



Collagen Turnover Biomarkers Associate with Active Psoriatic Arthritis and Decrease with Guselkumab Treatment in a Phase 3 Clinical Trial (DISCOVER-2)

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ABSTRACT

Introduction: Guselkumab, a novel interleukin-23p19 subunit monoclonal antibody, has been shown to effectively improve the

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diverse manifestations of active psoriatic arthritis (PsA) in two phase 3 trials (DISCOVER-1, DISCOVER-2). Serum concentrations of extracellular matrix (ECM) biomarkers at baseline and following treatment with guselkumab were evaluated in patients with active PsA, and the relationship of these biomarkers with baseline PsA characteristics and clinical response to guselkumab treatment was explored.

Methods: Serum samples were collected at weeks 0, 4, 24, and 52 from a selected subset ($N = 260$) of the 739 biologic-naïve patients with PsA treated with guselkumab 100 mg every 4 or 8 weeks or placebo in DISCOVER-2. Demographically matched healthy controls ($N = 76$) were used for comparison. The samples were analyzed for ECM biomarkers associated with collagen degradation (C1M, C2M, C3M, C4M, C6M, C10C) and collagen formation (PRO-C1, PRO-C2, PRO-C3, PRO-C4, PRO-C6).

Results: Baseline concentrations of collagen degradation biomarkers C1M, C3M, C4M, and C6M and collagen formation biomarkers PRO-C3 and PRO-C6 were significantly higher (i.e., ≥ 1.25 -fold and false discovery rate adjusted $p < 0.05$) in PsA patients than in healthy controls. Serum C1M, C3M, C4M, and C6M levels declined from baseline in guselkumab-treated patients in both dosing regimens. In addition, guselkumab-treated ACR20 responders ($\geq 20\%$ improvement in American College of Rheumatology response criteria) had significantly lower C1M levels than ACR20 nonresponders.

Conclusion: These data demonstrate that serum collagen biomarkers are elevated in patients with PsA compared with healthy controls and that treatment with guselkumab decreases levels of C1M, C3M, C4M, and C6M. Importantly, C1M serves as a biomarker that associates with improvement of joint signs and symptoms.

Trial Registration: ClinicalTrials.gov identifier: NCT03158285.

Keywords: Biomarkers; Collagen turnover; Extracellular matrix; Guselkumab; Psoriatic arthritis

Key Summary Points

Why carry out this study?

The identification of biomarkers that are associated with psoriatic arthritis (PsA) disease and could potentially serve as predictors of treatment response in patients with PsA is an unmet need.

What did the study ask?

The effect of guselkumab treatment on serum concentrations of biomarkers associated with collagen degradation and collagen formation was evaluated in patients with active PsA.

What was learned from the study?

At baseline, serum concentrations of collagen degradation biomarkers (C1M, C3M, C4M, and C6M) and collagen formation biomarkers (PRO-C3 and PRO-C6) were significantly higher in patients with PsA than in healthy controls.

In the guselkumab every-4-weeks and every-8-weeks groups, serum levels of C1M, C3M, C4M and C6M decreased as early as week 4 and were lower in guselkumab-treated patients compared with placebo patients at week 24.

Among guselkumab-treated patients, ACR20 responders had significantly lower C1M levels at week 24 than did ACR20 nonresponders.

INTRODUCTION

Psoriatic arthritis (PsA) is a progressive, joint-degenerative inflammatory disease characterized by swelling, pain, fatigue, and stiffness in the joints [1, 2]. Joints affected by PsA often show evidence of cartilage destruction, bone erosions, and synovitis, as well as increased new bone growth as evidenced by active osteoblasts and hypertrophic chondrocytes [1, 3]. Several therapies are available for PsA with differing mechanisms of action, and treatment guidelines have focused on individualized treatment to address as many relevant disease domains as possible [2, 4, 5]. Conventional and biologic disease-modifying antirheumatic drug (DMARD) options for PsA include methotrexate and inhibitors of tumor necrosis factor, interleukin-17A (IL-17A), IL-12/23p40, IL-23p19, phosphodiesterase 4, T cell activation, and the Janus kinase pathway [1, 2, 6].

Guselkumab, a monoclonal antibody targeting the IL-23p19 subunit, demonstrated efficacy compared with placebo in reducing skin and musculoskeletal signs and symptoms in patients with active PsA in two phase 3 trials, DISCOVER-1 and DISCOVER-2 [7, 8]. In DISCOVER-2, guselkumab 100 mg every 4 weeks (Q4W) or every 8 weeks (Q8W) improved joint symptoms, physical function, skin symptoms of psoriasis, and overall health and quality of life in biologic-naïve patients with active disease [7, 8]. In addition, evidence of inhibition of structural damage was also demonstrated for the guselkumab 100 mg Q4W dose regimen at week 24. At 1 year, radiographic progression from baseline was similar between the 100 mg Q4W and Q8W dose regimens [9].

