Gene Expression Profiles Normalized in Psoriatic Skin by Treatment with Brodalumab, a Human Anti–IL-17 Receptor Monoclonal Antibody

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The IL-17 pathway is an established driver of psoriasis pathogenesis. We examined the detailed molecular and cellular effects of blockade of IL-17 signaling in human psoriatic skin before and following treatment with brodalumab, a competitive inhibitor of the IL-17 Receptor A subunit. Thousands of aberrantly expressed genes in lesional skin normalized within 2 weeks following brodalumab treatment, with conversion of the lesional psoriasis transcriptome to resemble that seen in nonlesional skin. Keratinocyte-expressed genes appeared to normalize rapidly, whereas T cell–specific normalization occurred over six weeks. The three IL-17 ligand genes that are upregulated in lesional skin, IL17A, IL17C, and IL17F, were all downregulated in a dose-dependent manner following brodalumab treatment. Cellular measures also showed a similar pattern with dramatic decreases in keratinocyte hyperplasia within one week, and decreases in infiltrating leukocytes occurred over a longer timescale. Individuals with the highest brodalumab exposure showed normalization of both IL-17–responsive genes and the psoriasis transcriptome, whereas subjects with lower exposures showed transient or incomplete molecular responses. Clinical and molecular response appeared dependent on the extent of brodalumab exposure relative to the expression of IL-17 ligand genes, and reduction of IL-17 signaling into the nonlesional range was strongly correlated with normalization of the psoriasis transcriptome. These data indicate that blockade of IL-17 signaling in psoriatic skin leads to rapid transcriptomal changes initially in keratinocyte-expressed genes, followed by normalization in the leukocyte abnormalities, and demonstrates the essential role of the IL-17R on keratinocytes in driving disease pathogenesis.


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oriat is a chronic debilitating disease that affects 1–9% of the population, varying according to age and geographic region (1–3). Psoriasis is characterized by recurrent episodes of red, scaly, well-demarcated skin plaques (4) and histological changes including a thickened hyperproliferative keratinocyte layer, a reduced or absent granular layer, dilatation of dermal blood vessels, and proinflammatory leukocyte infiltration of both dermal and epidermal skin. Recent work has indicated significant increases in lesional skin of both CD4+ and CD8+ T cell subsets that produce the innate and adaptive cytokine IL-17A (5, 6).

The IL-17 cytokine family consists of six cytokines (IL-17A–IL-17F) and five receptors (IL-17RA–IL-17RE) (7). Of these, only levels of IL-17A, IL-17C, and IL-17F mRNA are elevated in lesional skin from patients with psoriasis, and these cytokines have been demonstrated to induce expression of proinflammatory target genes in keratinocytes (8, 9). The IL-17A, IL-17F, and IL-17 A/F heterodimer ligands act through an IL-17RA/RC complex, and IL-17C acts through an IL-17RA/RE complex (9), with anti–IL-17RA Abs able to block activities of all four ligand complexes (9).

Several biological agents with activity in psoriasis are associated with suppression of the IL-17 pathway. Cyclosporin and anti–IL-12p40 agents interfere with either psoriasis-related T cell development (10) or activity and anti-TNF agents may impact IL-17 by reducing synergistic costimulation (11). The suppression of the IL-17 pathway in psoriasis following anti-TNF treatment with etanercept was demonstrated by Zaba et al. (11), where they compared clinical response with the molecular effects on genes downstream from the TNF and IL-17 receptors. More recently, investigational agents directly targeting the IL-17A pathway have shown very high levels of efficacy in the clinic (12–15). Treatment with brodalumab, which targets the common receptor subunit IL-17RA, has been demonstrated to result in high percentages of individuals achieving total skin clearance (14, 15). Molecular and cellular reductions in psoriatic lesional skin markers occurred rapidly after both anti–IL-17R and anti–IL-17A ligand treatment (16), though detailed molecular comparisons between nonlesional and treated lesional skin have not yet been presented.

In this study, we show that blocking IL-17RA with brodalumab, a human IgG2 mAb that selectively binds and blocks signaling through IL-17RA, in patients with psoriasis results in rapid and significant improvements in molecular and cellular abnormalities of disease. Detailed microarray analysis of gene expression suggests that IL-17R blockade blunts inflammatory circuits emanating from the multiple IL-17 ligands that bind and trigger signaling through this receptor. Global changes in transcriptional expression within psoriatic lesions to levels observed in nonlesional skin include reductions in
numerous inflammatory factors from keratinocytes as well as multiple leukocyte subsets, and these changes are accompanied by improvements in clinical and histologic metrics. The expression of many disease-related genes was strongly suppressed, down to nonlesional levels, and this normalization was tightly correlated with successful competitive inhibition of IL-17 signaling by brodalumab.

