Treatment of Psoriasis Patients with IMO-3100 Shows Improvement in Gene Expression Patterns of Meta-Analysis Derived-3 Transcriptome and IL-17 Pathway

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Abstract

Background/Purpose: IMO-3100, an antagonist of TLRs 7 and 9, has shown significant improvement in PASI scores in a randomized, double-blind, placebo-controlled Phase 2 trial in patients with moderate-to-severe plaque psoriasis. In the Phase 2 clinical trial, 44 patients were randomized to receive 4 once-weekly injections (Days 1, 8, 15, and 22) of IMO-3100 at 0.16 or 0.32 mg/kg or placebo; 40 patients were clinically evaluable, and clinical data were presented at IID 2013. To evaluate immunological changes following treatment with IMO-3100, biopsy samples were obtained on Days 1 (lesional and normal skin) and 29 (lesional skin). Biopsy samples from six patients in the 0.16-mg/kg group with positive clinical responses and six placebo patients also were used for gene expression analysis by DNA microarray.

Methods: Meta-analysis derived (MAD)-3 transcriptome represents a gene-expression profile associated with psoriasis[1]. The overall effect of IMO-3100 vs. placebo treatment on the psoriasis transcriptome was compared by gene set enrichment analysis (GSEA).

Results: Principal component analysis of the microarray data showed clear distinction between lesional and normal skin within the twelve patients. Analysis showed that IMO-3100-treated patients had significant improvement in MAD-3 gene profile compared to placebo-treated patients. Placebo treatment did not significantly modulate gene expression, whereas genes up-regulated in the psoriasis transcriptome were significantly ($p<10^{-16}$) improved by IMO-3100 treatment. GSEA identified strong improvements in genes regulated in keratinocytes by IL-17 and the combination of IL-17 and TNF. In addition to the DNA microarray analysis, gene expression targets were analyzed by qPCR, which showed IL-17 was down-regulated in IMO-3100-treated patients with PASI improvements.

Conclusion: In summary, treatment of patients with psoriasis with IMO-3100 leads to down regulation of the IL-17 pathway, which is central to the pathogenesis of psoriasis.

Methods

Phase 2 Clinical Trial

TLR7 and TLR9 inflammatory responses to immune complexes containing self-DNA or self-RNA include induction of the IL-17 pathway (Figure 1). A Phase 2 clinical trial was conducted with four once-weekly subcutaneous doses of IMO-3100 to evaluate the treatment effect of IMO-3100 versus placebo. IMO-3100 produced a clinical response of significant improvement in PASI scores and, subject to the limitations of biopsies of partially resolved psoriatic plaques, correlated with histological improvement (Figures 2 and 3). [2]

Patient and Sample Selection for DNA Microarray

Biopsy samples were selected after data unblinding from 6 patients treated with 0.16 mg/kg/week IMO-3100 and 6 placebo patients.

At baseline: lesional skin (LS) and non-lesional skin (NL)

One week after the last dose: post-treatment LS

The median [range] PASI changes (Day 29 vs Day 1) in the selected patients were (-14.5%) [-28% to 0%] for placebo patients and (-40%) [-68% to -237%] in IMO-3100 patients. Biopsy samples were hybridized to Affymetrix HGU133 plus 2.0 chips.

Material and Methods

Quality control of Microarray chips was assessed using ArrayQualityControl and Harshlight packages. Expression values were obtained using gcrma algorithm. Probes with at least 2 samples with more than 5 expression and standard deviation larger than 0.1 were kept for further analysis. Changes with treatment was modeled using Mixed effect models with Treatment and Time effect and a random intercept for each patient. Moderated t-test was used to assessed the significance of the differences under R' limma framework.

This study was funded by Idera Pharmaceuticals Inc.

