($n = 67$) regardless of target joint status or previous treatment regimen, mean (95% CI) improvements of −3.22 (−5.40, −1.04) and −3.04 (−5.12, -.97) were observed at Week 49 for total HJHS and the HJHS joint-specific domain, respectively (Figure 2B). These changes were maintained through Week 97. No change in mean total HJHS was observed for participants who were ≥40 years old ($n = 42$). The MMRM analysis assessed the effects of age on estimated HJHS at Weeks 49 and 97 of emicizumab prophylaxis. The improvements in joint health (as shown by decreased HJHS from baseline) were greater in younger versus older participants, with the model predicting HJHS (95% CI) at Week 49 for PwHA age: 25 years, −3.08 (−5.25, .90); 40 years, −1.36 (−3.09, .37) and 55 years, .36 (−2.60, 3.32; Figure 2C). In addition, HJHS levels across age categories were maintained from Weeks 49 to 97, indicating continuation of improved joint health through approximately 2 years of emicizumab prophylaxis. The percentage of participants in different subgroups with HJHS or Sum of Joint scores of zero also increased slightly over time (Table S1), although this analysis was not sensitive enough to demonstrate significant benefit.

An analysis of Arm C participants ($n = 17$) predicted changes from baseline (Week 25) in estimated total HJHS (95% CI) following emicizumab: with changes at Week 49 of −1.16 (−4.95, 2.63) and at Week 73 of −1.42 (−7.83, 5.00). The numerical joint health improvements in this small group that switched to emicizumab at Week 25 were consistent with the observations in Arms A, B and D.

### 3.4 | Biomarker analyses at baseline

At baseline, large variability in bone and joint health biomarkers was observed between individual HAVEN 3 participants (Table 2). With the exception of sRANKL and IL-6, and despite some individual high and low values, median biomarker concentrations at baseline were within laboratory- or literature-based healthy reference ranges. Values below the limit of quantification (BLQ) were imputed with half of that limit of quantification; 57% of values for sRANKL and 49% of values for IL-6 were BLQ. All IL-1$\beta$ samples, except for one, were BLQ. No reference range was available for CS846.

At baseline, participant age was negatively correlated with biomarkers evaluated for bone formation (OC, P1NP) and bone resorption (CTX-I), driven by higher concentrations of those biomarkers observed in adolescents (ages <18 years). Participant age was positively correlated with biomarkers for cartilage turnover (COMP) and osteoblasts/osteoclastogenesis (OPG/sRANKL; $p < .001$ for Pearson and Spearman correlations; Table 3). No clear association was evident for biomarkers and body mass index. No significant differences in baseline values were observed in the biomarkers of PwHA previously on FVIII prophylaxis versus episodic treatment (Table S2), or in PwHA with target joints versus those without (Table S3).

### 3.5 | Biomarker analyses after emicizumab prophylaxis

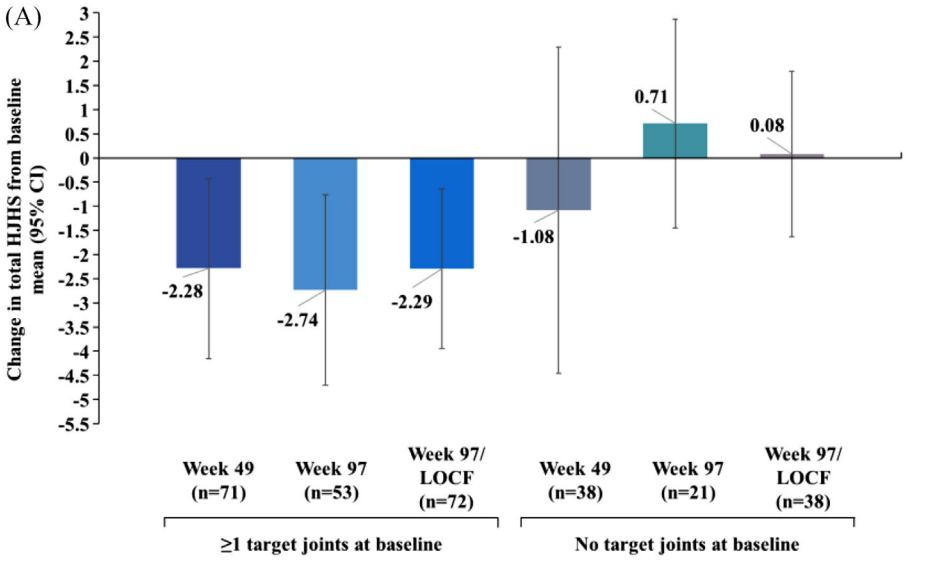
Through Week 73, no significant changes from baseline in bone and joint health biomarker concentrations were observed (Figure 3). However, higher concentrations of biomarkers for bone formation (P1NP, OC) and bone resorption (CTX-I) were observed in adolescents than in adults (>18 years) at all timepoints (Figure 3).