Materials and Methods

Study population

Twenty-five patients with moderate to severe plaque psoriasis were treated with a single dose of brodalumab (n = 4, 140 mg s.c.; n = 8, 350 mg s.c.; n = 8, 700 mg i.v.) or placebo (n = 5). The study (clinicaltrials.gov NCT00867100) was reviewed and approved by appropriate institutional review committees at each study site. The study design and clinical results have been reported previously (15). To investigate the molecular and cellular changes in the skin following IL-17RA blockade with brodalumab, biopsies were obtained from a single nonlesional site at baseline, and from three locations in a single predesignated lesion that was sampled at baseline, wk 2 and 6 for most subjects. To assess even earlier molecular and cellular changes, posttreatment biopsies were obtained at baseline, wk 1, and wk 2 from four subjects in the 350-mg group and one receiving placebo. Biopsies were divided for analysis with both histology and RNA expression profiling.

Brodalumab concentrations were measured in blood at multiple intervals, exposures calculated as area under the curve (AUC), and fit to a pharmacokinetic model (17). No anti-brodalumab Abs were observed during the biopsy study (15).

Biopsy processing

One portion of each biopsy was flash frozen in liquid nitrogen for RNA isolation as described previously (18). Briefly, while still frozen, biopsies were homogenized in lysis buffer with or without manual disruption and RNA was prepared using the mirVana miRNA Isolation kit (Applied Biosystems, Carlsbad, CA) with on-column DNase treatment (Qiagen, Valencia, CA). RNA quality was assessed using a Bioanalyzer 2100 (Agilent, Palo Alto, CA). RNA from one nonlesional sample from a placebo-treated individual failed quality control and was not subsequently used and therefore the total sample number is either 25 or 24 depending on the analysis. The other portion of the biopsy was immediately frozen in OCT and processed for histology as described previously (19).

RNA analysis

Fifty nanograms of total RNA was amplified (Ovation RNA Amplification system with WB reagent; Nugen, San Carlos, CA) and labeled (FL Ovation RNA Amplification system with WB reagent; Nugen, San Carlos, CA). RNA from one nonlesional sample from a placebo-treated individual failed quality control and was not subsequently used and therefore the total sample number is either 25 or 24 depending on the analysis. The other portion of the biopsy was immediately frozen in OCT and processed for histology as described previously (19).

Quantitative RT-PCR (qRT-PCR) was performed using primer sets from Applied Biosystems with a 7900HT Sequence Detection instrument (Applied Biosystems). Cycle threshold (Ct) values were converted to Delta C t values by subtracting the mean of two relatively invariant genes (β-actin and ubiquitin C, or β-actin and GAPDH). Comparative differences between each lesional and the baseline nonlesional sample from each subject were calculated as the DeltaDelta Ct. Statistical differences in gene expression between groups were calculated from the ΔΔCt values using the Student t test with heteroscedastic distributions. Log2 average expression of IL-17 ligand genes and pharmacokinetic AUC was calculated from samples collected in the first 2 wk and the three ΔΔCt values were added to create a three ligand summary value. Log2 (brodalumab exposure−IL-17 ligand mRNA) values were generated by subtracting the three-ligand ΔΔCt values from Log2 (brodalumab AUC) values.

Microarray data analysis

A core set of genes differentially expressed between independently procured nonlesional and lesional skin was identified (“Core PsO”) using 14 paired samples from the Astarden subset and 15 paired samples from the ZabaAmenogen subset of the publically available microarray data set of paired lesional and nonlesional psoriasis samples (GSE41664) (18). A table of probe sets, log ratios, and p values was created from estimated between group differences in probe-set intensities provided by the AffyPLM: three step function using R version 2.15.2 (20, 21). The table was ordered by p value, and the 5000 sequences with the lowest p values were selected as a “core” set, “Core PsO” (Supplemental Table I). Of these 5000 probe sets, 2628 were overexpressed, and 2372 were underexpressed in lesional skin relative to nonlesional skin. This Core PsO probe set was used to compare lesional and nonlesional samples in this study using R version 2.15.2 (20), with the AffyPLM: three-step function (21). The resulting log ratio differences for the 700-mg dose groups were used to generate the histograms in Fig. 1 using the ggplot2 package (20).