Biomarkers can be used to elucidate normal and pathological processes and to measure biological response to a therapeutic intervention [10]. In PsA, there is a clear unmet medical need for biomarkers that can characterize patients and identify which therapy may provide the most benefit for a particular patient [10, 11]. Tissue turnover is shifted in the affected joints of patients with PsA, resulting in the release of neo-epitopic fragments from the extracellular matrix (ECM). Collagen proteins

are the predominant structural element of the ECM, with the most abundant collagens of the joint tissue being types I, II, III, IV, VI, or X collagen. Markers quantifying the breakdown and formation peptides of these collagens may be useful as biomarkers of PsA [3, 12–16]. Type I collagen is the most abundant collagen in the body as it is the main structural protein of bone. The remodeling of type I collagen has been assessed by biomarkers for decades, as exemplified by the C-terminal telopeptides of type I collagen (CTX-I), a specific bone resorption marker [17]. Type II collagen is the main collagen found in the articular cartilage [18]. Types III, VI, and IV collagens are found in the interstitial matrix, the intermediate tissue layer, and the basement membrane, respectively, the latter being found in the synovial tissue, skin and lungs (as examples) [14, 19]. Type X collagen is produced by hypertrophic chondrocytes as tissues become calcified [3]. Markers that reflect tissue turnover may be useful in evaluating the tissue-associated disease activity.

Degradation metabolites of these collagens are elevated in other diseases with musculoskeletal involvement, such as rheumatoid arthritis (RA), ankylosing spondylitis (AS), and systemic sclerosis (SSc). In RA, serum C1M and C4M (markers for degradation of type I and type IV collagen, respectively) are associated with bone and synovium turnover, respectively [20]. Additionally, C1M and C3M, markers for degradation of type III collagen, are associated with inflammatory interstitial matrix tissue degradation in RA and inflammatory status in osteoarthritis [21]. C6M, a marker for degradation of type VI collagen, is elevated in AS as well as in SSc [22, 23].

The collagen formation biomarkers PRO-C1 (type I collagen), PRO-C3 (type III collagen), and PRO-C6 (type VI collagen) are used to assess the release of propeptides as the newly formed collagens are integrated into the tissue after being produced by activated fibroblasts. PRO-C1 (N-terminal propeptide of type I procollagen [PINP]) has been shown to be a biomarker of bone formation [24]. PRO-C3 and PRO-C6 more closely associate with fibrogenesis [25]. PRO-C4 (type IV collagen formation biomarker) associates with basement membrane turnover [25].

PRO-C2, C2M, and C10C (biomarker of cathepsin-degraded type X collagen) were originally used to assess articular cartilage turnover and chondrocyte differentiation induced by growth factors [25–27]. These growth factors are involved in dedifferentiation of joint cells in PsA and, thus, the biomarkers may reflect ongoing damage to the cartilage.

Given the need for predictive and pharmacodynamic/response biomarkers for PsA treatment and increased collagen turnover in PsA [12, 13], serum samples collected from a subset of individuals with PsA who participated in DISCOVER-2 were compared to those from healthy controls. We examined the relationship between serum concentrations of tissue-derived ECM product biomarkers with PsA disease phenotypes and with guselkumab treatment response.

METHODS

DISCOVER-2 Participants and Study Design

DISCOVER-2 was a double-blind, randomized, placebo-controlled, phase 3 study of guselkumab in adults with active PsA [8]. Details of the DISCOVER-2 study design and patient populations have been reported elsewhere [8, 9]. Briefly, participants had active PsA, with at least five tender and five swollen joints, and a C-reactive protein (CRP) concentration of at least 0.6 mg/dL despite standard therapies, and were naïve to biologic therapies. Patients were randomized (1:1:1) to receive subcutaneous injections of guselkumab 100 mg Q4W; guselkumab 100 mg at weeks 0 and 4, then Q8W; or placebo. At week 24, patients in the placebo group crossed over to receive guselkumab Q4W.

Biomarker Study Participants

Of the 739 treated patients in DISCOVER-2, these exploratory analyses were limited to a subset of 260 participants (Q4W, $n = 82$; Q8W, $n = 92$; placebo, $n = 86$). Serum collagen biomarkers were evaluated for patients for

Table 1 Baseline demographic and disease characteristics of biomarker subset of DISCOVER-2 patients

Baseline demographic and disease characteristics	DISCOVER-2 biomarker subset			Total	DISCOVER-2 study cohort ^a
	Guselkumab 100 mg		Placebo		
	Q4W	Q8W			
Patients, <i>N</i>	82	92	86	260	739
<i>Demographics</i>					
Age (years)	45.1 (10.8)	45.1 (11.6)	46.1 (10.9)	45.4 (11.1)	45.7 (11.7)
Male	51 (62.2%)	49 (53.3%)	42 (48.8%)	142 (54.6%)	388 (52.5%)
White	81 (98.8%)	87 (94.6%)	85 (98.8%)	253 (97.3%)	724 (98.0%)
Weight (kg)	87.0 (18.5)	82.3 (18.5)	80.6 (18.4)	83.2 (18.6)	84.3 (19.5)
<i>Disease characteristics</i>					
PsA disease duration (years)	6.2 (5.9)	5.5 (5.1)	7.0 (5.9)	6.2 (5.6)	5.5 (5.7)
DAS28-CRP	5.3 (1.0)	5.2 (1.0)	5.5 (0.9)	5.3 (1.0)	5.2 (1.0)
Swollen joint count (0–66)	14.5 (9.1)	12.1 (7.8)	13.1 (7.0)	13.2 (8.0)	12.3 (7.2)
Tender joint count (0–68)	24.5 (14.4)	20.0 (12.5)	22.5 (13.1)	22.3 (13.4)	21.3 (12.9)
Psoriasis Area Severity Index (0–72)	11.5 (11.6)	8.9 (10.9)	9.9 (11.4)	10.1 (11.3)	9.9 (11.1) ^b
Body surface area affected by psoriasis, %	18.5 (19.3)	17.4 (21.3)	17.6 (20.9)	17.8 (20.5)	17.4 (20.4)
<i>Concomitant medications</i>					
MTX	48 (58.5%)	56 (60.9%)	55 (64.0%)	159 (61.1%)	443 (59.9%)
Oral corticosteroids	11 (13.4%)	21 (22.8%)	16 (18.6%)	48 (18.5%)	145 (19.6%)
NSAIDs	66 (80.5%)	62 (67.4%)	58 (67.4%)	186 (71.5%)	504 (68.2%)