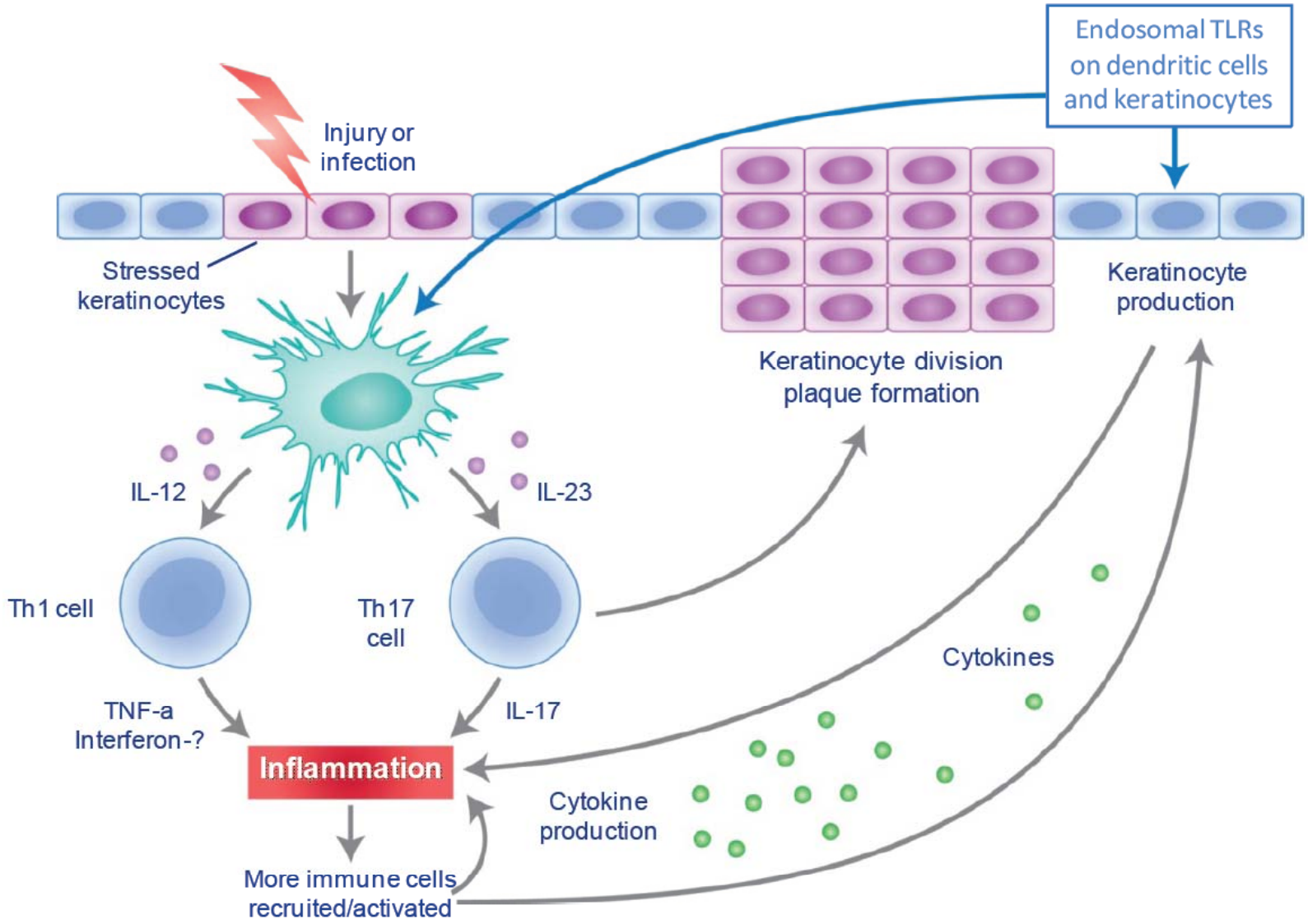
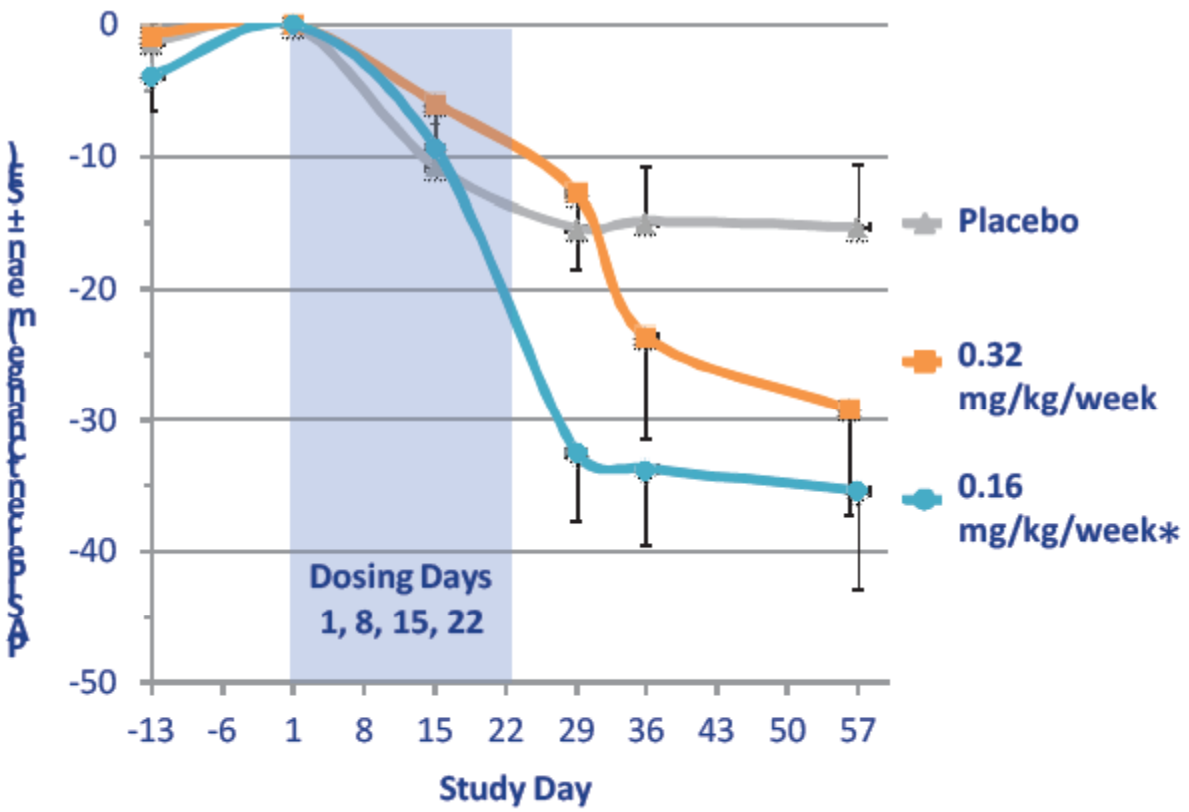