The IL-17 and IFN-γ transcriptional scores were computed by averaging the log2 intensity of the probe sets from the sequences in each of the IL-17 and IFN-γ gene sets (Supplemental Table II). These sequences were selected from those induced by the respective cytokines in keratinocytes (22) and paired to those well measured on the Nugen/Affymetrix platform. A reduced set of Core PsO sequences were produced by removing the IL-17 and IFN-γ gene set members to reduce specific bias from the expression of genes that were tightly regulated by IL-17 or IFN-γ when calculating correlation coefficients with the IL-17 and IFN-γ gene sets. The psoriasis transcriptional index (PsTI) was defined based on the reduced Core PsO by subtracting the average log2 intensity of the probe sets from the sequences higher in nonlesional skin from the average log2 intensity of the probe sets higher in lesional skin. Therefore, the PsTI does not directly depend on the intensities of any of the genes in the IL-17 and IFN-γ gene sets. As a comparison of two different sets of intensities on an individual microarray chip, it also does not depend on comparison with any reference samples other than minimal effects that will have occurred during array normalization. Although lesional samples from individuals always scored higher than nonlesional samples from the same individuals, PsTI values ranged between individuals, with potential influences from genetics, which were not analyzed.

Results

Predose lesional and nonlesional skin biopsies, and two postdose biopsies were collected from each of 25 patients with moderate to severe plaque psoriasis enrolled in a single dose study of brodalumab (n = 4, 140 mg s.c.; n = 8, 350 mg s.c.; n = 8, 700 mg i.v.) or placebo (n = 5). Details of the study design and clinical results have been reported previously (15). Pharmacokinetic analysis showed that individuals in the 700-mg group all had high exposures lasting for ≥4 wk, whereas subjects in the lower dose groups had variable exposure (15, 17). All eight individuals in the 700-mg cohort had AUC values >1300 μg × day/ml and psoriasis area and severity index (PASI) improvements ≥70%. Pharmacokinetic variability was greater in the s.c. cohorts, ranging from ~10 to 325 μg × day/ml. Two subjects in the 140-mg s.c. cohort had higher exposure than that of the lowest exposure subject in the 350-mg group. Thus, single doses of 140 and 350 mg s.c. represent a continuum from low to moderate exposure lasting for as short as one week but up to 4 wk. To investigate the molecular effects of extended high-level blockade through the IL-17 R, we focused on the 700-mg group, whereas dose-dependent effects and incomplete blockade were examined in samples from the entire set.

IL-17R blockade with brodalumab normalizes the psoriasis transcriptome

A 5000 probe set was identified (Core PsO), based on significant differential expression between nonlesional and lesional skin regardless of fold changes, using data from two other studies on the same microarray platform (18). Of the 5000 probe sets selected, 2628 were usually overexpressed and 2372 were underexpressed in lesional skin, as compared with nonlesional skin. This same distribution of aberrant gene expression was replicated in the predose samples from this study, with separated bimodal distribution of sequences (Fig. 1A). This separated bimodal distribution of the up-in-lesional and down-in-lesional sequence sets was ob-
Normalization of T cell genes following brodalumab treatment occurs more slowly than keratinocyte-expressed genes

Because the low nonlesional expression levels of some cytokine genes are near the limit of detection on the microarray platform, full differences between postdose and nonlesional levels can be difficult to estimate and fold change estimates can suffer data compression (18). To accurately characterize the time course and dose dependence of key members of the Th1, Th17, and keratinocyte cytokine networks, we used qRT-PCR to measure eight cytokine genes known to be dysregulated in psoriasis. These included the genes for IL-17C and the Th17/22 cytokines, which have the highest and most consistent overexpression in psoriasis lesional skin (1, 8) as well as the gene for the Th1 cytokine IFN-γ and the genes for the upstream regulators IL-23 (for Th17/22) and IL-12 (Th1). At baseline, all eight genes were significantly different between nonlesional and lesional (p < 0.01), in the overall group (n = 24) or in either of the 350- or 700-mg group (n = 8 each). IL25 (IL17E), another IL-17R ligand gene that is not expressed by T cells, was <1.5-fold overexpressed in lesional skin and rapidly moved toward nonlesional levels as measured by microarray (data not shown).