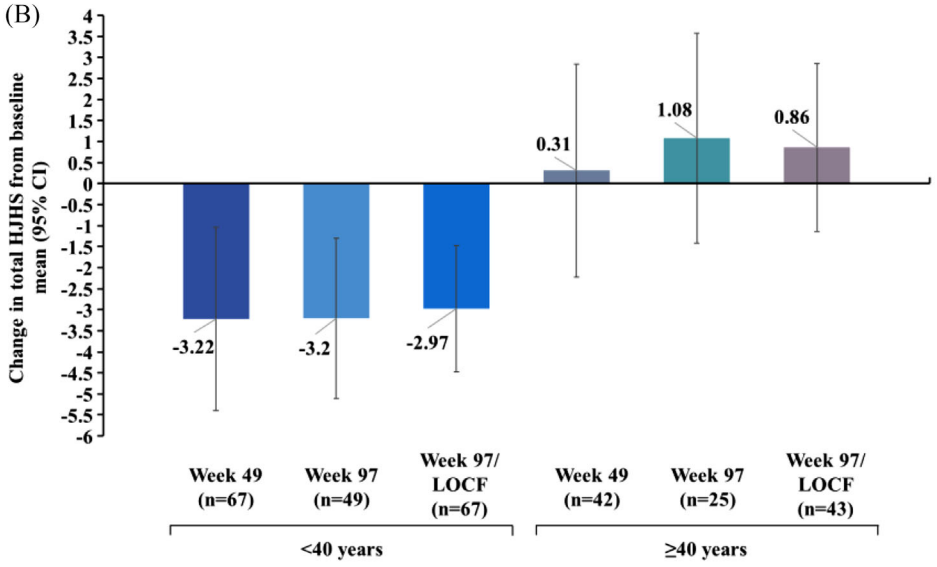
## 4 | DISCUSSION

This HAVEN 3 exploratory analysis showed that emicizumab prophylaxis led to an overall improvement in joint health status, as measured by changes in total HJHS and joint-specific domain scores in younger PwHA (ages 12–39 years) or PwHA with target joints at baseline. Improvements relative to baseline were maintained up to Week 97, indicating sustained joint health benefits with emicizumab prophylaxis. This trend is consistent with the long-term efficacy observed for emicizumab in a pooled analysis of HAVEN 1–4[13,20–22]: across a median (interquartile range) efficacy period of 120.4 (89.0–164.4) weeks, a model-based ABR (95% CI) of .9 (.7–1.2) and .5 (.4–.7) was observed for treated joint bleeds and treated target joint bleeds, respectively. Correspondingly, the proportion of PwHA reporting zero treated joint and treated target joint bleeds improved from 77.7% and 85.9% at Weeks 1–24 to 90.0% and 94.1% at Weeks 121–144.[23]

The improvements in joint health observed in adolescents and younger adults were clinically relevant (≥2-point reduction in HJHS joints domain[2]). This was expected given that emicizumab has demonstrated efficacy in reducing bleeds across all age groups,[13,23] which has translated into improved patient-reported QoL.[24] In participants aged ≥40 years, however, no change was observed, potentially due to degree of joint damage, which in some cases may be irreversible. Prophylaxis can effectively reduce joint bleeding in PwHA; however, the extent of subclinical bleeding not controlled by prophylaxis is unknown. Furthermore, prophylaxis does not resolve prior joint damage.[9,25,26] As such, the observed lack of effect of emicizumab on participants aged ≥40 years is expected.

