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CCL20 and *IL22* Messenger RNA Expression After Adalimumab vs Methotrexate Treatment of Psoriasis:

A Randomized Clinical Trial

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Abstract

IMPORTANCE—Methotrexate is a first-line systemic agent for treating of psoriasis, although its onset of effects is slower and overall it is less effective than tumor necrosis factor blockers.

OBJECTIVE—To differentiate the response of psoriatic disease to adalimumab and methotrexate sodium.

DESIGN, SETTING, AND PARTICIPANTS—Single-center, randomized, assessor-blind, 2-arm clinical trial of 30 patients from the outpatient dermatology center of Tufts Medical Center, enrolled from August 18, 2009, to October 11, 2011. Patients aged 18 to 85 years with chronic plaque-type psoriasis, a minimum Physician Global Assessment score of 3 (higher scores indicate more severe disease), and a psoriatic plaque of at least 2 cm were randomized in a 1:1 fashion to receive subcutaneous adalimumab or oral methotrexate. Skin biopsy specimens obtained at baseline and weeks 1, 2, 4, and 16 were given a histologic grade by blinded assessors to evaluate treatment response. Analyses were conducted from April 16, 2013, to January 5, 2015.

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Study concept and design: Krueger, Gottlieb.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Goldminz, Suárez-Fariñas, Krueger, Gottlieb.
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Additional Contributions: Biljana Bazdar-Vinovrski, MS, PharmD, acted as the study pharmacist. No financial compensation was given for these services.

INTERVENTIONS—A 16-week course of subcutaneous adalimumab (40 mg every 2 weeks after a loading dose) or low-dosage oral methotrexate sodium (7.5–25 mg/wk).

MAIN OUTCOMES AND MEASURES—Changes in genomic, immunohistochemical, and messenger RNA (mRNA) profiles.

RESULTS—Methotrexate responders experienced significant downregulation of helper T-cell–related (T_H1, T_H17, and T_H22) mRNA expression compared with methotrexate nonresponders. Comparisons among adalimumab-treated patients were limited by the number of nonresponders (n = 1). Between adalimumab and methotrexate responders, we found no significant differences in gene expression at any study point or in the expression of T-cell–related mRNA at week 16. Adalimumab responders demonstrated early downregulation of chemokine (C-C motif) ligand 20 (*CCL20*) mRNA (mean [SE] at week 2, −1.83 [0.52], $P < .001$; week 16, −3.55 [0.54], $P < .001$) compared with late downregulation for methotrexate responders (week 2, 0.02 [0.51], $P = .96$; week 16, −2.96 [0.51], $P < .001$). Similar differences were observed with interleukin 22 (*IL22*) mRNA showing early downregulation for adalimumab responders (week 2, −3.17 [1.00], $P < .001$; week 16, −3.58 [1.00], $P < .001$) compared with late downregulation for methotrexate responders (week 2, −0.44 [0.68], $P = .64$; week 16, −5.14 [0.68], $P < .001$). Analysis of variance findings for key mRNA and immunohistochemical marker expression over the study course were significant only for *CCL20* ($P = .03$) and *IL22* ($P = .006$) mRNA comparing adalimumab and methotrexate responders.

CONCLUSIONS AND RELEVANCE—Methotrexate is an immunomodulator with effects on helper T-cell signaling in psoriasis. Similar genomic and immunohistochemical response signatures and levels of mRNA downregulation at study completion among adalimumab and methotrexate responders suggest a disease-driven instead of therapeutic-driven pathway regulation. Adalimumab and methotrexate responses are differentiated by patterns of normalization of *CCL20* and *IL22* mRNA expression and may explain the varied onset and degree of clinical responses by each treatment.

TRIAL REGISTRATION—clinicaltrials.gov Identifier: NCT00932113

Psoriasis is a chronic immune-mediated disorder of dysregulated T-cell signaling that affects approximately 3% of the global population. Methotrexate sodium was first evaluated as a treatment for psoriasis in the 1950s, whereas newer biological agents, including adalimumab, were introduced in the 1990s. Studies have demonstrated the superior clinical efficacy and faster onset of the clinical response of adalimumab in the treatment of psoriasis compared with methotrexate.[1,2] However, to our knowledge, no investigations have profiled the features of genomic, immunohistochemical, and messenger RNA (mRNA) expression that determine and differentiate responses of psoriatic disease to methotrexate and adalimumab. The primary objective of our study was to characterize the genomic, immunohistochemical, and mRNA signatures associated with methotrexate and adalimumab in the treatment of plaque-type psoriasis.

Methods

We performed a single-center, randomized, assessor-blind, 2-arm clinical trial. We consecutively enrolled patients attending the tertiary-referral, academic dermatology clinic

at Tufts Medical Center from August 18, 2009, through October 11, 2011. Samples were sent to The Rockefeller University in New York, New York, for further analysis. The study received approval from the internal review boards at both institutions and followed CONSORT recommendations for reporting of randomized clinical trials (Figure 1). The study protocol is found in the Supplement 1.

Patients

Each patient provided written informed consent for the study before initiation of study participation. Eligible patients were men or women aged 18 through 85 years with chronic plaque-type psoriasis. A minimum Physician Global Assessment (PGA)[3] score of 3, based on erythema, induration, and scale (composite score range, 0–5; higher scores indicate more severe disease), and a psoriatic plaque of at least 2 cm in an area that could undergo repeated biopsy were also required for enrollment.