Data are reported as the mean with the standard deviation (SD) in parenthesis, or as *n* with the percentage (%) in parentheses, unless otherwise noted

DAS28-CRP Disease Activity Score in 28 Joints using C-reactive protein, *MTX* methotrexate, *NSAID* nonsteroidal anti-inflammatory drug, *PsA* psoriatic arthritis, *Q4W* every 4 weeks, *Q8W* every 8 weeks

^a*p* values were calculated for comparisons of the total biomarker cohort vs. the overall DISCOVER-2 study cohort using two-tailed Fisher's exact test for categorical variables and the two-tailed *t* test for continuous variables. All *p* values were > 0.05 with the exception of the mean DAS28-CRP score, which had a *p* value of 0.049

^b*N* = 738

whom serum samples were available at baseline and at least one post-baseline visit, with priority given to those who had samples available at weeks 0, 4, 24, and 52. Patients were further selected by baseline demographic and disease characteristics to ensure that the biomarker subset was reflective of the total study population (Table 1).

Healthy Controls

Serum samples from healthy controls ($N = 76$) were selected from an internal biobank at Nordic Bioscience (Herlev, Denmark). The biobank consists of samples acquired from commercial sources, including from BioIVT (West Sussex, UK) and Discovery Life Sciences (San Luis Obispo, CA, USA), and samples were collected according to the European Union General Data Protection Regulation and U.S. ethical regulations and privacy act. These samples were matched for age and gender to the DISCOVER-2 PsA biomarker subset ($n = 260$). The median age of the healthy control population was 45 (range 20–69) years, and there were 38 (50%) males.

Biomarker Analyses

Blood samples were collected from the subset of DISCOVER-2 participants ($N = 260$) for biomarker analyses at weeks 0 (pre-treatment, referred to here as baseline), 4, 24, and 52. The samples were collected in standard serum separation tubes, prepared into aliquots, frozen at less than or equal to $-20\text{ }^{\circ}\text{C}$, and then shipped frozen on dry ice to Covance Central Laboratory Services (Indianapolis, IN, USA) for storage prior to biomarker measurement.

For this study, serum protein concentrations of 11 serum biomarkers of ECM collagen formation and degradation were measured at Nordic Bioscience following methods previously described [12, 13]. The biomarkers assayed were collagen formation biomarkers PRO-C1, PRO-C2, PRO-C3, PRO-C4, and PRO-C6 and collagen degradation biomarkers C1M, C2M, C3M, C4M, C6M, and C10C [22, 23, 27]. All biomarkers were measured by competitive immunoassays using manual enzyme-linked

immunosorbent assays (ELISAs) or the Immunodiagnostic Systems robotic platform (IDS-i10; Immunodiagnostic Systems (IDS), Bolden, Tyne & Wear, UK). Samples were rerun if the duplicate coefficient of variation was $> 15.0\%$. Intra- and inter-assay variation was $< 15.0\%$. All runs included the same three quality control samples, which were to be within 20% range of the set target value.

Statistical Methods

Biomarker data were \log_2 -transformed, and Spearman rank correlation or general linear models (GLMs) were used for analyses. For correlations, statistical significance was defined as GLM-adjusted $p < 0.05$. The strength of the correlation was defined using Spearman rank correlation coefficient (r_s) as weak ($|r_s| < 0.25$), modest ($0.60 > |r_s| \geq 0.25$), or strong ($|r_s| \geq 0.60$). Significance of associations of demographic and clinical variables across treatment groups was tested by analysis of variance F tests and Chi-square tests for continuous and categorical variables, respectively. For comparison of biomarkers between patients with PsA and healthy controls, between guselkumab-treated and placebo-treated PsA patients, and between PsA clinical responders and non-responders, GLM was used and significance was defined by false discovery rate adjusted (adj) $p < 0.05$ and fold difference of ≥ 1.25 . Clinical response was defined as achieving $\geq 20\%$ improvement in American College of Rheumatology response criteria (ACR20 response), the primary endpoint of DISCOVER-2, ACR50 response ($\geq 50\%$ improvement in ACR criteria), and $\geq 75\%$ improvement in the Psoriasis Area and Severity Index (PASI75 response) at week 24. For response analyses, guselkumab arms were combined to increase the power of the analyses.

Compliance with Ethics Guidelines

This study (ClinicalTrials.gov identifier: NCT03158285) was conducted according to the Declaration of Helsinki of 1964 and its later amendments and the International Committee on Harmonisation good clinical practices.

Protocols were approved by ethics committees at each site (Sterling IRB approval number for US sites: 5910C), and all patients provided written informed consent.