Participants had better joint health and function if they were previously on prophylaxis before switching to emicizumab and had no target joints at baseline; therefore, these groups had less opportunity for improvement. The relationship between joint health and type of bleed


(A)
Change in total HJHS from baseline mean (95% CI)
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
-5.5
-2.28
-2.74
-2.29
-1.08
0.71
0.08
Week 49 (n=71)
Week 97 (n=53)
Week 97/ LOCF (n=72)
Week 49 (n=38)
Week 97 (n=21)
Week 97/ LOCF (n=38)
≥1 target joints at baseline
No target joints at baseline


(B)
Change in total HJHS from baseline mean (95% CI)
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
-3.5
-4
-4.5
-5
-5.5
-6
-3.22
-3.2
-2.97
0.31
1.08
0.86
Week 49 (n=67)
Week 97 (n=49)
Week 97/ LOCF (n=67)
Week 49 (n=42)
Week 97 (n=25)
Week 97/ LOCF (n=43)
<40 years
≥40 years

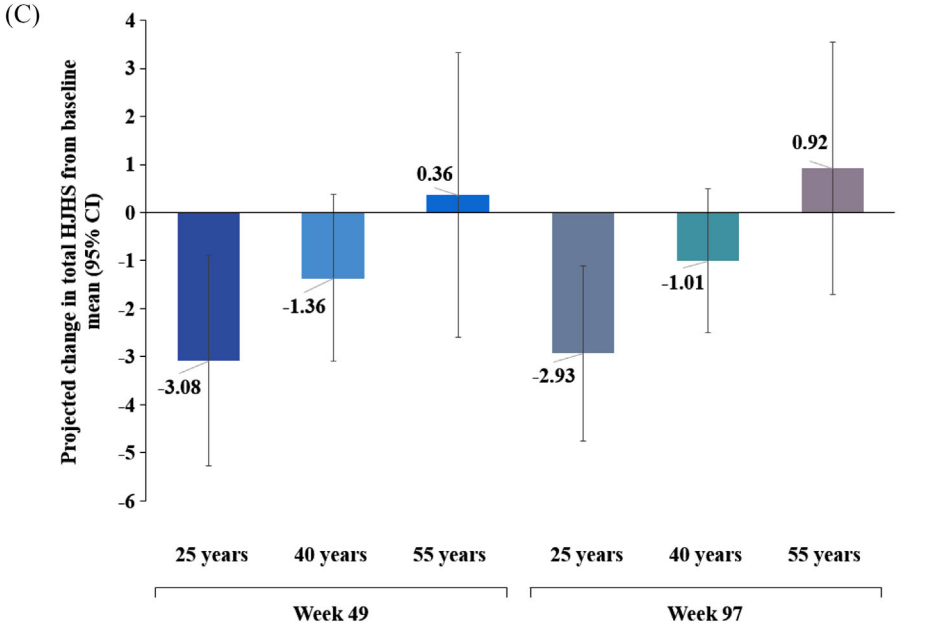

(C)
Projected change in total HJHS from baseline mean (95% CI)
4
3
2
1
0
-1
-2
-3
-4
-5
-6
-3.08
-1.36
0.36
-2.93
-1.01
0.92
25 years
40 years
55 years
25 years
40 years
55 years
Week 49
Week 97

**TABLE 3** Correlation analysis of bone and joint biomarker concentrations and age in evaluable HAVEN 3 participants

| Parameter | Role in bone and joint health | *N* | *r* (Pearson correlation) | *p*-value (Pearson correlation) | *r* (Spearman correlation) | *p*-value (Spearman correlation) |
|---|---|---|---|---|---|---|
| OC | Bone formation | 116 | −.49 | <.0001 | −.50 | <.0001 |
| P1NP | Bone formation | 117 | −.41 | <.0001 | −.52 | <.0001 |
| CTX-I | Bone resorption | 116 | −.49 | <.0001 | −.47 | <.0001 |
| COMP | Cartilage turnover | 117 | .59 | <.0001 | .60 | <.0001 |
| CTX-II | Cartilage degradation | 117 | .04 | .65 | .07 | .4366 |
| CS846 | Cartilage synthesis/repair | 117 | -.02 | .85 | −.08 | .3673 |
| OPG/sRANKL[a] | Osteoblasts/osteoclastogenesis | 117 | .44 | <.0001 | .49 | <.0001 |
| TNFα | Inflammation | 117 | .26 | .0043 | .30 | .0011 |
| IL-6 | Inflammation | 117 | .12 | .2070 | .11 | .2349 |

Abbreviations: COMP, cartilage oligomeric matrix protein; CS846, aggrecan chondroitin sulphate epitope 846; CTX-I, *C*-terminal telopeptide of type I collagen; CTX-II, C-terminal telopeptide of type II collagen; IL, interleukin; OC, osteocalcin; OPG, osteoprotegerin; P1NP, *N*-terminal pro-peptide of type I procollagen; sRANKL, soluble receptor activator of nuclear factor-κβ ligand; TNFα, tumour necrosis factor alpha.