The keratinocyte-expressed inflammatory cytokine IL17C returned to nonlesional levels of expression by week 2 in the 700-mg group (Fig. 2C). The IL12A and IL23A genes, which code for the unique subunits of each cytokine, rapidly returned to nonlesional levels, consistent with some expression from keratinocytes, or other cells directly responsive to IL-17RA activation (Fig. 2C). The Th17/22 cytokine genes IL17A, IL17F, and IL22 are regulated downstream of IL-23 (5), and expression of these genes is partially reduced at week 2 and reduced to nonlesional levels in the 700-mg group at week 6, which occurs after normalization of IL-23 gene expression (Fig. 2C). Downstream of IL-12, the mRNA expression for the Th1 cytokine IFN-γ was minimally changed at week 2, and only slightly decreased at week 6 in the 700-mg group, consistent with changes observed in IL-12 subunit genes, where the IL12B gene was only partially reduced at week 2, while expression of the IL12A gene increased toward the nonlesional level. In the lower dose brodalumab groups, similar effects were observed, although with less pronounced magnitude of response (Fig. 2C). Of note, IL17C and the IL-23 subunit genes were strongly reduced within 1 wk, whereas the T cell cytokine genes were only partially reduced at that time point. Thus, the upstream regulators IL-23 and IL-12 respond first to IL-17R blockade, with changes in the downstream T cell–specific genes for IL-17A, IL-17F, IL-22, and IFN-γ following.

**Brodalumab induces changes in inflammatory cellular measures**

The normalization of global RNA abnormalities was mirrored in cellular analysis of the skin by immunohistochemistry (IHC). Changes in keratinocyte markers were notable within 1 wk (Fig. 3A, 350-mg cohort) (15), including reductions in proliferation markers (Ki67 cell counts), and returned to nonlesional levels at week 2 (earliest day examined) for the 700-mg group. In contrast, inflammatory leukocyte infiltrates decreased less rapidly over 6 wk, including cell counts for T cells (both CD3 and CD8 [data not shown]), CD11c myeloid cells, and DC–lysosome-associated membrane glycoprotein (DC-LAMP) expressing DCs (Fig. 3B–D). Reductions in these leukocytes were notable at week 2, but the full effect of brodalumab in both the dermis and the epidermis was not noted until week 6. Representative IHC photomicrographs are shown for one subject in the 700-mg group (Fig. 3E).
Global aberrant psoriasis-induced gene expression and PASI rapidly change in a brodalumab dose-dependent manner and track with changes in the IL-17 signature

The PsTI was defined as the difference between the average intensity of two defined sets of upregulated and downregulated genes in individual skin samples (see Materials and Methods). By incorporating the intensities from thousands of genes without requiring direct comparison with reference samples, the PsTI represents a broad summary measure of the molecular inflammation state of individual skin biopsies, to enable comparison...
with scores for IL-17– and IFN-γ–responsive genes and with clinical responses (e.g., PASI). More inflamed samples have higher PsTI values. Most lesional samples clustered around a PsTI of +0.9 (mean [SEM]: 0.89 [0.19], median 0.97, n = 25), whereas nonlesional samples clustered approximately −0.9 (mean [SEM]: −0.92 [0.16]; median −0.94, p < 0.001, n = 24). Thus, molecular resolution of disease would be expected to move the score from positive to negative for an individual’s lesional skin samples. All but two of the predose lesional samples (all cohorts including placebo) had PsTI above 0.45 compared with the nonlesional samples of which all but two have PsTI below 0.45 (Fig. 4A). Thus postdose samples with PsTI below −0.5 represent local conversion from a lesional transcriptional phenotype to a nonlesional phenotype. Following treatment with brodalumab, all the 700-mg samples had index values below 0 at week 2 and below −0.5 at week 6. Samples from seven of the eight 350-mg subjects had subzero scores at either weeks 2 or 6. Of these, four subjects achieved scores below −0.5, although for one subject, it was transient, with a return to the lesional range at week 6. In the single-dose 140-mg group, there was a less pronounced effect, although the PsTI fell below −0.5 for one of the four subjects. Three subjects in the 140-mg group (n = 4) and one in the 350-mg group (n = 8) had PsTI scores that remained above +0.2 throughout. The four early samples obtained from week 1 in the 350-mg group showed rapid and intermediate normalization, with continued decreases in the index for each subject at week 2. Thus, the PsTI, which measures global changes in the psoriasis transcriptome, responds to brodalumab treatment similarly to the keratinocyte-expressed cytokines.