RESULTS

Baseline Characteristics and Efficacy of DISCOVER-2 Biomarker Subset

The baseline characteristics of the biomarker subset of DISCOVER-2 PsA patients were assessed (Table 1). As with the overall DISCOVER-2 population [8], demographics, baseline clinical characteristics, and baseline collagen biomarker levels were generally balanced across treatment arms within the subset (Table 1; Electronic Supplementary Material [ESM] Table 1). In addition, baseline demographic and disease characteristics were similar between the biomarker subset and the overall DISCOVER-2 study population (Table 1) [8].

Similar to the reported findings from the full clinical cohort in DISCOVER-2 [8], the biomarker subset had significantly higher rates of clinical response in guselkumab-treated patients compared to placebo-treated patients (measured at week 24 by ACR20 [67% in the Q4W group, 59% in the Q8W group, and 26% in the placebo group; adj $p < 0.0001$] and by PASI75 [78% in the Q4W and Q8W groups, and 24% in the placebo group; adj $p < 0.0001$]) (ESM Table 2).

Collagen Biomarkers at Baseline in PsA Patients Versus Healthy Controls

Differences in the serum concentrations of collagen biomarkers in patients with PsA versus healthy controls were assessed (Fig. 1; ESM Fig. 1). At baseline, concentrations of collagen degradation biomarkers C1M, C3M, C4M, and C6M and collagen formation biomarkers PRO-C3 and PRO-C6 were significantly higher (i.e., ≥ 1.25 -fold and adj $p < 0.05$) in patients with PsA compared with matched healthy controls. C10C and PRO-C1 also trended toward upregulation in PsA patients compared with healthy controls, but the difference did not reach

statistical significance (1.17-fold; ESM Fig. 1). Serum C2M, PRO-C2, and PRO-C4 concentrations at week 0 did not differ between PsA patients and healthy controls (ESM Fig. 1).

Collagen Biomarkers and Baseline Clinical Features in PsA Patients

Collagen biomarkers were assessed for their association with baseline clinical features in patients with PsA, including PASI score, baseline body surface area (BSA) with psoriasis, CRP, and joint manifestations (Table 2). Significant and modest correlations were seen between baseline levels of collagen degradation biomarkers C1M, C3M, C4M, and C6M and collagen formation biomarker PRO-C4 and baseline PASI score ($r_s = 0.25$ – 0.32 ; $p < 0.05$). Significant and modest correlations were also seen between C3M, C4M, and C6M and baseline BSA with psoriasis involvement ($r_s = 0.27$ – 0.30 ; $p < 0.05$). Regarding PsA joint manifestations, baseline levels of C1M, C3M, C4M, and C6M were positively correlated with baseline Disease Activity Score in 28 Joints using CRP (DAS28-CRP) ($r_s = 0.29$ – 0.35 ; $p < 0.05$) and baseline CRP levels ($r_s = 0.65$ – 0.74 ; $p < 0.05$). C10C was inversely correlated with baseline CRP ($r_s = -0.27$; $p < 0.05$). Correlations between baseline levels of select collagen degradation biomarkers and baseline swollen joint and tender joint counts and enthesitis and dactylitis scores were weak (all $r_s < 0.25$), but statistically significant ($p < 0.05$). Other than baseline PRO-C4 levels modestly correlating with PASI score ($r_s = 0.28$; $p < 0.05$), correlations between collagen formation biomarkers and baseline clinical features of PsA were generally weak or not statistically significant.

Effect of Guselkumab on Collagen Biomarkers

The effect of guselkumab on collagen biomarkers was measured at weeks 4, 24, and 52 (Fig. 2). Beginning at week 4, serum C1M, C3M, C4M, and C6M levels declined from baseline in guselkumab-treated patients in both dosing