Before study initiation, patients were required to receive (1) no investigational drugs or biological agents within the previous 12 weeks; (2) no methotrexate within the previous 6 weeks; (3) no psoralen–UV-A or oral systemic treatments within the previous 4 weeks; and (4) no UV-B or topical therapies within the previous 2 weeks. However, a continued, stable regimen of classes I or II topical corticosteroids applied to the scalp, axilla, or groin was permitted during study participation. We excluded patients who were previously treated with adalimumab, who had a primary nonresponse to methotrexate, infliximab, or etanercept, or who had experienced safety-related issues secondary to treatment with methotrexate or anti–tumor necrosis factor (TNF) agents. The remaining exclusionary criteria consisted of a history of internal malignant neoplastic disease within 5 years, alcohol or other drug abuse, pregnancy or active nursing, known seropositivity for human immunodeficiency virus, an untreated positive tuberculosis test result, chronic hepatitis B or C virus infection, multiple sclerosis, transverse myelitis, optic neuritis, epilepsy, or a severe infection or receipt of a live vaccine within the previous 30 days.

Study Design

Thirty patients were enrolled in the trial. Through restricted randomization, the patients were distributed in a 1:1 fashion by the study pharmacist to receive adalimumab or methotrexate. The role of the study pharmacist was limited to randomizing patients to treatment and dispensing the study drug; the pharmacist otherwise had no access to patient information or study data. Methotrexate sodium was given as an oral dosage starting at 7.5 mg/wk that was increased based on published protocols.[1] Folic acid was also administered as a 1-mg oral dose, 5 days per week, during study participation. Adalimumab was given as an 80-mg subcutaneous loading dose at baseline and then administered as a 40-mg dose at week 1 and every 2 weeks thereafter. The total treatment course was 16 weeks for each group.

Biopsy specimens of lesional and nonlesional skin (LS and NLS, respectively) were obtained before the first dose of each study drug (baseline). During the course of treatment, LS biopsy specimens were also obtained at weeks 1, 2, 4, and 16, from a single area of psoriatic skin selected at baseline (the target lesion). Each patient was scheduled to undergo a total of 6 biopsies.

Clinical Efficacy and Safety Monitoring

The primary clinical efficacy end points included the proportion of patients achieving a Psoriasis Area Severity Index (PASI)[3] of 75, defined as a 75% improvement in PASI from baseline. The PASI values range from 0 (no disease) to 72 (severe disease) and measure the severity of psoriasis (erythema, induration, and scale) and the total body surface area of disease involvement. Other clinical end points included improvement in body surface area (calculated using the patient's palm size as a 1% equivalent), PGA score, and the target lesion site (the PGA score for a single plaque selected at baseline for each patient). Routine laboratory tests and monitoring of vital signs and adverse events were performed throughout the study.

Statistical Analysis

Sample Size Calculation for Microarray Experiments—The R package ssize.fdr[4] was used to calculate the sample size needed for a microarray experiment powered to detect differentially expressed genes among patients who responded to treatment (responders) at week 16 (end of treatment).[5] Estimates for sample size calculations were based on previous experiments with anti-TNF agents that used a similar study design.[6] A sample size larger than 30 guaranteed that we could detect differentially expressed genes at a false discovery rate (FDR) of less than 0.05 and a fold change (FCH) of greater than 2.0 with a power higher than 80% for week 16.

Assessor Blinding—Assessors of clinical (A.B.G.) and histologic responses (M.S.-F. and J.G.K.) were blinded. All nonclinical assessments were performed after study completion.

Microarray Analysis

Preprocessing and Illumina-Based Psoriasis Transcriptome: Classic quality control steps excluded 3 samples (patient 18 at week 16, patient 13 at baseline [NLS], and patient 11 at baseline [LS]). Data were preprocessed using R package lumi,[7] quantile normalization, and variance stabilization ($\log_2$) transformation. Expression values were filtered to eliminate probe sets with low variation or intensity. The disease profile (psoriasis transcriptome) was defined by the differentially expressed genes between LS and NLS samples from each patient with an FDR of less than 0.05 and an FCH of greater than 1.5.

Modeling Response-Profile to Treatment: Response to treatment was determined by assessor-blinded, histologic scores of keratin 16 expression and epidermal thickness compared with LS.[8,9] Each patient was classified as a responder, a nonresponder, or a partial responder by these criteria.[10] Histologic response was characterized by elimination of suprabasal keratin 16 staining, normalization of epidermal differentiation, and reduced acanthosis. Nonresponse was characterized by retained acanthosis, abnormal epidermal differentiation, and positive keratin 16 staining among suprabasal keratinocytes. Epidermal, dermal, and overall expression of CD3 and CD11c were also evaluated, and other immunohistochemical markers were replaced in favor of additional array analyses. Complete data analysis was performed on the patients from each treatment group who were classified as responders or nonresponders. A mixed-effect linear model was used to model gene expression for the clinical trial's time course design. This model estimated the variables of

interest even with 3 samples excluded based on poor quality control. Model fitting and hypothesis testing were conducted using the *limma* package from Bioconductor.[11] We used a moderated *t* statistic for differences in time and calculated a moderated *F* score for composite comparisons. *P* values were calculated with adjustment for multiple hypotheses using the Benjamini-Hochberg approach.[12]

Reverse Transcription–Polymerase Chain Reaction Analysis

Expression of mRNA was measured for chemokines, cytokines, and antimicrobial peptides, which are known to be altered among patients with psoriasis and in response to treatment.[13,14] These included mRNA expression related to the following sets of helper T cells: T_H1 (interferon γ [*IFNG*; OMIM 147570] and myxovirus resistance 1 [*MX1*; OMIM 147150]), T_H17 (cathelicidin antimicrobial peptide [*CAMP*; OMIM 600474], chemokine [C-C motif] ligand 20 [*CCL20*; OMIM 601960], chemokine [C-X-C motif] ligand 1 [*CXCL1*; OMIM 155730], defensin β4A [*DEFB4A*; OMIM 602215], interleukin 17A [*IL17A*; OMIM 603149], *IL17F* [OMIM 606496]; and *IL23A* [OMIM 605580]), and T_H22 (*IL22*; OMIM 605330). Values were normalized to the housekeeping gene hARP, $\log_2$-transformed for statistical analysis, and modeled using mixed-effect models. Several other mRNAs of interest were not evaluated by reverse transcription–polymerase chain reaction analysis owing to their high sensitivity on gene arrays. Analyses were conducted from April 16, 2013, to January 5, 2015.