[a]Ratio of OPG and sRANKL biomarkers. OPG and sRANKL are essential for regulation of bone remodelling and exert their effect by controlling the activation state of RANK on osteoclasts; as such, the ratio of these biomarkers (OPG/sRANKL ratio) enables consideration of possible synergistic effects.[4]

management used/presence of target joints has been demonstrated previously in children and adults with haemophilia, with early use of prophylaxis leading to less joint damage.[27–29]

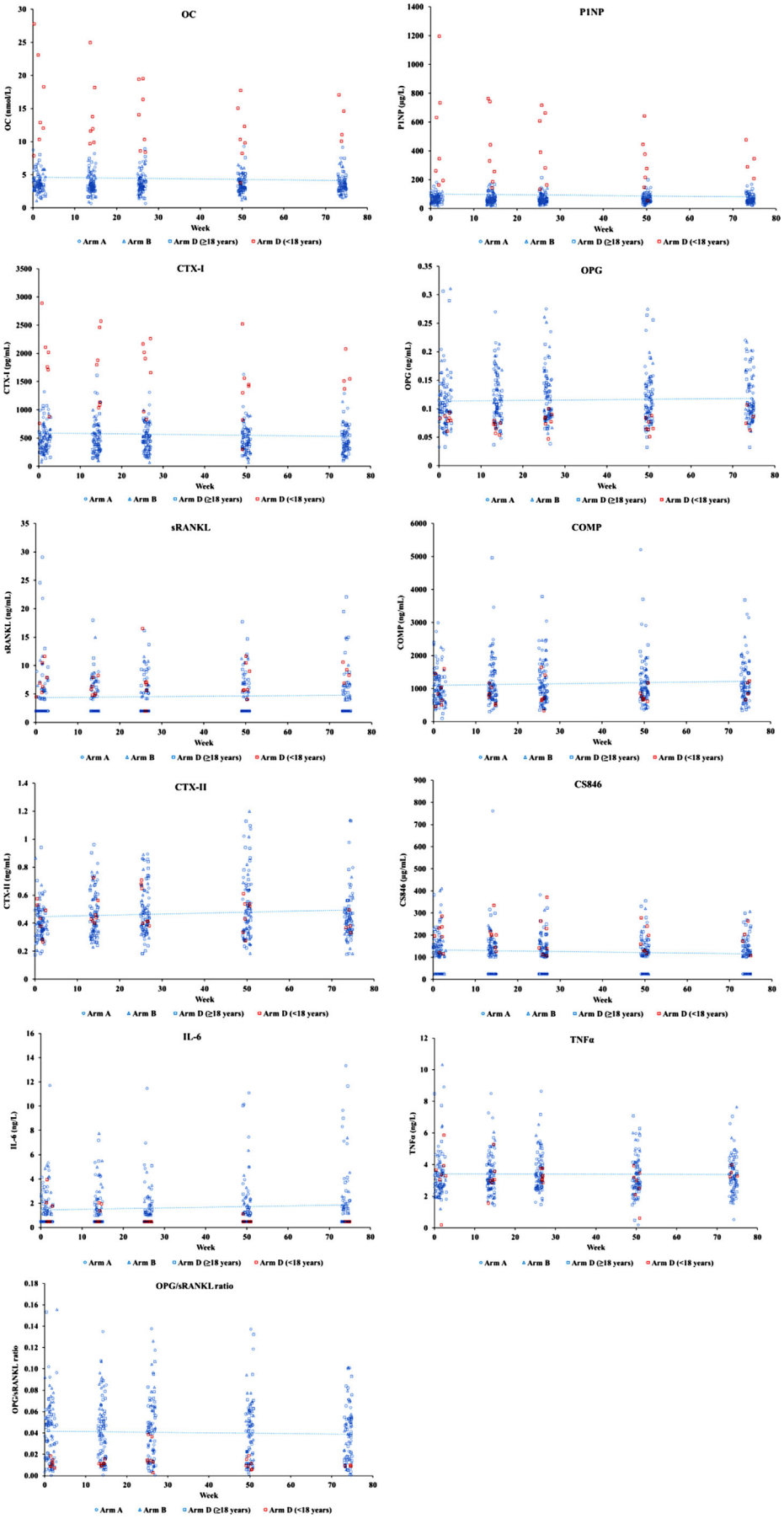
The impact of emicizumab prophylaxis was generally consistent across individual joints, although the knee joints were the most responsive and demonstrated slightly greater improvement. This is aligned with a previous analysis in people with moderate and severe haemophilia aged ≥16 years, which found that ankle and elbow joints were more prone to deterioration.[2] However, improvements in individual joints did not reach the threshold for clinical relevance.