Figure 1. Endosomal TLRs in the Pathogenesis of Psoriasis



* p=0.04, repeated measures analysis;
p<0.02 for plaque induration and lower limb regional PASI score

Figure 2. Clinical response shows a significant reduction in PASI score compared to placebo. Patients receiving 0.16 mg/kg/week dosing showed a significant improvement in PASI score when compared to placebo (p=0.04). (From IID 2013[2])

[2]Kimball, A.B. et al., IID 2013

Treatment Related Immunological Changes

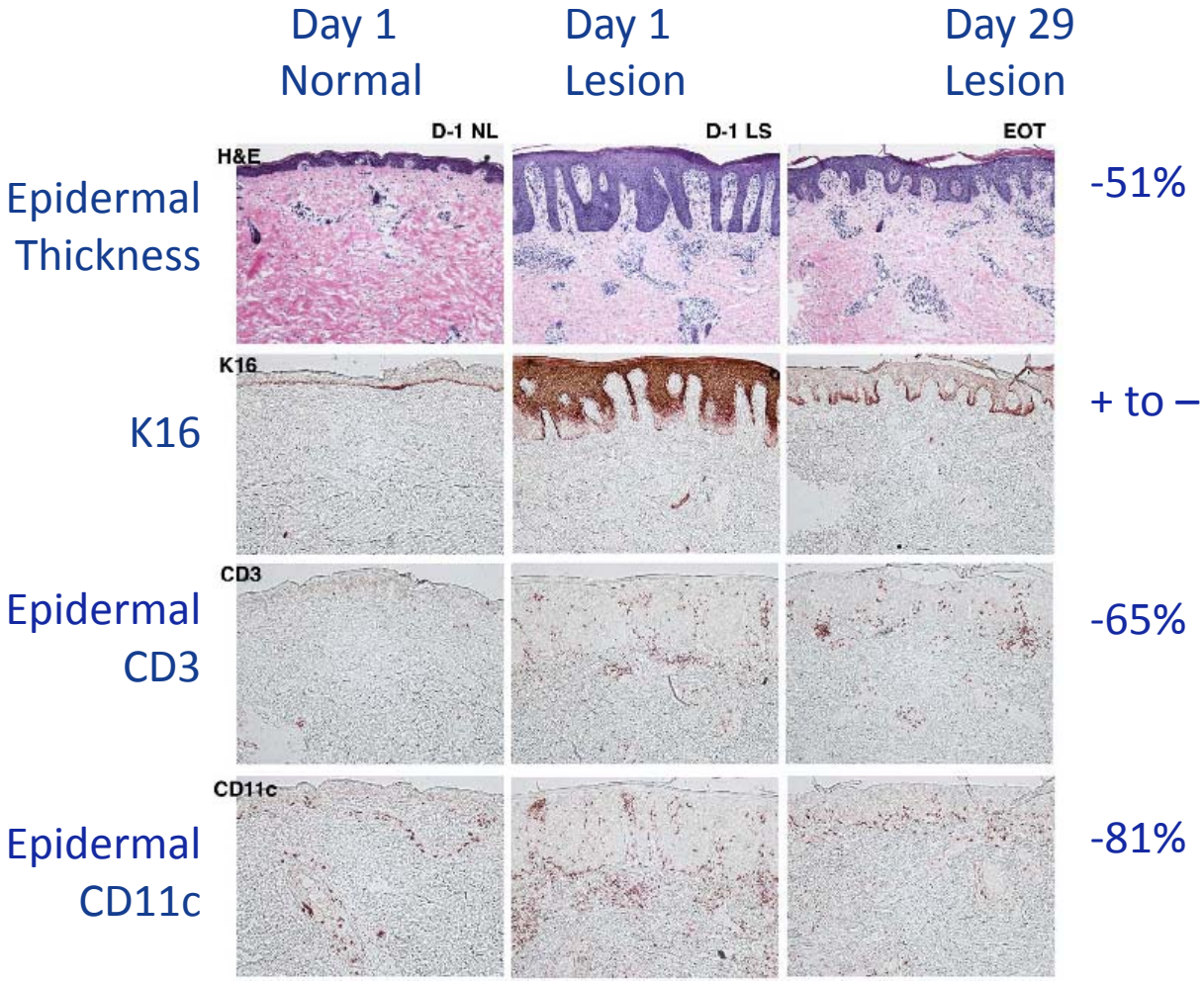


Figure 3. Histological improvement is seen at day 29 compared to day 1 (representative shown). H&E and IHC staining for K16, CD3, and CD11c showing reductions in epidermal thickness, keratin 16 expression, infiltrating T-cells and myeloid DCs. (From IID 2013[2])