**IL-17 and IFN-γ score**

IL-17 and IFN-γ scores were generated as summary averages of the intensity of sequence sets (modified for our microarray platform) that were identified as keratinocyte genes induced by IL-17 (14 genes) or IFN-γ (18 genes), respectively, similar to that of Nograles et al. (22). Although the component genes may not each drive pathogenic processes, these summary scores provide surrogate for the level of IL-17– and IFN-γ-driven signaling in each biopsy. Changes in the IL-17 score following brodalumab treatment closely mirrored the global PsTI including subjects with smaller changes in the low and middose cohort (Fig. 4B). Furthermore, the IL-17 score completely discriminated between the lesional and nonlesional samples. Like the overall PsTI, there was some variation including a single outlier nonlesional sample. The IFN-γ score also decreased after brodalumab treatment (Fig. 4D); however, the reduction was partial and only into the top of the nonlesional range (similar to changes in IFN-γ mRNA). Taken together with the continued slight elevations of IFN-γ mRNA above nonlesional expression, this suggests that the low levels of persisting IFN signaling are insufficient to drive other aspects of psoriasis in the presence of IL-17RA blockade.

Interestingly, although PASI decreases generally mirrored the transcriptional scores, there was continued decrease in the 700-mg group between week 2 and week 6 (Fig. 4C). However, it should be noted that PASI is a global index that considers a patient’s total skin, resulting in an averaging of the resolution of some lesions and continued inflammation of other lesions. In contrast, the three transcriptional scores are specific to individual biopsies, therefore, reflecting the common biology at that lesion.

**Correlation between the IL-17 score and the PsTI**

Correlation between the IL-17 score and the PsTI was very high (r = 0.95; Fig. 5) across all samples, underscoring the tight coupling of the overall psoriasis transcriptional profile to IL-17 signaling. Correlation between the IFN-γ score and the PsTI was not quite as strong (r = 0.73), in line with postbrodalumab reduction of IFN signaling, but incomplete coupling of IFN-γ with the psoriasis molecular mechanisms. The IL-17 score and the IFN-γ score also were correlated at about the same level, suggesting that IFN-γ signaling has no greater specific dependence on IL-17 than on other inflammatory signals in psoriasis.

**Correlations between scores and identification of molecular nonresponders**

The PsTI and IL-17 scores of the pre- and postdose lesional skin samples correlated well with changes in the PASI scores (r = 0.73, r = 0.75, both p < 0.001). Predose lesional samples clustered in the upper right area of each plot with both high PASI and high molecular scores (Fig. 6A). Placebo samples did not show improvement in either PsTI or IL-17 score, and this mirrors the lack of improvement in the PASI score. In contrast, the vast majority of samples from individuals after brodalumab treatment moved into the lower left portion of each plot with low molecular (PsTI and IL-17) and low PASI scores. This supports the concept that normalizing the transcriptional response is a direct consequence of IL-17 signaling blockade and is correlated to subsequent clinical response.

Subjects were classified as clinical responders, based on the lowest PASI achieved during the study. Likewise, molecular responders (MR) were defined based on the lowest PsTI and lowest IL-17 (thus, the minimal PsTI or minimal IL-17 score) score achieved by each subject within the study. MR include subjects with subzero PsTI in...
one \((n = 3)\) or both \((n = 13)\) posttreatment biopsies. There was a strong correlation \((r = 0.92, p < 0.001)\) between minimal PsTI and minimal IL-17 score achieved by subjects during the study (Fig. 6B). Of note, four subjects that received brodalumab maintained high IL-17 ligands and high PsTI, clustering with the placebo-treated individuals (Fig. 6B), and were classified as molecular non-responders (MNR).

**Figure 5.** PsTI and IL-17 score are tightly correlated across lesional, NL, and treated samples and are more correlated than either is with IFN-\(\gamma\) score.

Molecular resolution in psoriatic lesions is dependent on sufficient brodalumab exposure to compete with the local level of IL-17 ligands

MNR may result from failure to fully block IL-17 signaling, possibly because of much higher local ligand concentrations, lower local brodalumab concentration, or a combination of the two. In contrast to the lack of correlation between molecular response and predose lesional IL-17 ligand gene expression levels (data not shown), there was a correlation between molecular response and averaged IL-17 ligand gene expression levels over the first 2 wk (Fig. 7). MNR tended to have elevated levels of IL-17 ligand mRNAs and low concentration of brodalumab (AUC) (Fig. 7A).