Overall, no significant changes from baseline in bone and joint health biomarker concentrations were observed following 72 weeks of emicizumab. While the timeframe of biomarker changes in HA has not been well characterised, in other settings, biomarker changes have been observed within a year[15–17]; therefore, if bone and joint health decline had occurred in HAVEN 3, we would have expected it to be reflected in the biomarker levels at the end of the study period. It is possible, however, that the biomarkers measured in plasma and serum are not sensitive enough to capture changes in bone/joint health due to emicizumab prophylaxis. Median baseline concentrations of the biomarkers measured were within normal ranges or similar to published levels in healthy individuals, leaving limited room for improvement,[15–17] and they remained within these normal ranges after switching to emicizumab and discontinuing FVIII treatment. In a previous analysis of potential biomarkers for haemophilic arthropathy (including CTX-I, COMP and CS846), normal mean biomarker concentrations were also reported in 117 PwHA without FVIII inhibitors.[15]

The stable trend in bone biomarker levels observed in this exploratory analysis may indicate no detrimental effect on bone formation/resorption resulting from reduced/no FVIII exposure. The positive correlation with age observed for baseline OC, P1NP and CTX-1 biomarkers was largely driven by adolescents, who had higher levels of these biomarkers. This observation is consistent with reported typical increases in these biomarkers during skeletal growth.[30,31] In addition to age, circadian rhythm and sex can influence biomarker concentrations; however, these factors should not have impacted the consistency of measurements reported herein[32–34] since all participants in HAVEN 3 were male. Further, since CTX and OC concentrations are known to peak in the early morning due to circadian rhythm,[35] and bone resorption levels decrease post-prandially,[32] blood samples were collected from participants before noon and prior to emicizumab administration, where possible, to avoid unintended influence on CTX and OC levels.

Age and presence of target joint(s) at baseline appear to be predictors of HJHS improvement from baseline; further study is required to confirm these findings. The data derive from the HAVEN 3 study, which enrolled adolescent and adult PwHA without FVIII inhibitors only. A limited number of timepoints were included in these analyses. HJHS assessments were not performed in a blinded manner and were not controlled. The majority of this cohort were >18 years of age; therefore, bone development in younger ages is not well represented in

**FIGURE 2** Mean improvement from baseline in total HJHS (including gait score) after 48, 96 and 97/LOCF weeks of emicizumab prophylaxis in evaluable HAVEN 3 participants with versus without target joints (A) and participants aged <40 years versus ≥40 years (B) (Groups A, B and D) and MMRM analysis of estimated improvement from baseline in total HJHS after 48 and 96 weeks of emicizumab prophylaxis in PwHA by age (C). A higher HJHS score indicates worse joint health. Clinically relevant improvements are defined as a ≥4-point reduction in Total HJHS.[2] Mean (SD) total HJHS at baseline: 25.6 (20.9; for $n = 90$ participants with ≥1 target joint), 15.9 (16.8; for $n = 43$ participants with no target joints), 14.8 (13.5; for $n = 78$ participants aged <40 years) and 33.4 (22.9; for $n = 55$ participants aged ≥40 years). (A) and (B) exclude Arm C and include only those with an evaluable HJHS score at both baseline and Weeks 49 and 97. CI, confidence interval; HJHS, haemophilia joint health score; LOCF, last observation carried forward; MMRM, mixed-effect model repeated measure; PwHA, people with haemophilia A; SD, standard deviation


OC
OC (nmol/L)
P1NP
P1NP (µg/L)
CTX-I
CTX-I (pg/mL)
OPG
OPG (ng/mL)
sRANKL
sRANKL (ng/mL)
COMP
COMP (ng/mL)
CTX-II
CTX-II (ng/mL)
CS846
CS846 (µg/mL)
IL-6
IL-6 (ng/L)
TNFα
TNFα (ng/L)
OPG/sRANKL ratio
OPG/sRANKL ratio
Week
Arm A
Arm B
Arm D (≥18 years)
Arm D (<18 years)

this analysis. Additionally, there is a lack of representation for participants >50 years. Due to participant variability, data from sub-group analyses where sample numbers are small should be interpreted with caution (e.g., Arm C). Biomarker analysis can be negatively impacted by sample stability, for example, IL-6 can be affected by sample type, extraction methods, storage temperature and long-term storage[36]; however, there is no evidence that longer sample storage negatively affected these data. Additionally, healthy reference ranges have not yet been established for all biomarkers.

