Tofacitinib attenuates pathologic immune pathways in patients with psoriasis: A randomized phase 2 study

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Background: Tofacitinib is an oral Janus kinase inhibitor being investigated for psoriasis.

Objective: We sought to elucidate the molecular mechanisms underlying the clinical efficacy of tofacitinib in patients with psoriasis.

Methods: Twelve patients with plaque psoriasis were randomized (3:1) to receive 10 mg of tofacitinib or placebo twice daily for 12 weeks. Biopsy specimens were taken from nonlesional (baseline) and lesional (baseline, days 1 and 3, and weeks 1, 2, 4, and 12) skin. Biopsy specimens were examined for psoriatic epidermal features (thickness, Ki67$^+$ keratinocytes and keratin 16 [KRT16] mRNA expression, and phosphorylated signal transducer and activator of transcription [pSTAT]$^+$ nuclei) and T-cell and dendritic cell (DC) subsets by using immunohistochemistry, and mRNA transcripts were quantified by using a microarray.

Results: In lesional skin keratinocyte pSTAT1 and pSTAT3 staining was increased at baseline but reduced after 1 day of tofacitinib (baseline, median of 1290 pSTAT1$^+$ cells/μm^2; day 1, median of 332 pSTAT1$^+$ cells/μm^2; and nonlesional, median of 155 pSTAT1$^+$ cells/μm^2). Epidermal thickness and KRT16 mRNA expression were significantly and progressively reduced after days 1 and 3 of tofacitinib administration, respectively (eg, KRT16 decreased 2.74-fold, day 3 vs baseline, $P = .016$). Decreases in DC and T-cell numbers were observed after weeks 1 and 2, respectively. At week 4, significant decreases in IL-23/T_H17 pathways were observed that persisted through week 12. Improvements in clinical and histologic features were strongly associated with changes in expression of psoriasis-related genes and reduction in IL-17 gene expression.

Conclusions: Tofacitinib has a multitiered response in patients with psoriasis: (1) rapid attenuation of keratinocyte Janus kinase/STAT signaling; (2) removal of keratinocyte-induced cytokine signaling, leading to reductions in pathologic DC and T-cell numbers to nonlesional levels; and (3) inhibition of the IL-23/T_H17 pathway. (J Allergy Clin Immunol 2016;137:1079-90.)

Key words: *IL-17, IL-22 family, IL-23, inflammation, Janus kinase, keratinocyte, psoriasis, phosphorylated signal transducer and activator of transcription, T_H17 cell, tofacitinib*

Psoriasis is a chronic immune-mediated disease characterized by epidermal hyperplasia.[1] This is driven by infiltration of T cells and dendritic cells (DCs) and associated increased cytokine levels, leading to the formation and persistence of skin plaques.[1]

Current opinion on the pathogenesis of psoriasis emphasizes the role of cytokine signaling to drive a pathogenic cycle, in which inflammatory T-cell and DC infiltrates release IL-17, IFN-γ, IL-22, and TNF, leading to the activation and proliferation of keratinocytes.[2] The stressed and dysregulated keratinocytes release chemokines, cytokines, and antimicrobial peptides (AMPs).[2] The chemokines recruit additional myeloid DCs and T_H1 and T_H17 cells, and cytokines (eg, IL-1β, IL-6, and TNF-α) enhance the proinflammatory interactions between the recruited DCs and T cells. The AMPs, including cathelicidin antimicrobial peptide (LL-37), activate pathologic DCs to produce IFN-α/β and myeloid DCs to secrete IL-12 and IL-23. These activated DCs and T cells complete the pathogenic cycle by continuing to activate keratinocytes.[2]

Tofacitinib is an oral Janus kinase (JAK) inhibitor that is being investigated for psoriasis. Tofacitinib is a small molecule with an intracellular mechanism of action against JAKs. Phase 3 studies in patients with moderate-to-severe chronic plaque psoriasis have demonstrated the efficacy of 5 and 10 mg of tofacitinib twice daily in improving clinical outcomes.[3-5]

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Abbreviations used
AMP: Antimicrobial peptide
DC: Dendritic cell
DEFB4A: Defensin beta 4a
FDR: False discovery rate
JAK: Janus kinase
KRT16: Keratin 16
MAD3: Meta-analysis of 3 psoriasis gene signatures
PASI: Psoriasis Area and Severity Index
pSTAT: Phosphorylated signal transducer and activator of transcription
STAT: Signal transducer and activator of transcription
TLDA: TaqMan Low Density Array
TPSS: Target Plaque Severity Score

In kinase assays tofacitinib inhibited JAK1, JAK2, and JAK3 and, to a lesser extent, tyrosine kinase 2.[6] Tofacitinib potently inhibited common γ-chain cytokines (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21), IFN-γ, IL-6, and, to a lesser extent, IL-12 and IL-23.[6] Tofacitinib also inhibited IFN-α/β and IL-22 signaling in isolated keratinocytes *in vitro* (data on file; Pfizer Inc, New York, NY). In preclinical models tofacitinib affected both innate and adaptive immune responses and inhibited pathogenic T_H17 cell differentiation by inhibiting expression of IL-23 receptors.[7] However, precisely how the JAK/signal transducer and activator of transcription (STAT) pathway interacts with the multiple pathways that are central to psoriasis pathogenesis in human subjects is not completely understood.