**Figure 6.** (A) PsTI and IL-17 scores correlated with PASI across pretreatment and posttreatment lesional samples. Samples from untreated conditions (predose lesional and placebo) cluster in the upper right of each plot with high molecular scores and high PASI, whereas most post-brodalumab samples cluster in the lower left of each plot with low scores (black diamonds). In four subjects, both postdose time-point samples from the same individuals remain within the untreated cluster (open diamonds) and therefore these subjects are considered MNR. Other cases, where one but not both postdose samples achieved a low transcriptional and PASI score, are marked as responders (gray/black diamonds). (B) The lowest PsTI and IL-17 score observed in samples from each individual are also correlated, and segregate 16 MR from 4 MNR and five placebo individuals in the upper right.
There appeared to be MR and MNR with similar levels of IL-17A and IL-17F ligand expression and brodalumab exposure. Because IL-17A, IL-17F, and IL-17C are all increased in lesional skin and signal through IL-17RA, we also looked at the combined expression of all three ligands (Fig. 7A, bottom panel). The pooled IL-17 ligand versus brodalumab concentration demonstrated a separation between the MR and MNR at a given concentration of brodalumab.

In addition to brodalumab exposure, the relationship between molecular response and the PsTI or IL-17 transcriptional scores was assessed. The minimal PsTI or IL-17 score attained following brodalumab treatment correlated to exposure, with a trend toward lower exposure in the MNR (Fig. 7B). To better model the relationship between brodalumab exposure and molecular changes, we examined the relationship between transcriptional scores and the ratio of brodalumab exposure to total ligand expression. A strong correlation was present between the brodalumab/IL-17 ligand ratio and both PsTI ($r = 0.80$, $p < 0.001$; Fig. 7C) and IL-17 score ($r = 0.78$, $p < 0.001$; data not shown).

**Discussion**

An emerging body of evidence is defining psoriasis as a Th17-driven disease, as supported by genetic (10), histologic (11, 23), and clinical data from multiple approved and investigational therapies (5, 24). Multiple IL-17 ligands are overexpressed in psoriatic lesions and many disease-related mRNAs induced by IL-17 signaling are also elevated (22, 25).

In this study, we demonstrated that IL-17RA blockade by brodalumab had rapid and extensive effects on inflammatory gene expression and cellular hallmarks of psoriasis. The molecular effects preceded slower, but still substantive, effects on inflammatory cell populations. The most immediate effects were seen in keratinocyte-associated genes, including decreased expression of genes involved in inflammation and hyperproliferation. By 2 wk, and most notably in the subjects with higher levels of IL-17R blockade, the gene expression profile in lesional skin largely resembled that of nonlesional skin as inflammatory circuits unwound following brodalumab treatment. The gene expression data following anti-IL17A ligand blockade also showed rapid changes in the high-dose group, though the extent of normalization to a nonlesional profile was not assessed (16). Gene expression changes following anti-TNF blockade were slower, with minimal changes by 2 wk, moderate changes after 4 wk, and gene expression was still not completely normalized after 12 wk (11). In addition, changes in the global inflammatory gene expression in psoriasis following IL-17R blockade in this study, or TNF blockade in Zaba et al. (11) were tightly correlated with the signature expression of IL-17-induced genes from keratinocytes, and changes following TNF blockade were more associated with the IL-17 stimulation signature than with the TNF signature. These data strongly suggest that IL-17R signaling is the major driver of the complex phenotype of psoriasis.

These data suggest that keratinocytes are a primary site of action for IL-17R blockade, with changes in inflammation-associated keratinocyte gene expression occurring more rapidly than effects on inflammatory T cell gene expression products. A large set of keratinocyte-expressed genes returned to nonlesional levels at the first biopsy time point (7–14 d after brodalumab). Ki67 staining, a marker of keratinocyte proliferation, decreased to nonlesional levels at the earliest time points in many subjects receiving brodalumab, indicating rapid cessation of the hyperproliferative phenotype. In conjunction with the rapid effects on keratinocyte-associated genes, there were variable and slower changes in inflammatory cytokine genes expressed by infiltrating leukocytes. These changes paralleled histologic disappearance of the T cell populations. Although brodalumab-induced normalization of many leukocyte populations responded on a slower time scale, CD11c DC were reduced to close to nonlesional levels by wk2. This may reflect the rapid reduction in a number of factors that can activate DC, including LL-37, S100 proteins, and the cytokines IL-36 and IL-23 (26), but may also reflect the loss of IL-17 activity on DC or other skin-resident myeloid cells, as well as keratinocytes. These data call into question the roles in the disease phenotype of Th1 and Th22 cells whose products (IFN-γ and IL-22) should still be active, whereas activity of the Th17 products (IL-17A and IL-17F) would be blocked by brodalumab. Broad molecular normalization occurred despite ongoing elevated expression of both IFNG and IL22 mRNA.