## 5 | CONCLUSIONS

Clinically relevant improvements in HJHS were observed in adolescents and younger adults and in those with target joints after 48 weeks of emicizumab in HAVEN 3; this effect was maintained through 96 weeks of emicizumab prophylaxis. Surrogate biomarkers of bone and joint health did not show significant changes over the first 72 weeks of emicizumab prophylaxis. This may reflect the effects on the measured biomarkers by factors other than joint health such as age and physical activity. For most, bone and joint biomarkers were already similar to levels reported in healthy individuals, with little possibility to demonstrate improvement; however, the lack of any demonstrated decline may provide some reassurance that removal of regular FVIII supplementation does not appear to adversely affect bone health. Additional data are needed to better understand the long-term effect of emicizumab prophylaxis on bone and joint health in PwHA, especially those starting emicizumab at a young age.

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### CONFLICTS OF INTEREST

A.K. is an employee of F. Hoffmann-La Roche Ltd and holds stock in the company. M.N. is an employee of F. Hoffmann-La Roche Ltd. C.K. has received honoraria for participation in advisory boards for Sanofi US, Takeda, Genentech, Inc. and Spark Therapeutics. G.C. has received honoraria from uniQure, Bayer, Sobi, CSL Behring, Novo Nordisk, Kedrion, LFB, Grifols, Werfen, BioMarin, Sanofi, Takeda, and F. Hoffmann-LaRoche Ltd. T.C. is an employee of Spark Therapeutics, a member of the Roche Group, and a former employee of Genentech, Inc., and holds stock in F. Hoffmann-La Roche Ltd. I.P-P. is a former employee of Genentech, Inc. J.A. is an employee of Genentech, Inc., a member of the Roche Group and holds stock in F. Hoffmann- La Roche Ltd. G.L. is a former employee of Genentech, Inc., holds stock in F. Hoffmann-LaRoche Ltd and is a current employee of Spark Therapeutics.

### AUTHOR CONTRIBUTIONS

Anna Kiialainen, Ido Paz-Priel, and Gallia G. Levy contributed to the study design, study conduct, recruitment and follow-up of patients, data collection, data analysis and interpretation. Markus Niggli, Christine L. Kempton, and Giancarlo Castaman contributed to the study conduct, recruitment and follow-up of patients, data collection, data analysis and interpretation. Tiffany Chang contributed to the data analysis and interpretation. Joanne I. Adamkewicz contributed to the study design, data analysis and interpretation. All authors critically reviewed progressive drafts of the manuscript and approved the final version. All authors had access to the relevant data for the manuscript.

### DATA AVAILABILITY STATEMENT

Qualified researchers may request access to individual patient level data through the clinical study data request platform (https://vivli.org/). Further details on Roche's criteria for eligible studies are available here (https://vivli.org/members/ourmembers/). For further details on Roche's Global Policy on the Sharing of Clinical Information and how to request access to related clinical study documents, see here (https://www.roche.com/research_and_development/who_we_are_how_we_work/clinical_trials/our_commitment_to_data_sharing.htm)

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**FIGURE 3** Change in biomarker concentration from baseline until after 72 weeks of emicizumab prophylaxis. Trend lines correspond to total population; all participants included in Arms A and B were aged ≥18 years. OPG and sRANKL are essential for regulation of bone remodelling and exert their effect by controlling the activation state of RANK on osteoclasts; as such, the ratio of these biomarkers (OPG/sRANKL ratio) enables consideration of possible synergistic effects.[19] COMP, cartilage oligomeric matrix protein; CS846, aggrecan chondroitin sulphate epitope 846; CTX-I, *C*-terminal telopeptide of type I collagen; CTX-II, C-terminal telopeptide of type II collagen; IL, interleukin; OC, osteocalcin; OPG, osteoprotegerin; P1NP, *N*-terminal pro-peptide of type I procollagen; sRANKL, soluble receptor activator of nuclear factor-$\kappa\beta$; TNF$\alpha$, tumour necrosis factor alpha

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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