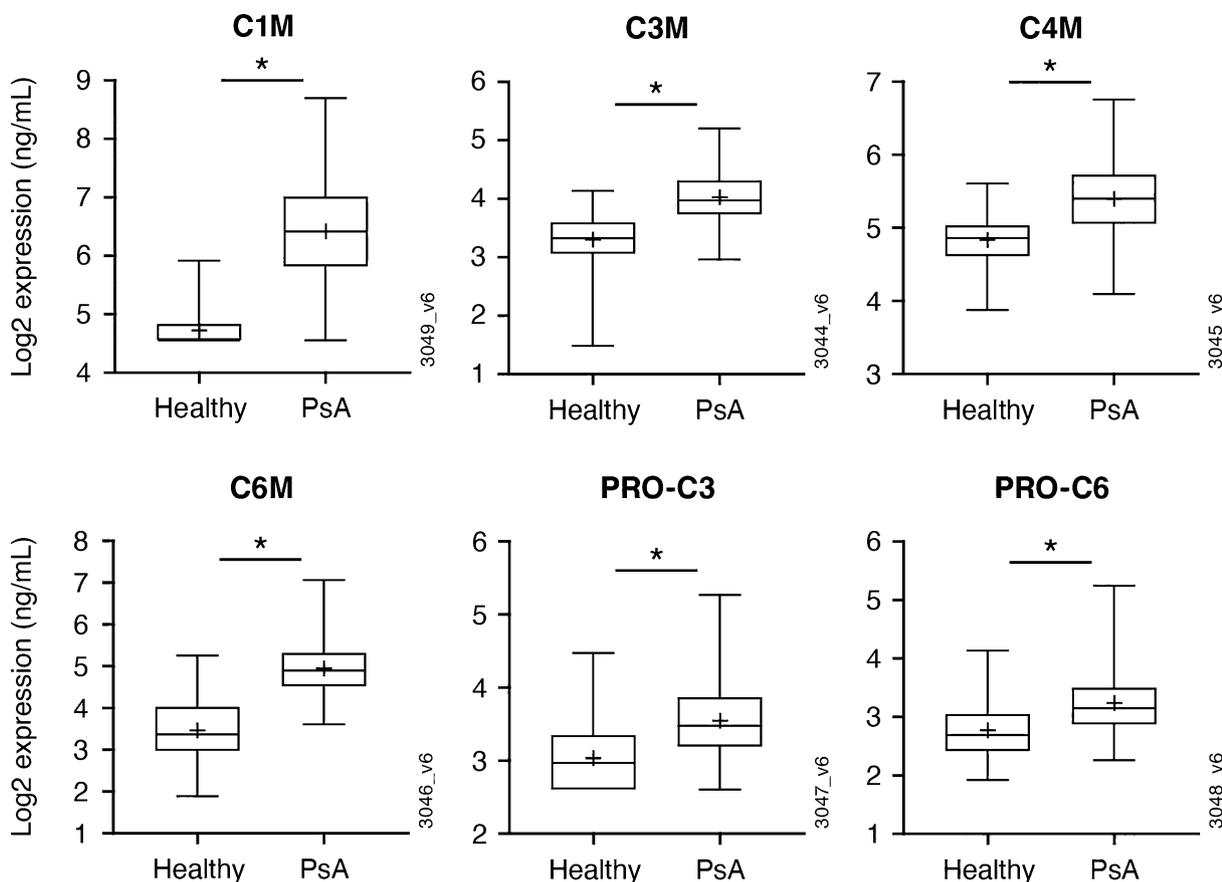


Fig. 1 Upregulation of collagen degradation and formation biomarkers in the serum of PsA patients (PsA biomarker subset: $N = 260$) compared to healthy controls (healthy control subset: $N = 76$). Healthy controls were demographically matched (age and sex) to the PsA biomarker subset. Values are shown as the median (mean is shown as a plus [+] sign). Boxes represent the interquartile range, while whiskers represent the minimum

and maximum values. The asterisk indicates significance defined by a fold change ≥ 1.25 and adjusted (adj) $p < 0.05$. *C1M*, *C3M*, *C4M*, *C6M* collagen degradation biomarkers, *PRO-C1*, *PRO-C3* collagen formation biomarkers, *PsA* psoriatic arthritis

regimens, with further decreases from baseline levels noted at week 24. At week 24, serum C1M, C3M, C4M, and C6M levels were lower in both guselkumab groups versus placebo (C1M: Q4W adj $p < 0.05$, Q8W adj $p = 0.05$; C3M and C4M: adj $p < 0.05$ for each dose arm; C6M: Q4W adj $p = 0.066$, Q8W adj $p < 0.05$) (Fig. 2; ESM Table 3). Trends were consistent for both guselkumab dosing regimens, although patients receiving guselkumab 100 mg Q4W exhibited a trend for greater decline in C1M. Reduced serum C1M, C3M, C4M, and C6M levels were maintained at week 52. Following crossover to

guselkumab Q4W at week 24, patients randomized to placebo also demonstrated a reduction in C1M, C2M, C3M, and C6M levels by week 52 (adj $p < 0.0001$) (ESM Table 3), thus confirming the pharmacodynamic effect of IL-23p19 inhibition on these collagen biomarkers.

No significant changes in other biomarkers, including collagen formation biomarkers PRO-C1, PRO-C2, PRO-C3, PRO-C4, and PRO-C6 and degradation biomarkers C2M and C10C, were observed following guselkumab or placebo treatment (data not shown).

Table 2 Correlations between collagen biomarkers and baseline clinical features of PsA (r_s values)

Collagen biomarkers	PSO BSA	PASI	DAS28-CRP	SJC66	TJC68	Baseline CRP	Enthesitis Score	Dactylitis Score
C1M	<i>0.24</i>	<u><i>0.25</i></u>	<u><i>0.30</i></u>	<i>0.13</i>	0.09	<u><i>0.74</i></u>	0.10	0.11
C2M	– 0.02	– 0.04	0.07	0.04	0.04	– 0.05	0.01	0.03
C3M	<u><i>0.28</i></u>	<u><i>0.31</i></u>	<u><i>0.29</i></u>	<i>0.18</i>	0.08	<u><i>0.65</i></u>	0.10	<i>0.15</i>
C4M	<u><i>0.30</i></u>	<u><i>0.32</i></u>	<u><i>0.34</i></u>	<i>0.17</i>	0.12	<u><i>0.73</i></u>	<i>0.13</i>	<i>0.14</i>
C6M	<u><i>0.27</i></u>	<u><i>0.27</i></u>	<u><i>0.35</i></u>	<i>0.22</i>	<i>0.16</i>	<u><i>0.68</i></u>	<i>0.14</i>	<i>0.15</i>
C10C	– 0.08	– 0.09	0.01	0.03	0.01	<u><i>– 0.27</i></u>	– 0.02	– 0.05
PRO-C1	– 0.05	0.01	– 0.01	0.08	– 0.01	0.02	– 0.07	0.07
PRO-C2	<i>0.14</i>	<i>0.13</i>	– 0.02	– 0.03	– 0.04	– 0.12	– 0.04	0.02
PRO-C3	0.03	0.05	0.09	0.05	0.01	0.11	0.01	0.04
PRO-C4	<i>0.23</i>	<u><i>0.28</i></u>	0.07	0.08	0.02	<i>0.15</i>	0.05	<i>0.13</i>
PRO-C6	<i>0.15</i>	<i>0.15</i>	0.04	0.04	– 0.06	<i>0.14</i>	– 0.03	0.01