Results

Forty-three patients underwent assessment for eligibility, and 30 were randomized to each treatment group (Figure 1). Thirteen patients were excluded, including 6 lost to or unavailable for follow-up after screening, 4 with an inadequate PGA score or target lesion site at baseline, 2 who were seropositive for hepatitis B or C virus, and 1 with a history of clinical nonresponse to methotrexate. No significant difference in baseline demographic data was found between treatment groups (Table 1). All patients underwent evaluation at each designated study visit except 1 adalimumab-treated patient who was unavailable for follow-up at week 12. The mean maximum dosage of methotrexate sodium was 22 (range, 15–25) mg/wk.

Clinical and Histologic Efficacy

At weeks 8 and 16, 5 (33%) and 10 (67%) adalimumab-treated patients, respectively, achieved a PASI of 75 compared with 1 (7%) and 4 (27%) methotrexate-treated patients, respectively. Among adalimumab-treated patients, 11 (73%) achieved a PGA score of clear or almost clear (0–1) at week 16 compared with 4 (27%) methotrexate-treated patients. Adalimumab responders had a faster reduction in PASI from baseline and a greater percentage reduction in PASI at week 16 (mean, 84.5% [interquartile range, 76.8%–94.7%]) than methotrexate responders (mean, 64.4% [interquartile range, 58.0%–80.7%]) (eFigure, A in Supplement 2). Percentage of improvement in PASI and in target lesion site were strongly correlated for adalimumab-treated (Pearson $r = 0.81$) and methotrexate-treated (Pearson $r = 0.75$) patients. Based on histologic criteria, adalimumab-treated patients included 8 responders (53%), 6 partial responders (40%), and 1 nonresponder (7%)

compared with 7 responders (47%), 3 partial responders (20%), and 5 nonresponders (33%) among methotrexate-treated patients (eFigure, B and C, Supplement 2).

The adverse events in order of frequency included upper respiratory tract infection (n = 8), flulike illness (n = 2), dizziness (n = 2), and injection site reaction (n = 2) for adalimumab-treated patients. They included upper respiratory tract infection (n = 11), gastrointestinal tract upset (n = 8), and minor infection (n = 4) for methotrexate-treated patients.

Psoriasis Transcriptome

A psoriasis transcriptome of 671 upregulated and 624 down-regulated transcripts (representing 526 and 521 genes, respectively) was generated to compare baseline gene expression among LS and NLS samples from all 30 patients before treatment (FDR, <0.05; FCH, >1.5). This transcriptome was consistent with study findings among patients with psoriasis described previously.[6,15] Before initiation of therapy, no differentially expressed genes were found between the 2 treatment groups (LS-to-NLS sample comparison).

Methotrexate Responders and Nonresponders

Genomic Profile—Overall gene expression was plotted as a GG plot of $\log_2$-transformed gene expression against time (Figure 2A). Methotrexate responders demonstrated greater normalization of psoriasis transcriptome gene expression compared with nonresponders at all study points after baseline. A heat map of gene expression showed normalization of psoriasis transcriptome genes during the 16-week study for methotrexate responders but not for nonresponders (Figure 2B). The differential treatment effect (response to methotrexate therapy) was defined as genes in which the treatment effect (week_i compared with LS at each time point *i*) was significantly different between responders compared with nonresponders at the set FDR and FCH criteria of less than 0.05 and greater than 1.5, respectively. A differential treatment effect was observed only for the comparison of week 16 with LS.

mRNA Response Signatures—Among methotrexate responders, our study showed significant downregulation of T_H1, T_H17, and T_H22 pathway mRNA expression compared with nonresponders at week 16 (Figure 3). To further assess the role of T_H-specific cell lineages, we performed repeated-measures analysis of variance (ANOVA) on mRNA expression and demonstrated significance among T_H17 ($P < .01$ for all except *CAMP* [$P = .10$]) and T_H22 ($P < .001$) axis expression (Table 2). For the T_H1 axis, results of the ANOVA for *MX1* ($P = .03$), but not *IFNG* ($P = .09$) mRNA expression was statistically significant. Results of the ANOVA were not significant for immunohistochemical markers, including epidermal, dermal, and overall expression of CD3 and of CD11c ($P > .05$ for all) when we compared methotrexate responders and nonresponders.

Genomic Profile and mRNA Response Signatures

Adalimumab Responders and Nonresponders—No statistically significant differences between adalimumab responders and nonresponders in individual gene expression during the study course were found through microarray analysis. A GG plot and heat map of gene expression demonstrated normalization of psoriasis transcriptome genes

during the 16-week study course among adalimumab responders but not among nonresponders (Figure 2). Compared with adalimumab nonresponders, responders demonstrated significant downregulation of *CAMP, CXCL1, DEFB4A*, and *MX1* mRNA expression during the study course (Table 2). Results of the ANOVA were also significant for immunohistochemical markers, including epidermal, dermal, and overall CD11c expression ($P < .001$ for all) and epidermal ($P < .001$) but not dermal or overall CD3 expression ($P > .70$). We observed greater downregulation among adalimumab responders than nonresponders.