**FIGURE 7.** Local molecular resolution is dependent on sufficient brodalumab exposure to compete with the local level of IL-17 ligands. (A) MNR have higher levels of IL-17 ligand expression than MR with the same brodalumab exposure. The x-axis is the brodalumab exposure for each subject ($\mu g \times$ days/ml), and the y-axis is the average log2 expression of each ligand (inverted qRT-PCR cycle threshold units) over the first 2 wk or the sum for all three units in the bottom panel. (B) Response is correlated to exposure, but exposure alone does not account for molecular response. The x-axis is the brodalumab exposure for each subject, and the y-axis is the lowest IL-17 signature score or the lowest PsTI score achieved for each subject. (C) Molecular response is correlated with sufficient brodalumab exposure to outcompete IL-17 ligand expression.

**IFNG** was one of the few genes examined that did not reach nonlesional levels after brodalumab treatment, possibly because of comparatively incomplete suppression of IL-12, an upstream regulator of Th1 cells, as compared with IL-23 an upstream regulator of Th17 cells. Both cytokines are composed of two subunits including a shared p40 subunit encoded by the IL12B gene, a risk gene for psoriasis that influences IFN-γ production. The other IL-23 p19 subunit is encoded by the IL23A gene. Both IL23A and IL12B were upregulated in psoriatic lesional skin and normalized following brodalumab treatment. The IL23A gene was normalized at week 2, and the IL12B gene was normalized at week 6. IL-12 is the product of the shared IL12B gene and the IL12A gene, coding for a p35 subunit. In contrast to most inflammatory cytokine genes, IL12A is downregulated in psoriasis lesional skin relative to both nonlesional and healthy skin. The combined counterdirectional regulation of IL12A and IL12B results in moderately increased expression of the complete IL-12 cytokine (27),
and the expression of IL12A and IL12B at close to nonlesional levels at week 6 in the 350- or 700-mg groups would be expected to result in moderate decreases in IL-12 cytokine. However, immediately following brodalumab treatment, the expression of IL12A increased more rapidly than IL12B decreased, making it difficult to predict changes in IL-12 cytokine in the interval before week 6, and how these changes might affect IFN-γ levels. Added complications include the possible contributions from IL12A expression in regulatory T cells where it can combine with the EBV-induced 3 gene to express IL-35 (28), and the potential for some IFN-γ expression from IL-17/IFN-γ double-positive T cells under other control mechanisms. IFN-γ directly injected into human skin has been shown to induce a large gene expression signal but without the molecular and cellular characteristics seen in psoriasis (29). Although seen at elevated levels in psoriasis lesions, IFN-γ appears not to be a major contributor to ongoing psoriasis in the absence of other inflammatory signals. The T cell cytokine IL-22 also has been implicated as key driver of psoriasis pathology (30, 31). Injected IL-22 can cause a psoriasis-type skin lesion in mice (32). However, elevated levels of IL22 mRNA in many responder subjects were unchanged 2 wk after brodalumab treatment, despite broad resolution of global gene expression abnormalities, nonlesional levels of keratinocyte proliferation and KRT16 gene expression, decreased PASI scores, and resolving inflammatory infiltrate. This aligns with pathway/response data from anti–IL-17 ligand treatment (16) and suggests that in the absence of IL-17 pathway signaling, neither IFN-γ nor IL-22 is a major driver of human psoriasis. IL-22 is known to synergize with IL-17A in stimulation of keratinocytes (33), and it is possible the loss of IL-17 pathway signaling reduces the natural level of signaling downstream of IL-22.

All biopsies in this study were collected by week 6, limiting the analysis of long-term effects in this single dose study. T cell counts were decreasing in the week 2 to week 6 interval, as were the expression levels of T cell–associated genes. IFNG and the genes in the IFN-γ signature were downregulated by ~2-fold following brodalumab treatment. Analysis of these data cannot distinguish between whether the small set of incompletely normalized genes have some IL-17-pathway–independent drivers, or whether more extended exposure is necessary for normalization. Many of these genes are also found in the residual genomic profile following anti-TNF treatment (28).