$N = 260$

Values presented in italics are statistically significant (GLM adusted [adj] $p < 0.05$). Values presented in italics with underlining are statistically significant (GLM adj $p < 0.05$) for modest ($0.60 > |r_s| \geq 0.25$) or strong ($|r_s| \geq 0.60$) correlation. BSA Body surface area, GLM general linear model, PASI Psoriasis Area and Severity Index, PSO psoriasis, r_s Spearman rank correlation coefficient, SJC66 swollen joint count (0–66), TJC68 tender joint count (0–68), Enthesitis Score Leeds Enthesitis Index 0–6, Dactylitis Score Dactylitis Severity Score 0–60

Relationships Between Collagen Biomarker Levels and Clinical Outcomes

Levels of C1M at baseline, week 4, and week 24 were analyzed by ACR20 response at week 24, the primary endpoint for the DISCOVER-2 study (Fig. 3). Expression of collagen biomarkers at baseline and at week 4 was similar between week 24 ACR20 responders and nonresponders across treatment groups. In the combined guselkumab-treated Q4W and Q8W group at week 24, ACR20 responders had significantly lower C1M levels than did ACR20 nonresponders (– 1.26 fold; adj $p = 0.0065$). Similar results were observed for ACR50 responders versus ACR20 nonresponders (data not shown).

No significant differences in biomarker levels between PASI75 responders and nonresponders were seen at any timepoint (including baseline; data not shown), indicating a lack of association between these biomarker changes and PsA skin response.

DISCUSSION

This work demonstrates that collagen biomarkers are detectable and elevated in the serum of patients with PsA compared with healthy controls [12, 28] and that guselkumab treatment reduces the levels of specific collagen biomarker proteins.

The collagen biomarkers assessed in the current study are released from the ECM of connective tissues. In healthy tissues, there is a delicate balance between the formation and degradation of ECM components, such as collagens. The imbalance that occurs in inflammatory and fibro-proliferative diseases results in increased degradation and/or formation, leading to loss of tissue, altered tissue composition, or thickening and stiffening of the tissue [25]. To our knowledge, there are limited reports on how the tissue balance is altered in PsA compared to the homeostasis maintained between formation and degradation in healthy