Adalimumab and Methotrexate Responders—Adalimumab demonstrated greater normalization of psoriasis transcriptome gene expression compared with methotrexate, with the largest difference observed at week 4 (Figure 2A). From weeks 4 to 16, the rate of change for methotrexate surpassed that for adalimumab, although overall normalization of gene expression favored adalimumab at study completion. A heat map similarly showed faster and more complete normalization of gene expression for adalimumab responders compared with methotrexate responders (Figure 2B). During the entire study, however, we observed no differential response effect genes, defined as individual genes with a treatment effect significantly different when we compared the responders of each study group ($week_i$ compared with LS; FDR, <0.05; FCH, >1.5).

Log_2-transformed FCH mRNA expression, including T_H1-, T_H17-, and T_H22-related mRNA, was evaluated for adalimumab and methotrexate responders. The comparison of mRNA levels at week 16 with those of LS reached pretreatment NLS-to-LS expression levels for all transcripts studied (Figure 3). Adalimumab responders demonstrated early downregulation of *CCL20* mRNA (mean [SE] at week 2, −1.83 [0.52], $P < .001$; week 16, −3.55 [0.54], $P < .001$) compared with late downregulation for methotrexate responders (week 2, 0.02 [0.51], $P = .96$; week 16, −2.96 [0.51], $P < .001$). Similar differences were observed with interleukin 22 (*IL22*) mRNA showing early down-regulation for adalimumab responders (week 2, −3.17 [1.00], $P < .001$; week 16, −3.58 [1.00], $P < .001$) compared with late downregulation for methotrexate responders (week 2, −0.44 [0.68], $P = .64$; week 16, −5.14 [0.68], $P < .001$). Results of the ANOVA for mRNA expression during the 16-week study were significant only for *CCL20* ($P = .03$) and *IL22* ($P = .006$) (Table 2). Results of the ANOVA were not significant for immunohistochemical markers, including epidermal, dermal, and overall expression of CD3 and of CD11c (all $P > .40$), when we compared adalimumab and methotrexate responders.

Discussion

This assessor-blinded, 2-arm study investigates the effects of adalimumab and methotrexate in the treatment of psoriasis through clinical, genomic, immunohistochemical, and mRNA analyses. Similar to the findings of previous studies,[1,2] we demonstrate faster and more complete clinical disease responses among adalimumab-treated than methotrexate-treated patients. We show that methotrexate responders compared with methotrexate nonresponders experience significant down-regulation of mRNAs in the T_H1-, T_H17-, and T_H22-related signaling pathways and normalization of psoriasis transcriptome genes. Among adalimumab responders, normalization of *CCL20* and *IL22* mRNA expression is faster than among

methotrexate responders, but both groups share similar genomic and mRNA profiles at week 16, the study end point. Our findings highlight methotrexate's role as an immunomodulator and suggest that further investigations of CCL20-and IL22-related signaling in psoriasis are warranted.

Methotrexate is a first-line, systemic therapy in the treatment of psoriasis, and its use is typically required before prescription of biological agents. Despite methotrexate's significantly lower cost, current evidence suggests that biological agents are safe, have a faster onset of clinical response, and demonstrate an overall higher efficacy than methotrexate.[1,2] However, no known studies have evaluated methotrexate's effects on pathway regulation in psoriasis or compared the changes induced by methotrexate and biological agents at the genomic, immunohistochemical, and mRNA levels.

Our study shows that methotrexate is an immunomodulator with significant downregulatory effects on T_H1-, T_H17-, and T_H22-related signaling pathways in diseased psoriatic skin. Current research suggests that an aberrant T_H17 axis is a key component in the process leading to and maintaining inflammatory dysregulation in psoriasis.[16] In susceptible hosts, dendritic cell–driven IL23A production leads to activation of T_H17 and T_H22 subsets. Activated T_H17 produces IL17A and IL17F, which stimulate keratinocyte production of inflammatory mediators, including CCL20, CXCL1, and DEFB4A. Interleukin 22–producing T_H22 also plays a key role in mediating the epidermal changes observed in psoriasis.[17,18] As we demonstrated with methotrexate, effective treatment of psoriasis may be expected to normalize these aberrant signaling pathways. This hypothesis is supported by similarities between adalimumab and methotrexate responders in the differential response effect genes and in mRNA expression levels at week 16, suggesting disease-specific instead of treatment-specific pathway regulation. However, although quantitative gene and mRNA expression for responding patients appears to converge at week 16, adalimumab responders experienced faster downregulation of *CCL20* and *IL22* mRNA than methotrexate responders.

As a member of the IL10 cytokine family, IL22 is primarily produced by T lymphocytes, particularly T_H22 lymphocytes.[19] Interleukin 22 stimulation of barrier tissues, such as the skin, joints, and gastrointestinal tract, leads to regulation of chemokine and cytokine production within these tissues. Downstream processes, including cellular differentiation and proliferation, are essential for the maintenance of these physiologic barriers subject to frequent external insults.[20] However, dysregulated activity of these pathways can be pathogenic in disease states such as psoriasis. Psoriatic skin is characterized by increased expression of IL22 and other factors (eg, TNF, IFNγ) that increase keratinocyte and fibroblast expression of the IL22 receptor complex 1.[19] Within psoriatic skin, increased IL22 pathway signaling promotes characteristic findings, such as acanthosis and keratinocyte dedifferentiation.[21] Mutations in the *IL22* promoter site are known to lead to T-cell–mediated upregulation of IL22 expression and are associated with early-onset psoriasis.[22] In addition, the chronic course of psoriasis may in part be linked to persistent expression of IL22-producing, $CD4^+$, tissue-resident, memory T cells within the normal-appearing (NLS) psoriatic epidermis.[23] In patients with psoriatic arthritis, IL22 levels are significantly elevated in the synovial fluid and can regulate fibroblastlike synoviocyte proliferation.[24]

Osteoblast-dependent bone remodeling, a characteristic finding among patients with inflammatory arthritis, is also mediated in part by IL22 signaling.[25] Therefore, IL22-related signaling participates in the pathogenic inflammatory processes occurring in the skin and joints of patients with psoriasis.