Presented at ACR 2013, October 28, 2013

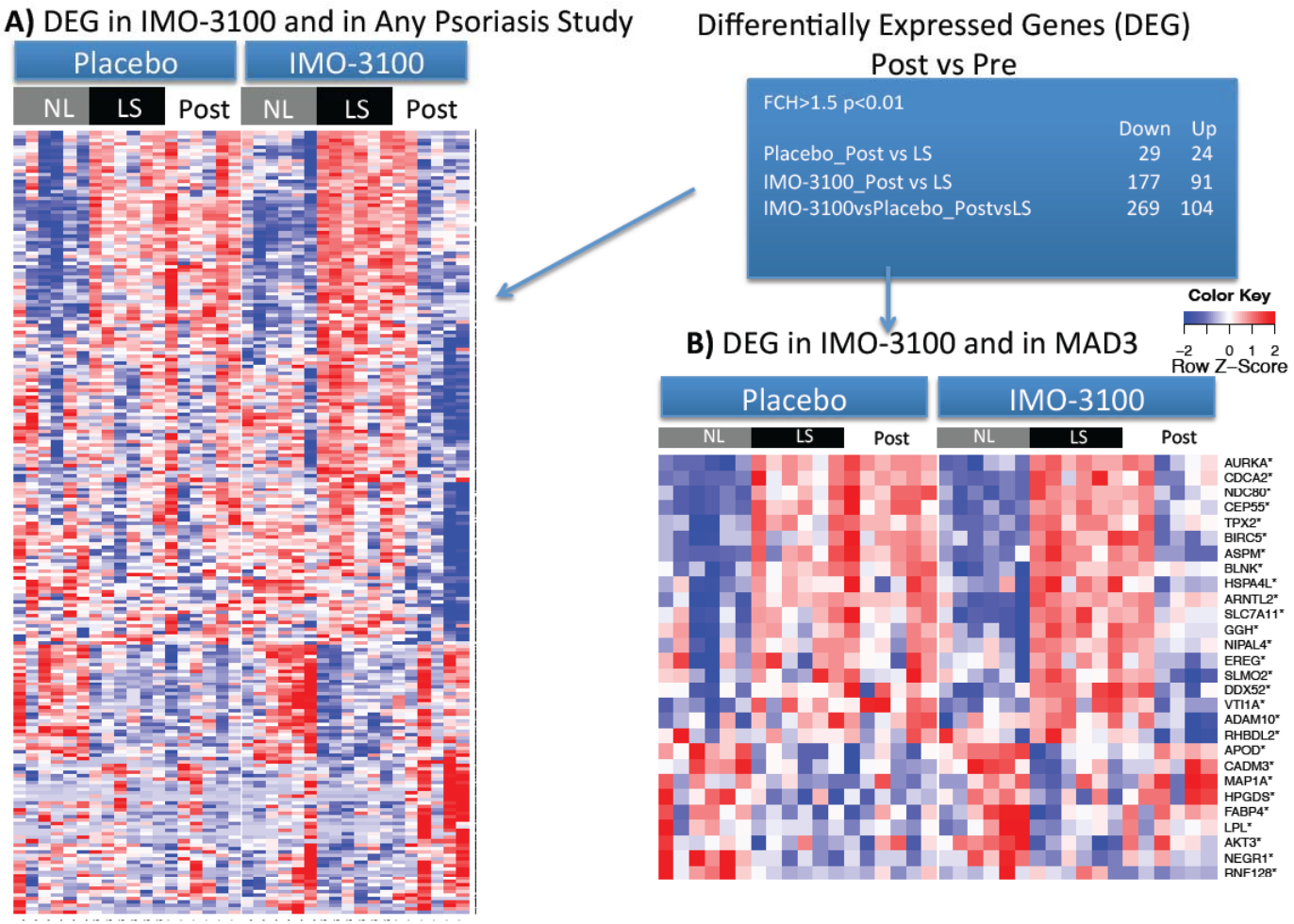


Figure 4. IMO-3100 modulates expression of genes that are known to be involved in psoriasis pathogenesis. Heat maps show the expression level of genes that are differentially expressed in IMO-3100 vs. placebo treated skin and A) any previously published psoriasis study and B) core pathogenesis psoriasis transcriptome genes that were meta-analysis derived (MAD3) [1]. Overall, placebo treatment did not modulate expression of genes that are up-regulated or down-regulated in psoriasis lesions (vs. background skin), but IMO-3100 treatment normalized expression of >370 genes associated with the psoriasis transcriptome. Thus IMO-3100 led to improvements in genes that are both up-regulated and down-regulated in psoriasis ($p < 10^{-6}$ for both effects). [1]Tian, S., *et al. PloS one* **7**, e44274 (2012).