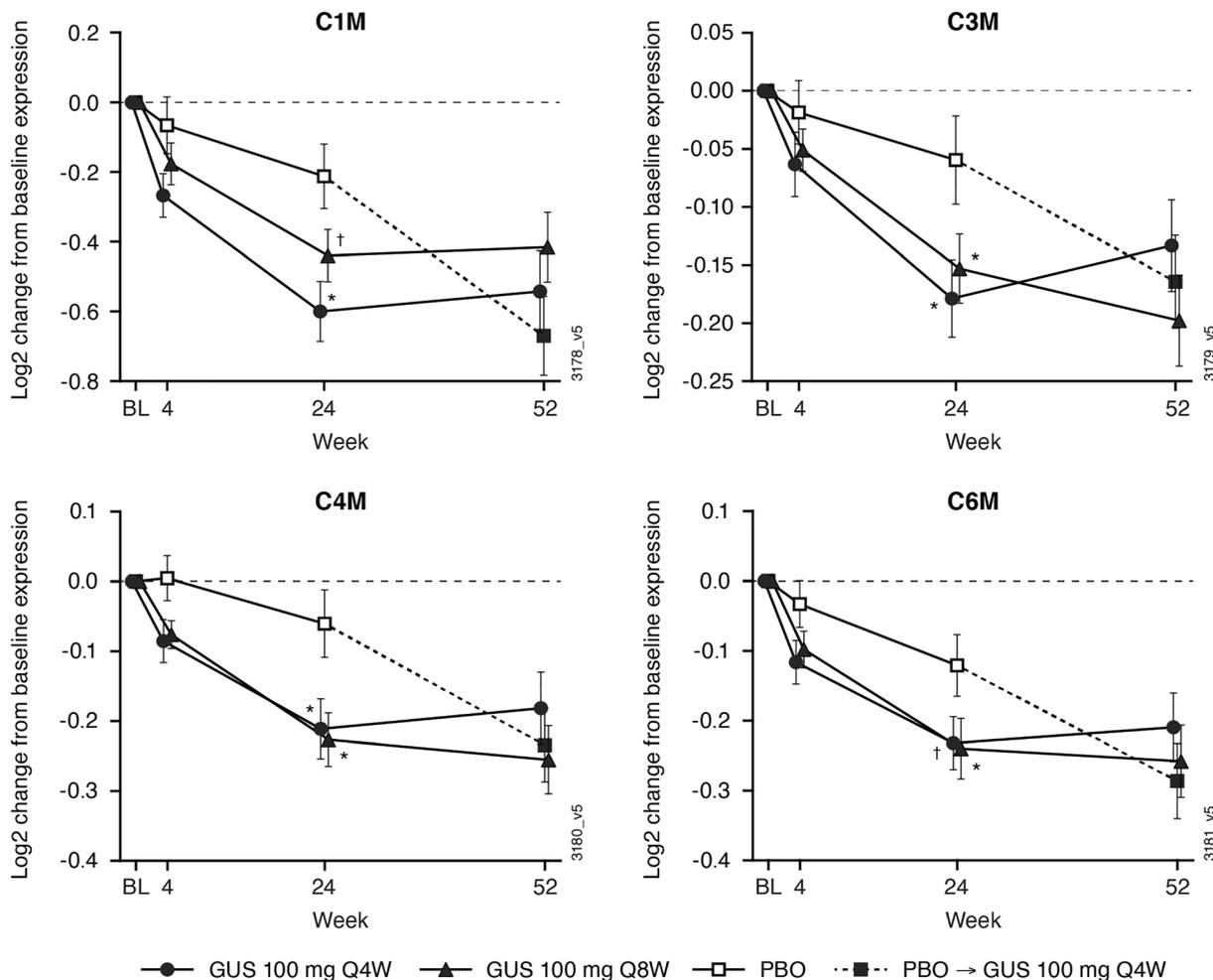


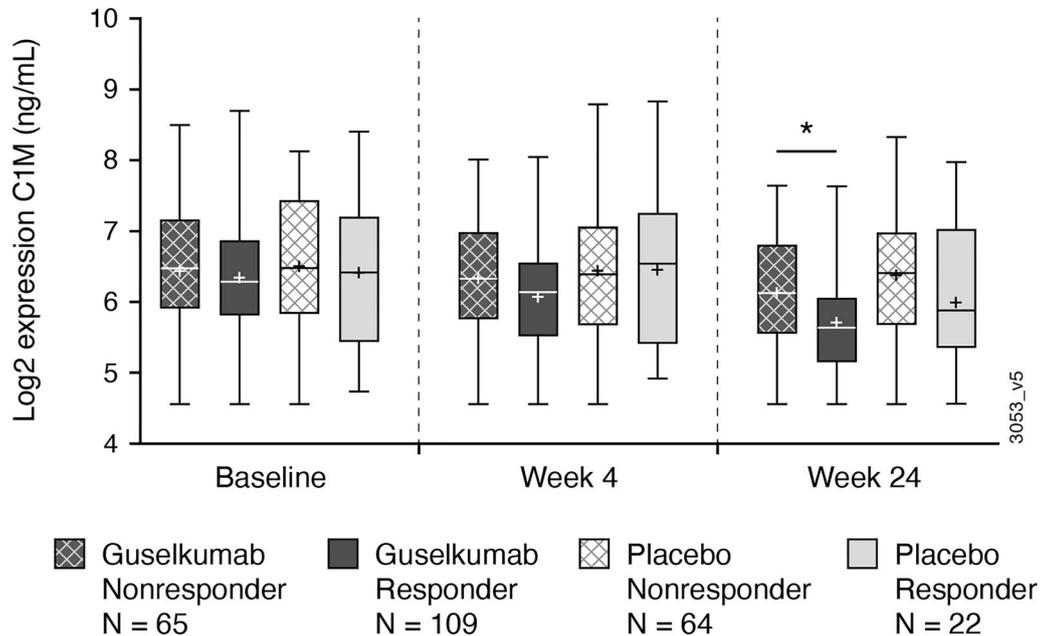
Fig. 2 Collagen degradation biomarkers (C1M, C3M, C4M, and C6M) significantly decrease with GUS vs. PBO at week 24. GUS treatment Q4W or Q8W significantly reduced collagen degradation biomarkers C1M, C3M, C3M, and C6M compared to PBO at 24 weeks. Asterisk indicates significant difference vs. PBO at adj $p_{GUSvsPBO} <$

0.05. Dagger indicates significant difference vs. placebo at $p_{GUSvsPBO} < 0.07$. Error bars represent 1 standard error. GUS Guselkumab, Q4W, Q8W every 4 weeks, every 8 weeks, PBO placebo

individuals. Therefore, in this study, biomarkers of both collagen formation (fibrogenesis) and collagen degradation (fibrolysis) were evaluated to gain greater insight into the shift in tissue balance in patients with PsA.

At baseline, serum concentrations of collagen degradation biomarkers C1M, C3M, C4M, and C6M and collagen formation biomarkers PRO-C3 and PRO-C6 were significantly higher in patients with PsA compared with matched healthy controls, which was indicative of the cartilage turnover experienced by patients with

PsA that often results in swollen and tender joints [28]. The collagen degradation markers C1M, C3M, C4M, and C6M were positively correlated with measures of baseline skin disease, DAS28-CRP, and general inflammation (CRP) in this study. In addition, weak, but statistically significant correlations were observed between these biomarkers and baseline swollen joint count. C3M and C4M have been implicated in synovitis, or inflammation of the joint synovial membrane, which is associated with symptoms of pain, swelling, and stiffness



* $p=0.0065$, significance between responder and non-responder defined by $p < 0.05$ and $|\text{fold difference}| \geq 1.25$.