The chemokine CCL20 is produced by multiple cell lines, including keratinocytes, neutrophils, and T cells, particularly T_H17 lymphocytes. Its chemokine receptor, C-C receptor 6 (CCR6), is strongly expressed by T cells and immature dendritic cells and therefore promotes the migration and entry of key psoriatic disease effector cells into target tissues. One of the immediate response psoriasis factors, CCL20, is upregulated by synergistic IL17/TNF signaling and attracts clusters of $CCR6^+$ dendritic cells and T cells to the psoriatic epidermis.[26,27] Administration of anti-CCL20 antibodies in a murine model reduce IL23-induced, psoriasiform inflammation and limit epidermal infiltration of IL22-producing $CCR6^+$ T cells, thereby disrupting the psoriatic phenotype.[28] The CCR6-knockout mice also experience significant reductions in IL23-induced *IL22* production compared with wild-type mice.

Two previous single-arm trials[29,30] have investigated the ability of adalimumab to regulate key inflammatory pathways in psoriasis on the mRNA level. In one study by Hendriks et al, [29] 10 clinically responding, adalimumab-treated patients demonstrated early downregulation of innate immune responses and epidermal complex–proliferation makers but late downregulation of adaptive immune responses. A study by Balato et al[30] showed significant reductions in *CCL20* and *IL22* mRNA expression, among others, in the skin and peripheral blood mononuclear cells isolated from 18 adalimumab-treated patients with psoriasis who had histologic evidence of response. In the study by Hendriks et al,[29] *CCL20* and *IL22* expression was not evaluated. Because both studies had a single arm, they were unable to evaluate qualitative differences between disease response (improvement in pathogenic axes associated with psoriasis) and medication response (how pathways are regulated by the therapy administered). Other single-arm studies have also demonstrated significant downregulation of *CCL20* and *IL22* lesional mRNA among treatment responders with psoriasis, including patients who receive cyclosporine or etanercept.[14,31] However, potential comparative evaluations of pathway regulation between these trials are limited by varied study methods.

Our trial suggests that CCL20 and IL22 signaling may be key determinants of the faster and more complete clinical responses observed with adalimumab compared with methotrexate in the treatment of psoriasis. Previous studies have investigated the anti-IL22 monoclonal antibody, fezakinumab, for the treatment of psoriasis (phase 1)[32] and rheumatoid arthritis (phase 2).[33] At present, no known, active clinical studies in psoriasis are ongoing, but targeting of IL22 alone may be too far downstream in the pathogenic mechanism of psoriasis to be an effective monotherapy. Although IL22 is a key regulator of epidermal hyperplasia and keratinocyte differentiation, other central effector cells and signaling path-ways in the disease process would not be targeted directly through IL22 inhibition. For example, blockade of IL22 receptor complex 1 may interfere with additional ligands and lead to more effective signaling disruption.[19] Signaling of CCL20/ CCR6 may also present an effective target in drug development because the signal links the innate (dendritic cells) and adaptive

(T cells) immune responses and is involved in chemotaxis and effector cell trafficking. Preclinical murine models investigating the blockade of these pathways have promising results, although no clinical studies have been initiated, to our knowledge.[28,34]

Our study has several limitations. Patterns of genomic and mRNA regulation may not reflect changes seen at the post-translational levels, including protein expression and post-translational modification. Additional studies investigating such expression may expand on the findings reported here. In addition, differences observed between adalimumab responders (n = 8) and nonresponders (n = 1) require further investigation because of the limited sample size. However, this study is one of the larger ones to characterize genomic and mRNA data from psoriatic skin samples. This study also represents, to our knowledge, the first known evaluation of methotrexate and comparison of methotrexate with an anti-TNF agent by these methods. Finally, methotrexate was dosed in a graduated fashion; therefore, delayed responses in clinical and mRNA expression may to some degree be expected. However, unlike *CCL20* and *IL22*, all other mRNA transcripts studied among methotrexate responders mirrored those of adalimumab responders in rates of normalization. Dose sensitivity in *CCL20* and *IL22* expression may be greater than those of the other transcripts studied, although this possibility is less likely, and would support the role of CCL20 and IL22 above the other chemokines and cytokines studied in the differential rates of disease response between adalimumab and methotrexate.