Although improvements in psoriasis can be monitored by means of clinical assessment or histologic analysis, a more detailed understanding of therapeutic responses can be attained through the profiling of disease-related and inflammatory mRNA. Previous studies have established that cytokine-targeted biologics (eg, TNF-α, IL-17, or IL-23 inhibitors) are efficacious for the treatment of psoriasis and have the ability to inhibit a central IL-23/T_H17 axis of psoriasis, with strong downregulation of a series of genes that are induced in keratinocytes and other cells by these proinflammatory cytokines.[8-12] Suppression of "core" disease-defining genes as a result of treatment can be demonstrated by gene profiling, which allows the relative suppression of different proinflammatory pathways to be quantitatively compared.[13-15] This molecular analysis allows residual expression of disease-related genes to be detected and quantified in lesions that have resolved clinically and histologically.

This phase 2 study aimed to elucidate the cellular and molecular mechanisms through which tofacitinib improves clinical manifestations of psoriasis by delineating the time course of changes in the pathogenic cycle of psoriasis.

METHODS

Patients

Eligible patients were 18 years or older with a diagnosis of moderate-to-severe plaque-type psoriasis for 12 or more months. Patients with a recent infection, current malignancy, or history of malignancy (except adequately treated or excised basal/squamous cell carcinoma or cervical carcinoma *in situ*) or evidence of active or latent tuberculosis infection were excluded.

Study design

This was a phase 2, randomized, placebo-controlled, double-blind study carried out in 6 centers in the United States from March 2013 to November 2013 (clinicaltrials.gov: NCT01710046). Patients were randomized 3:1 to receive 10 mg of oral tofacitinib or placebo twice daily for 12 weeks by using an automated Web or telephone randomization system.

At each study visit (baseline, days 1 and 3, and weeks 1, 2, 4, and 12), punch biopsy specimens were collected from each patient before the morning study drug dose (full details are provided in the Methods section in this article's Online Repository at www.jacionline.org). Baseline biopsy specimens were taken from lesional and nonlesional skin; at all later study visits, biopsy specimens were only taken from lesional skin. Clinical assessments at baseline and weeks 1, 2, 4, and 12 included the Psoriasis Area and Severity Index (PASI; overall measure of disease severity) and Target Plaque Severity Score (TPSS; individual lesion assessment of severity of erythema, induration, and scaling).

Immunohistochemistry and quantitative cell counting

Procedures for immunohistochemistry and quantitative cell counting are outlined in greater detail in the Methods section and Table E4 in this article's Online Repository. Biopsy specimens from lesional and nonlesional skin were stained for phosphorylated signal transducer and activator of transcription (pSTAT) 1 and pSTAT3, and numbers of epidermal and dermal reactive nuclei per square millimeter were quantified by using Definiens Tissue Studio software (Definiens AG, München, Germany). Skin biopsy specimens were also evaluated for expression of keratin 16 (KRT16); Ki67 (MKI67); cluster of differentiation antigens CD3, CD11c, and CD8; langerin (CD207); lysosomal-associated membrane protein 3 (LAMP3); S100 calcium-binding protein A7 (S100A7); MX dynamin-like GTPase 1/2 (MX1/MX2); human β-defensin (HBD2, defensin beta 4A [DEFB4A]); and S100A8/9 (S100A8), and the number of positive cells per square millimeter of epidermis was counted manually by using National Institutes of Health Image 6.1 software (http://rsb.info.nih.gov/nih-image).

Quantitative RT-PCR and microarray analyses

RNA was extracted from biopsy specimens by using the RNeasy Mini Kit (Qiagen, Valencia, Calif). The quality of extracted RNA was examined by using the Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, Calif). IL-23p40 (IL-12B) quantitative RT-PCR was performed with EZ PCR core reagents, primers, and probes (Life Technologies, Grand Island, NY), as previously published,[16] and the result was normalized to the ribosomal protein, large P0 (RPLP0) housekeeping gene. For microarray analysis, RNA was amplified, labeled, and hybridized by using a standard protocol (Ovation Whole Blood Solution and Encore Biotin Module; NuGEN Technologies, San Carlos, Calif) to GeneChip Human Genome U133A 2.0 Arrays (Affymetrix, Santa Clara, Calif) to measure relative gene expression. The data discussed in this publication have been deposited in the National Center for Biotechnology Information's Gene Expression Omnibus and are accessible through GEO Series accession number GSE69967.

TaqMan Low Density Array analysis

Samples for quantitative RT-PCR analysis using the Custom TaqMan Array Card (Life Technologies) were processed by Asuragen (Austin, Tex), according to the company's standard operating procedures (full details are provided in the Methods section and Fig E4 in this article's Online Repository at www.jacionline.org). A total of 192 inflammation/cytokine-related genes were analyzed, including 185 targets, 5 normalization genes, and 2 plate controls.

Statistical analysis

The study sample size was based on the objective of exploring the relationship between efficacy end points and exploratory gene markers and was not specifically powered for reaching statistical significance.