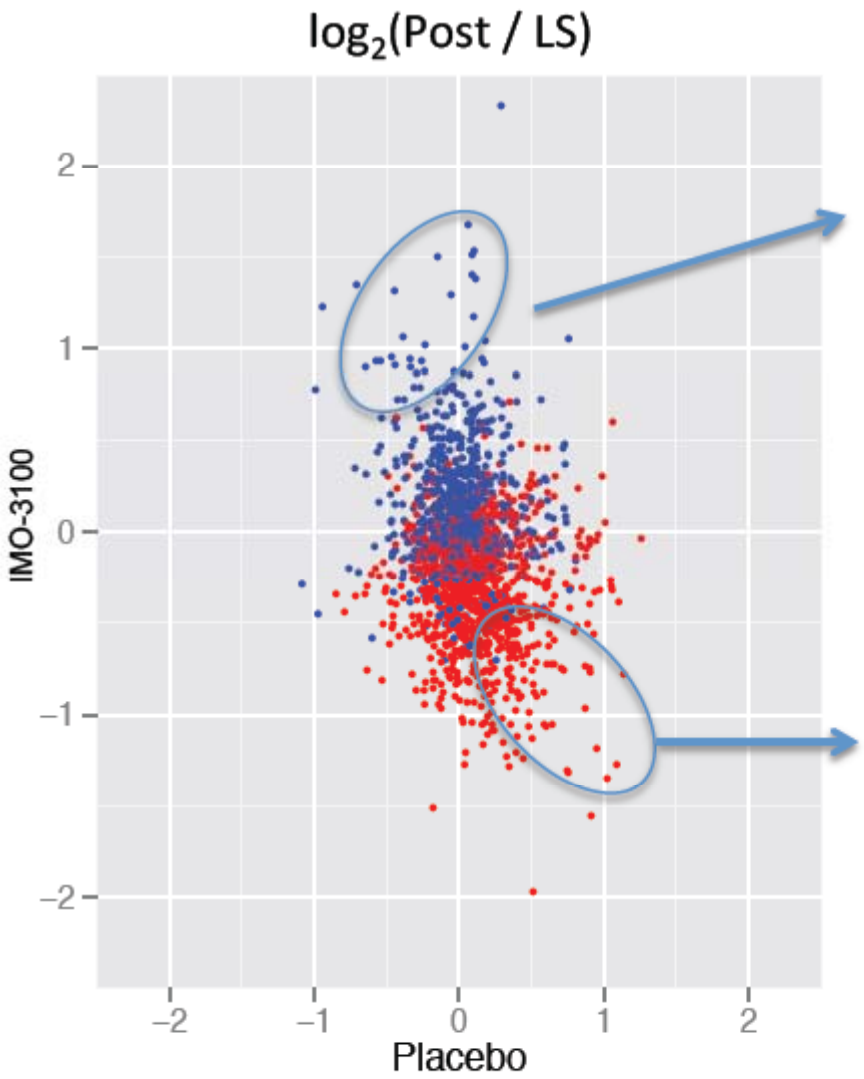


Figure 5. Scatter plot showing changes in gene expression in skin upon placebo or IMO-3100 treatment in MAD3 genes. Each dot represents a gene in the psoriasis transcriptome where blue are down- regulated and red are up-regulated.

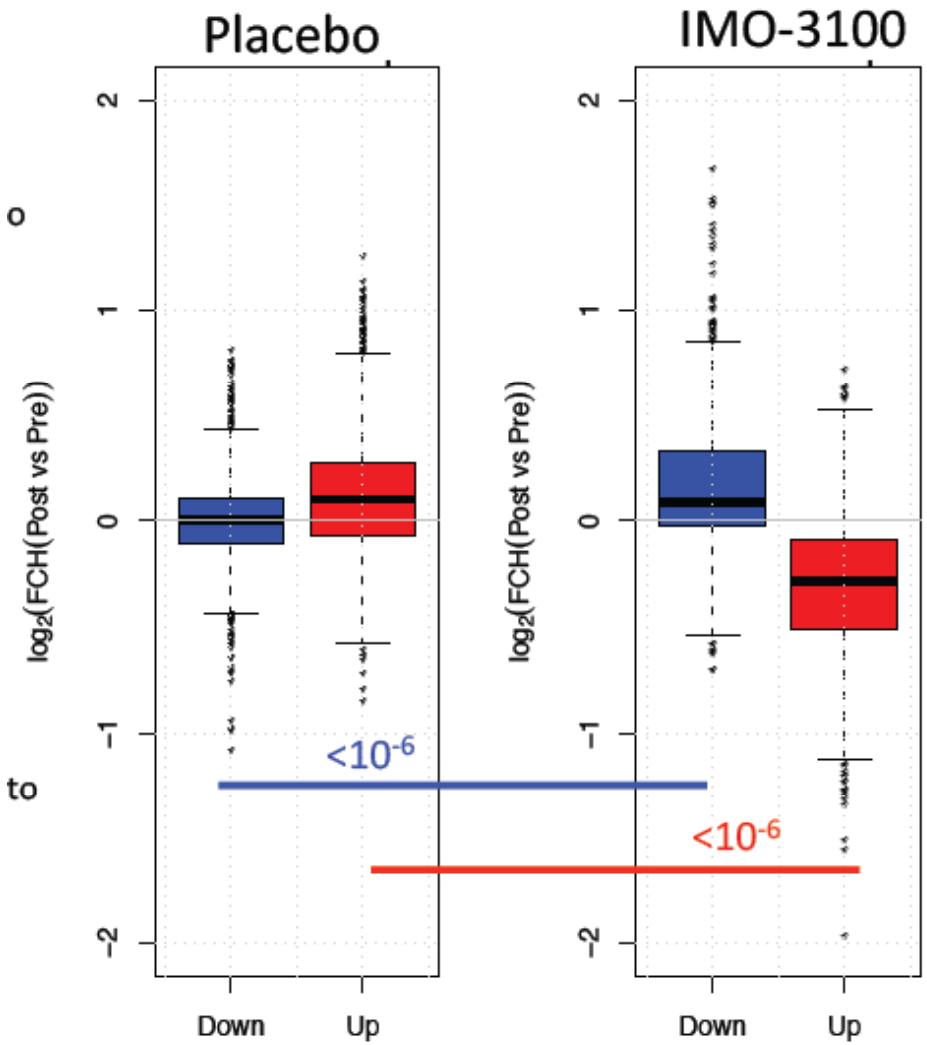


Figure 6. IMO-3100 has a greater treatment response on core pathogenesis psoriasis transcriptome genes that were meta-analysis derived (MAD3) compared to placebo. Blue are down- regulated and red are up-regulated