Conclusions

Clinical response to adalimumab in psoriasis is faster, more complete, and characterized by faster downregulation of *CCL20* and *IL22* mRNA compared with methotrexate. These cytokines are important disease mediators with downstream effects, including inflammatory cell migration, keratinocyte dysregulation, and joint space remodeling. Given the expression of CCL20 and CCR6 by the innate (dendritic cells) and adaptive (T cells) immune system and the role of IL22 in mediating crosstalk between keratinocytes and other immune cells, targets within these pathways may disrupt the psoriatic disease process at multiple critical points.[35] Therefore, IL22-and CCL20-related signaling pathways are candidates for further investigation to understand disease mechanisms and drug development in psoriasis.[28,35]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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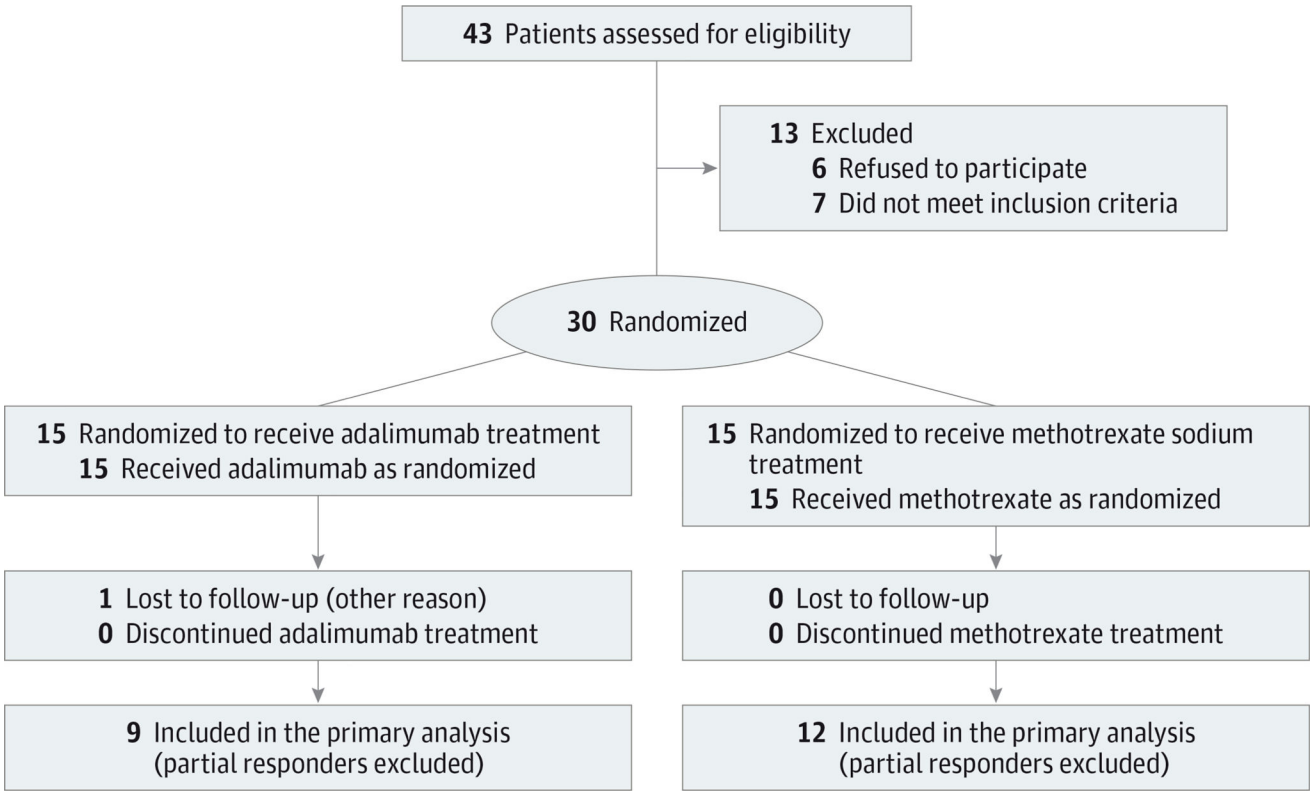

