



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

M. Suárez-Fariñas<sup>1</sup>, J. Belasco<sup>1</sup>, J. Fuentes-Duculan<sup>1</sup>, T. Sullivan<sup>2</sup>, R. Arbeit<sup>2</sup>, and J. Krueger<sup>1</sup>  
<sup>1</sup>Laboratory of Investigative Dermatology, The Rockefeller University, New York, NY; <sup>2</sup>Idera Pharmaceuticals Inc., Cambridge, MA

[www.iderapharma.com](http://www.iderapharma.com)



## 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<sup>1</sup>. 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).<sup>2</sup>

## *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)

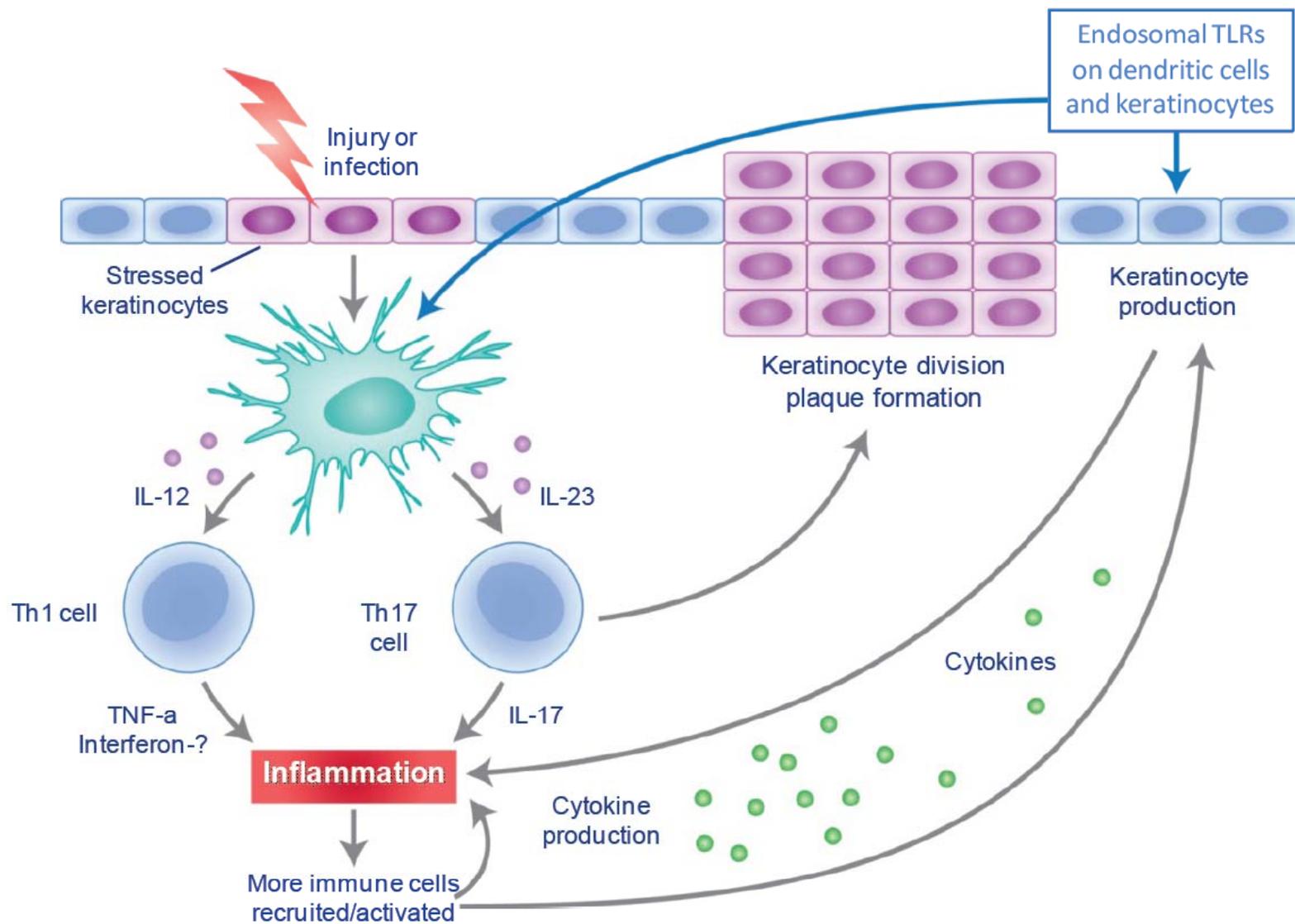
One week after the last dose: post-treatment LS