Fig. 3 Significantly lower C1M expression in ACR20 responders vs. nonresponders at baseline and weeks 4 and 24. Response was defined by ACR20 ($\geq 20\%$ improvement according to the American College of Rheumatology criteria) at week 24. Values are shown as the median (mean is shown as a plus [+]) sign). Boxes represent the

interquartile range, while whiskers represent the minimum and maximum values. Asterisk indicates significant difference at $p = 0.0065$ between responder and nonresponder defined by fold change ≥ 1.25 and $\text{adj } p < 0.05$

[29, 30], and type VI collagen plays a role in regulating synovial joint physiology [19]. Guselkumab treatment resulted in a decrease in collagen degradation biomarkers C1M, C3M, C4M, and C6M, suggesting that guselkumab may suppress inflammation-driven tissue degradation. On the other hand, collagen formation biomarkers PRO-C3 and PRO-C6 were not modulated in response to guselkumab. These biomarkers have been reported to be associated with the activation of dermal and lung fibroblasts and to be elevated in patients with active scleroderma [22, 31], suggesting that the biomarkers may be associated with skin remodeling; as such, they are interesting in the context of PsA and psoriasis. This raises two questions that cannot be answered in the current study. Are the elevated levels of the collagen formation biomarkers associated with increased fibrosis of, for example, the skin in

PsA, or are these biomarkers a reflection of the repair mechanism that is initiated to counteract the increased inflammation-driven tissue degradation reflected by the collagen degradation biomarkers?

C2M, a degradation product of type II collagen, is a biomarker of cartilage destruction [32]. Interestingly, in this set of PsA patients, we did not detect an increase in either C2M or PRO-C2 in comparison to healthy controls, potentially indicating a lack of cartilage involvement in this cohort.

This work identified C1M as an important biomarker for PsA as C1M levels were found to be elevated in the serum of the patients with PsA. C1M has been reported to be elevated in RA and associated with radiographic progression [33]. In contrast to the bone resorption marker C-terminal telopeptide of type 1 collagen, C1M is highly correlated with CRP and derived

through the action of matrix metalloproteinases, indicating that C1M is a direct marker of inflammation-driven bone degradation [34]. Thus, the increased levels observed in patients with PsA may reflect the relevance of this protein in the joint damage associated with PsA. Guselkumab treatment significantly reduced serum C1M levels at 24 weeks. For context, the log₂ reduction of -0.6 shown in Fig. 2 translates to a reduction of approximately 42% in C1M from baseline. In patients with RA, this level of reduction was correlated with improvement in joint space narrowing scores [35].

Importantly, this work has shown that C1M also serves as a biomarker that associates with joint response. Patients achieving ACR20 or ACR50 response at week 24 showed lower C1M levels than ACR20 nonresponders, further emphasizing the association of this biomarker with clinical disease activity. Specifically, diminution of inflammation-driven bone degradation—as reflected by lower C1M levels (Fig. 3)—may lead to improvements in ACR components (e.g., joint swelling and tenderness), as reflected by achievement of ACR response. ACR20 and ACR50 responders in the placebo group also had numerically lower C1M levels at week 24 compared with ACR20 nonresponders, suggesting that C1M may be associated with disease activity rather than the pharmacodynamic effect of guselkumab. No differences in serum protein levels were observed in patients who achieved PASI75 compared with those who did not realize this degree of skin response, indicating that collagen biomarker changes may not be associated with skin clearance.

There are several limitations to this work, which will require further exploration. First, this study evaluated collagen biomarker levels in the serum, and additional work is needed to understand, more directly, the collagen turnover in affected tissues. Also, while we showed that biomarkers may serve as a peripheral measure of underlying joint inflammation and damage, additional work is needed to further explore the relationship of these biomarkers to PsA. In addition, data on other characteristics that may influence these biomarker levels, such

as factors related to diet, environment, or genetics, were not collected for either the DISCOVER-2 study population or the healthy controls. The understanding of collagen turnover biomarkers, particularly in relation to rheumatic diseases, is an evolving field, and will require additional, large cohort studies to further delineate the role of these biomarkers.

CONCLUSIONS

In summary, results of this investigation indicate that serum collagen biomarkers C1M, C3M, C4M, C6M, PRO-C3, and PRO-C6 are elevated in patients with PsA compared with healthy controls. We also observed significant correlations between serum C1M, C3M, C4M, and C6M levels and PASI scores, DAS28-CRP scores, and CRP level at baseline. Levels of C1M, C3M, C4M, and C6M significantly decreased with guselkumab treatment at week 24, and lower C1M levels at week 24 were associated with achievement of ACR response at week 24, suggesting that collagen degradation biomarkers, individually or in combination, might be useful in evaluating changes in disease activity after treatment. The need for surrogate blood-based biomarkers of the joint to provide a more granular understanding of disease progression or improvement to support PsA patient care and clinical trials of new therapies is clear, and the results of this study suggest that serum collagen degradation and formation biomarkers have potential as PsA biomarkers. This work provides insight into the potential utility of ECM biomarkers in PsA patients and evidence that guselkumab may decrease collagen turnover.

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Data Availability. The data sharing policy of Janssen Pharmaceutical Companies of Johnson & Johnson is available at <https://www.janssen.com/clinical-trials/transparency>. As noted on this site, requests for access to the study data can be submitted through Yale Open Data Access (YODA) Project site at <http://yoda.yale.edu>.

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