Figure 1.
CONSORT Flow Diagram

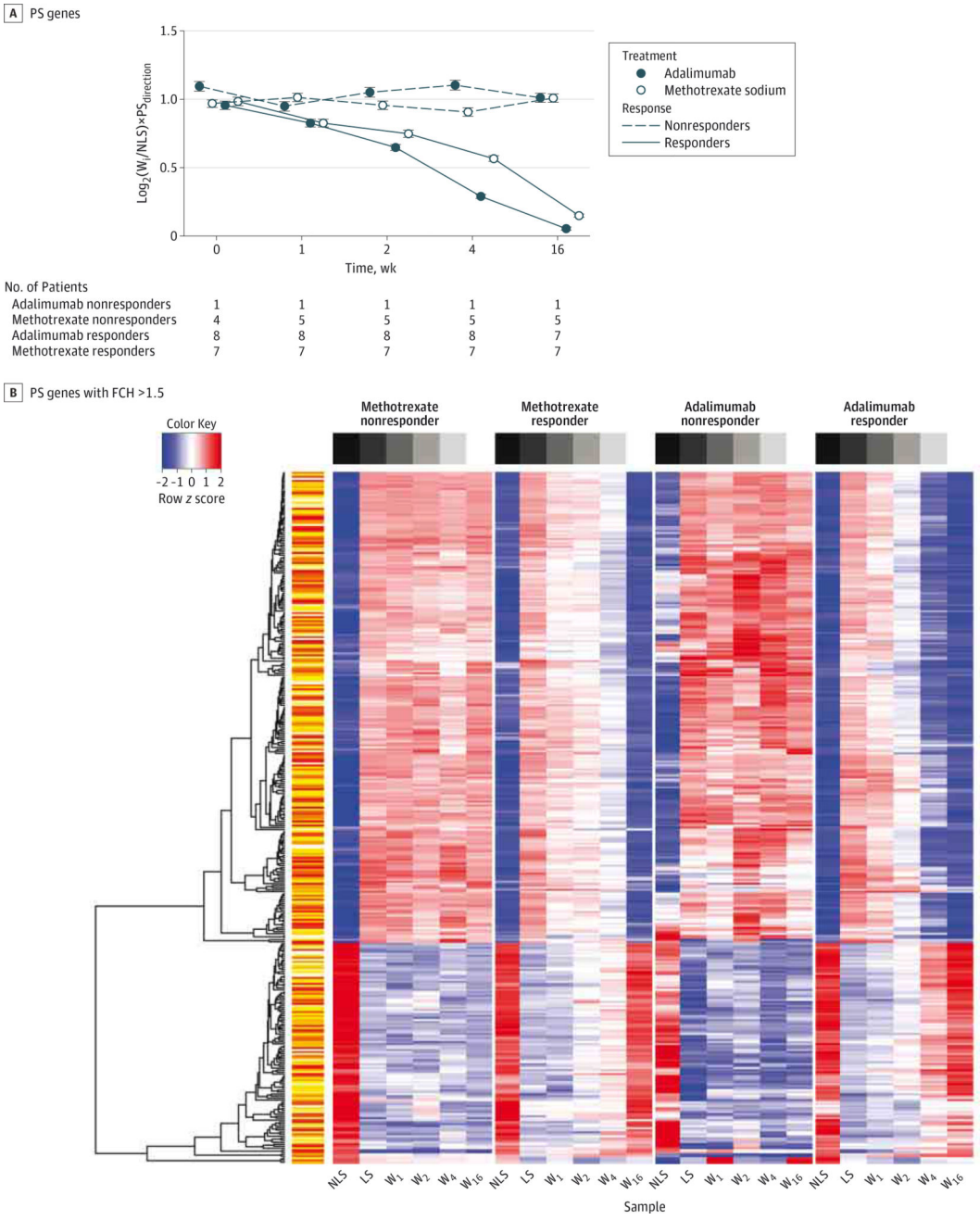

Figure 2. Changes in Genomic Data by Disease Response and Treatment Arm

A, gg plot showing variance stabilization (log_2) transformation of gene expression for psoriasis transcriptome (PS) genes (1295 probes/1047 genes) among patients who responded to adalimumab treatment (responders), patients who did not respond to adalimumab treatment (nonresponders), methotrexate sodium–treated responders, and methotrexate nonresponders at week (W) 1, 2, 4, and 16 compared with baseline expression in nonlesional skin (NLS). B, Heat map representing expression of PS genes for methotrexate nonresponders, methotrexate responders, adalimumab nonresponders, and adalimumab responders with fold change (FCH) of greater than 1.5 for baseline lesional and nonlesional

skin (LS and NLS, respectively) samples and samples at weeks 1, 2, 4, and 16.W_i indicates expression at any given week ($week_i$).