GSEA Analysis

		Rx16		Etanercept	
	NAME	ES	NES	ES	NES
Psoriasis Genes Up-reg	Gudjonsson (2009)	-0.66	-3.11	-0.84	-4.26
	S-F (2010)	-0.66	-3.07	-0.83	-4.33
	MAD-5	-0.63	-2.94	-0.82	-4.47
	S-F*(2012)	-0.60	-2.88	-0.74	-4.20
	Zhou (2005)	-0.64	-2.86	-0.78	-3.70
	MAD-3	-0.59	-2.81	-0.79	-4.40
	Yao (2007)	-0.57	-2.71	-0.73	-3.87
	NGS	-0.49	-2.33	-0.66	-3.44
	Down with all17 @ Week2	-0.60	-2.79	-0.78	-4.05
AD	AD NLVSNORMAL DOWN	-0.43	-2.04	-0.56	-2.97
	AD LSVSNL UP	-0.51	-2.26	-0.64	-2.99
Cytokine Pathways	KC IL17 (not TNF)	-0.69	-1.94	-0.81	-2.41
	RHE IL17 UP	-0.59	-2.63	-0.73	-3.46
	SYNERGISTIC IL17ANDTNFA KC	-0.46	-1.92	-0.60	-2.52
	SYNERGISTIC IL17ANDIL22 KC	-0.57	-1.86	-0.85	-2.72
	ADDITIVE IL17ANDTNFA KC	-0.53	-2.30	-0.67	-3.01
	KERATINOCYTES_AHCELLMAPS UP	-0.49	-2.14	-0.62	-2.91

Figure 7. Gene set enrichment analysis (GSEA) of published transcriptomes with our data showing major improvements in psoriasis-related genes (NES -2.33 to -3.11) and a major impact on genes modulated by IL-17 in keratinocytes (NES -2.63). ES=enrichment score, NES= normalized enrichment score