Blockade of the IL-17 pathway in psoriasis has shown impressive levels of clinical response, with median PASI improvements of >90% for anti-IL17A ligand blockade (13), and 100% for anti–IL-17R blockade with brodalumab (14). This study examined whether incomplete response to anti–IL-17 pathway therapeutics may be due to differential importance of IL-17 signaling in those individuals or differential levels of blockade. The brodalumab exposure–response data demonstrated a clear correlation between resolution of the IL-17 signature and clinical improvement. In subjects with low levels of brodalumab exposure, the expression of the characteristic psoriasis transcriptome was tightly correlated with the expression of the keratinocyte IL-17 signature. Low levels of brodalumab, coupled with high levels of IL17A, IL17F, and/or IL17C mRNAs, were observed in individuals with lower molecular and cellular responses. Thus, these data suggest that incomplete reduction in the IL-17 signature is the result of competition by locally high ligand levels. Because incomplete molecular and cellular responses were only seen in the presence of a continued high IL-17 signature, this supports the concept that effective blockade of the IL-17 axis will usually result in a clinical response as seen in recent phase 2 trials (13, 14) and suggests that incomplete blockade of IL-17 signaling may underlie the small percentage of low or incomplete PASI responses. No single IL-17 ligand was identified as being the key inflammatory player in this study. IL17A, IL17F, and IL17C all were overexpressed in psoriasis lesions, as has been seen by others (8, 34), and all were decreased after brodalumab treatment. Cumulative over expression of the ligands relative to brodalumab exposure was related to low molecular response. Neither IL17A nor IL17F was predominant, suggesting that both may contribute or that Thy17/Tc17 cells may not be the only mechanism driving IL-17 signaling, and IL-17C from keratinocytes also may be a significant contributor (8). The consistent high expression of IL17C is noteworthy (Fig. 7A) and suggests that IL-17C may be the dominant IL-17 ligand present in skin lesions. However, individuals with better clinical and molecular responses showed reduction in all three ligands following brodalumab treatment. The clinical and molecular responses after anti–IL-17A treatment with ixekizumab or secukinumab (12, 13, 16) suggest that reduction in signaling from IL-17A alone can reduce the inflammatory activity in most individuals. What is not clear is whether IL-17A can be assigned an exclusive role, as IL17F expression was also reduced following treatment with ixekizumab (16) or secukinumab (12). IL-17F also has significant dermatological activity as evidenced by the skin phenotype of dominant-negative mutations (35). So, although the contributions of the individual ligands remain to be fully defined and may even vary between individuals, the molecular changes after ixekizumab and brodalumab clearly indicate the central role of IL-17 signaling in psoriasis.

Many conceptual models of psoriasis pathogenesis are based on a positive-feedback inflammatory loop involving multiple cells and cytokines along with synergies between factors enabling a self-sustaining cycle (5, 36). The exact role of the individual cytokines initiating this cycle and those essential for maintenance or support of ongoing inflammation is not known. Synergistic interactions between cytokines may also amplify steps in the cycle (25). IL-17RA blockade with brodalumab rapidly reduced specific keratinocyte-derived mediators of inflammation (e.g., S100s, antimicrobial peptides, chemokines, and IL-36) and others that may be leukocyte-derived (e.g., IL-23). With a large number of rapid effects, reductions in IL-17–driven synergies (with factors such as TNF-α) may in turn lead to reductions in additional factors that themselves have synergistic effects, thus enabling the vicious inflammatory cycle to unwind. In this study, the results of IL-17RA inhibition included decreases in IL17C, IL17A, and IL17F mRNAs along with the upstream factor IL-23. These decreased ligand concentrations also may further reduce IL-17 pathway activation, even in the absence of continued high brodalumab exposure. Although clinical response ultimately waned for most subjects (15), individuals in the 350-mg group had continued low expression of keratinocyte-expressed genes and continued low clinical scores through 6 wk, even though brodalumab levels were all below the limit of detection. Although these data do not establish an initiating event, the rapidity of the molecular and clinical response to brodalumab suggests that IL-17R signaling in keratinocytes is a critical component of the inflammatory cycle, without which psoriasis pathogenesis cannot be maintained.

The treatment regimens for some biological agents targeting psoriasis have a higher initial exposure followed by a lower maintenance dose (37, 38). In these cases, initial improvements achieved with a higher initial induction dose in the first weeks can be subsequently sustained with a lower maintenance dose (37, 38). This also may be a result of downregulation of drug targets and competitors during the initial phase, allowing a lower exposure to still provide biochemical coverage. With the rapid decrease in the IL-17 ligand competitors observed in the first 2 weeks after brodalumab treatment, 2 weeks may be an analogous induction period.
In summary, blockade of IL-17RA with brodalumab lead to rapid molecular and histologic resolution of the inflammation circuits that characterize psoriasis. These molecular data from a single-dose study provide proof-of-concept for the key role of IL-17 ligands in the pathogenesis of psoriasis and support a mechanism for the high level of clinical response reported for brodalumab in a larger phase 2 study of patients with moderate-to-severe plaque psoriasis (14).

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Disclosures

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