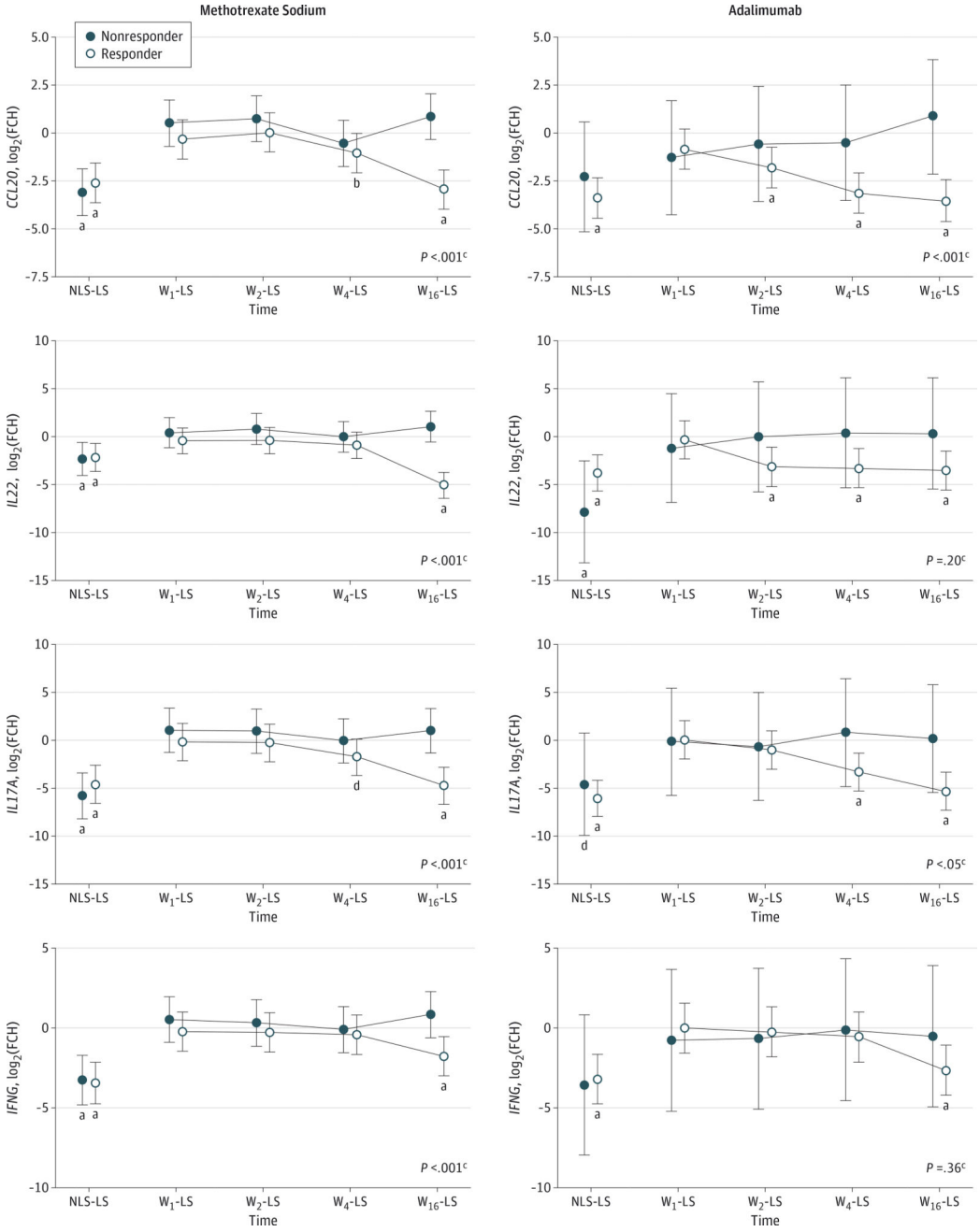

Figure 3. Changes in Select Messenger RNA (mRNA) Expression by Disease Response and Treatment Arm

Variance stabilization (log_2) transformation fold change (FCH) expression of mRNA for patients who responded (responders) and did not respond (nonresponders) to study treatments. Data points indicate mean; error bars, 95%CI. Chemokine (C-C motif) ligand 20 (*CCL20*), interleukin 22 (*IL22*), *IL17A*, and interferon-γ (*IFNG*) mRNA expression at baseline are compared for baseline lesional and nonlesional skin (LS and NLS, respectively), and week (W) 1, 2, 4, and 16 for methotrexate sodium (left column) and adalimumab (right column).

[a] $P < .001$.

[b] $P < .01$.

[c] P value for responders vs nonresponders, W_{16}-LS.

[d] $P < .05$.

Table 1

Baseline Characteristics for Methotrexate Sodium– and Adalimumab-Treated Patients[a]

Characteristic	Study Treatment		*P* Value
	Methotrexate (n = 15)	Adalimumab (n = 15)	
Male sex	13	11	.36
Age, mean (range), y	50.3 (22–69)	50.5 (22–69)	.97
Race, white	14	12	.28
Mean PASI[b]	15.9	16.8	.79
Target lesion location			
Upper extremity	3	3	.98
Lower extremity	6	5	
Trunk	5	6	
Buttock	1	1	
History of psoriatic arthritis	3	2	.62
Previous systemic therapy	4	6	.44
Mean BMI	35.0	32.1	.62
Disease duration, mean (range), y	21.5 (0–47)	17.3 (1–45)	.41
Age at onset, mean (range), y	29.1 (5–68)	33.3 (4–56)	.54

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); PASI, Psoriasis Area Severity Index.

[a] Unless otherwise indicated, data are expressed as number of patients.

[b] Values range from 0 (no disease) to 72 (severe disease).

Table 2

Reverse Transcription–Polymerase Chain Reaction Data for Key T_H17, T_H22, and T_H1 Mediators[a]

Pathway and Gene	Methotrexate Sodium			Adalimumab			Methotrexate Responders vs Adalimumab Responders, *P* Value[c]
	Responders	Nonresponders	*P* Value[b]	Responders	Nonresponders	*P* Value[b]	
T_H17							
CAMP	−2.83 (0.82) [.001]	0.87 (0.97) [.38]	.10	−3.85 (0.79) [<.001]	0.05 (2.2) [.98]	.03	.69
CCL20	−2.96 (0.51) [<.001]	0.84 (0.60) [.17]	<.001	−3.55 (0.54) [<.001]	0.84 (1.5) [.57]	.052	.03
CXCL1	−3.18 (0.70) [<.001]	1.22 (0.83) [.15]	<.001	−3.01 (0.61) [.002]	1.03 (1.6) [.48]	.02	.58
DEFB4A	−6.00 (1.1) [<.001]	1.83 (1.2) [.15]	.001	−6.96 (0.96) [<.001]	−0.07 (2.6) [.98]	.03	.50
IL17F	−3.24 (0.74) [<.001]	1.02 (0.87) [.25]	.009	−1.97 (0.99) [.054]	0.79 (2.8) [.78]	.87	.68
IL17A	−4.75 (0.96) [<.001]	1.00 (1.1) [.39]	<.001	−5.31 (0.98) [<.001]	0.18 (2.8) [.95]	.31	.74
IL23A	−2.72 (0.56) [<.001]	0.89 (0.67) [.19]	.003	−3.90 (0.79) [<.001]	−0.81 (2.2) [.72]	.36	.65
T_H22							
IL22	−5.14 (0.68) [<.001]	1.00 (0.80) [.22]	<.001	−3.58 (1.0) [.001]	0.25 (2.8) [.93]	.10	.006
T_H1							
IFNG	−1.77 (0.61) [.005]	0.82 (0.72) [.26]	.09	−2.66 (0.77) [.002]	−0.52 (2.2) [.81]	.84	.87
MX1	−2.00 (0.46) [<.001]	0.47 (0.54) [.39]	.03	−1.90 (0.31) [<.001]	0.04 (0.86) [.96]	.001	.10

Abbreviations: *CAMP*, cathelicidin antimicrobial peptide; *CCL20*, chemokine (C-C motif) ligand 20; *CXCL1*, chemokine (C-X-C motif) ligand 1; *DEFB4A*, defensin β4A; FCH, fold change; *IFNG*, interferon-γ; IL, interleukin; mRNA,messenger RNA; *MX1*,myxovirus resistance 1; T_H, helper T cell.

[a] Unless otherwise indicated, data are given as mean (SE) [*P* value] for the treatment effect at the week 16 study end point.

[b] *P* value for the interaction effect time × response comparing responders and nonresponders.

[c] *P* value for the interaction effect time × treatment.