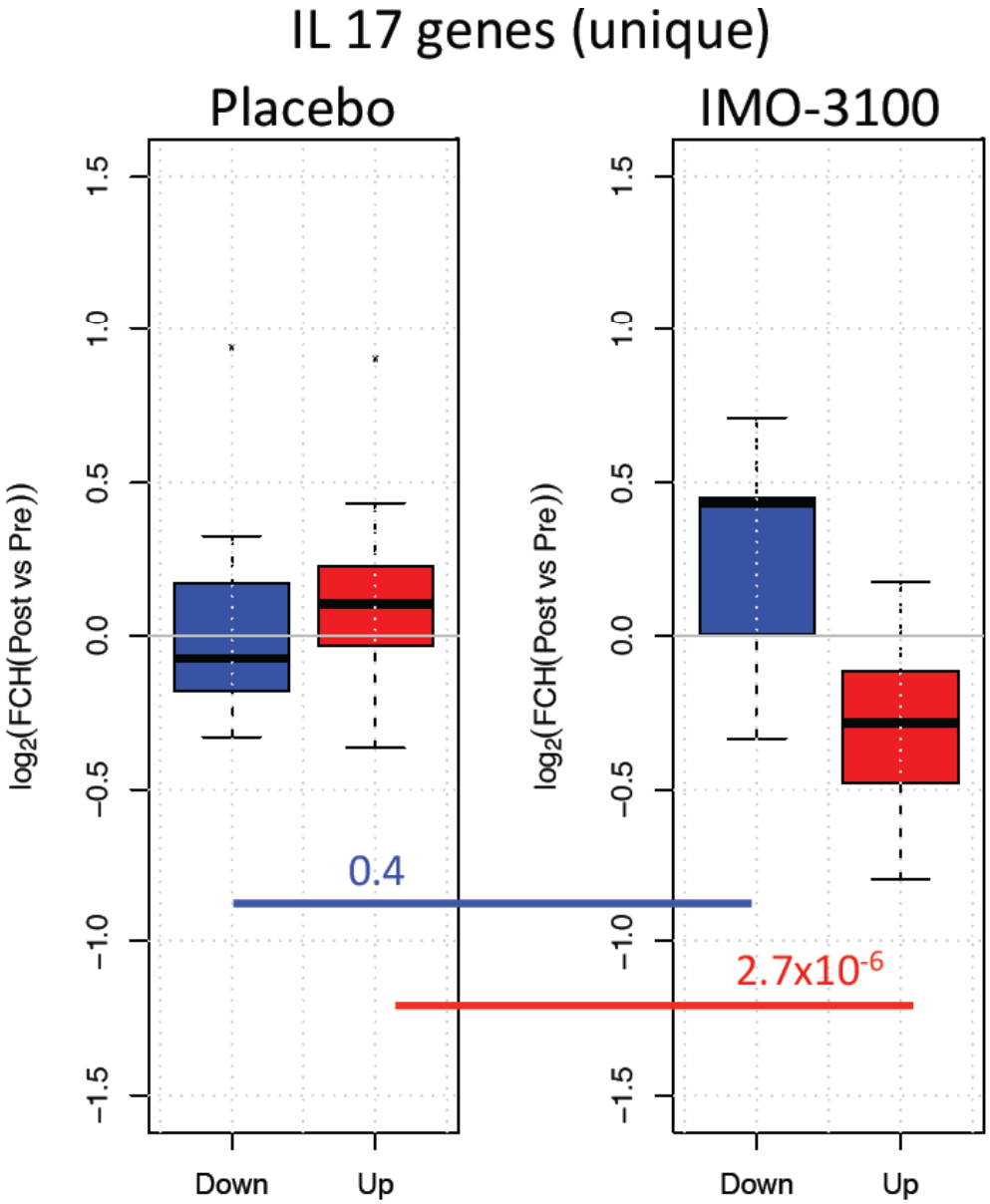


Figure 8. IMO-3100 has a greater treatment response on genes modulated by IL17 in keratinocytes compared to placebo. Blue are down- regulated and red are up-regulated

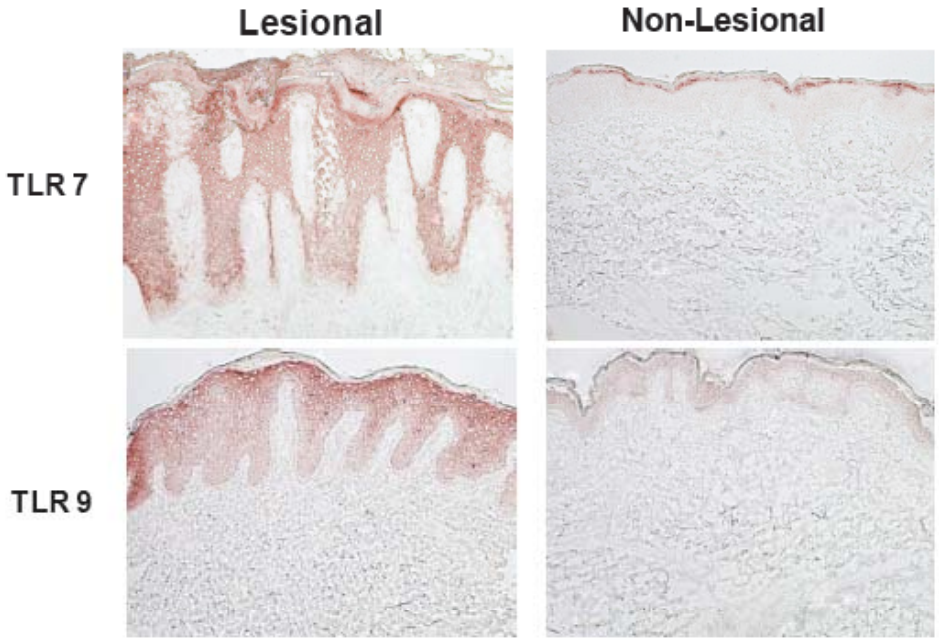


Figure 9. TLR 7 and TLR9 are strongly expressed in lesional skin keratinocytes.

Overall, these data suggest a new model for involvement of TLR7 and TLR9 in keratinocytes: TLR7/9 activation leads to activation of NFκB and links TLR pathways with both the TNF response in keratinocytes and with the IL-17 pathway response, where NFκB and C/EBPbeta or delta are the critical transcription factors that regulate keratinocyte expression of IL-17 response genes. Thus keratinocytes might respond directly to TLR7/9 inhibition by decreasing expression of IL-17 pathway products.

Conclusions

1. **IMO-3100, an antagonist of TLR7 and TLR9, significantly improved PASI scores in psoriasis patients after 4 once-weekly doses.**
2. **Histologic and gene expression analyses show treatment with IMO-3100 modulates hallmark indicators of psoriasis pathophysiology.**
3. **Specifically, genes unique to the IL-17 pathway were down-modulated by IMO-3100 treatment; this effect is similar to that produced by TNF-α blockers, consistent with both decreasing NF-κB.**
4. **TLR7 & TLR9 are strongly expressed in skin keratinocytes of active psoriatic lesions; thus IMO-3100 has two potential targets in the treatment of psoriasis – plasmacytoid dendritic cells and keratinocytes.**
   - **Myeloid dendritic cells also are targets for compounds that inhibit TLR8.**
5. **IMO-8400, an antagonist of TLR7, TLR8, and TLR9, is being evaluated in a Phase 2 trial in patients with psoriasis.**