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.

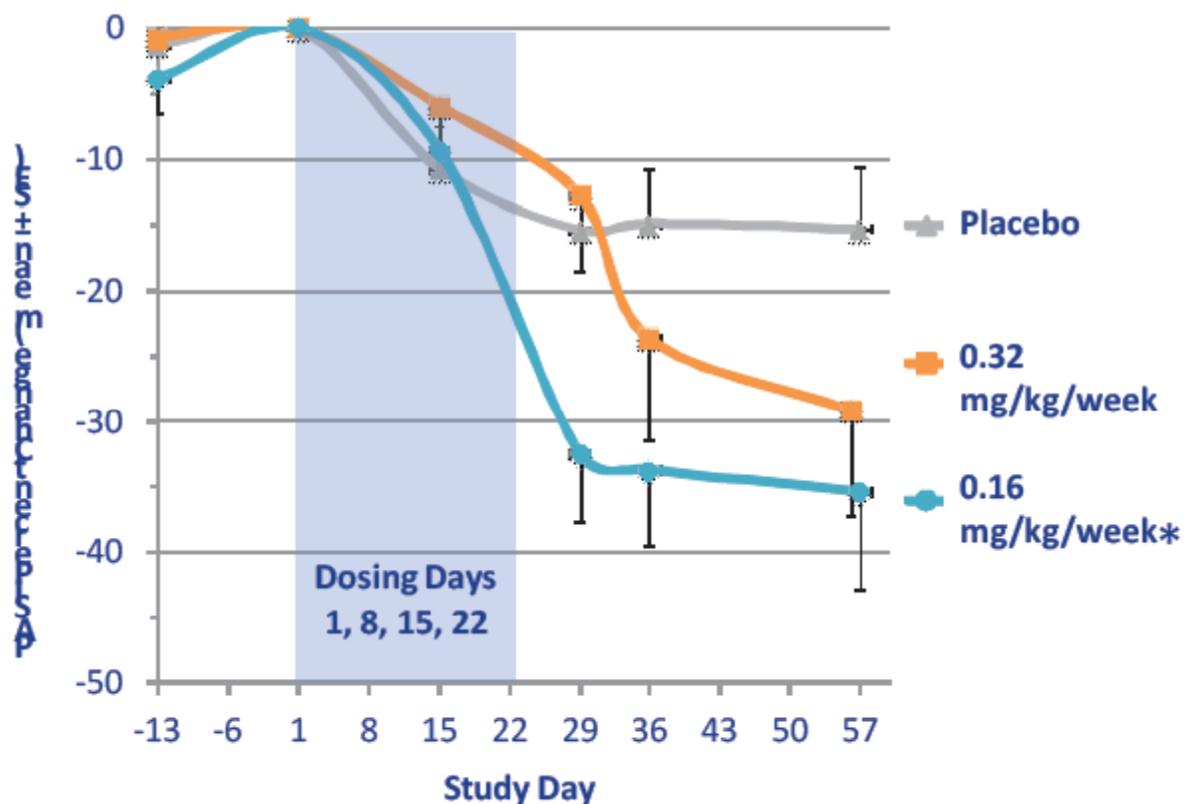
## *Material and Methods*

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 assess the significance of the differences under R' limma framework.

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**Figure 1. Endosomal TLRs in the Pathogenesis of Psoriasis**

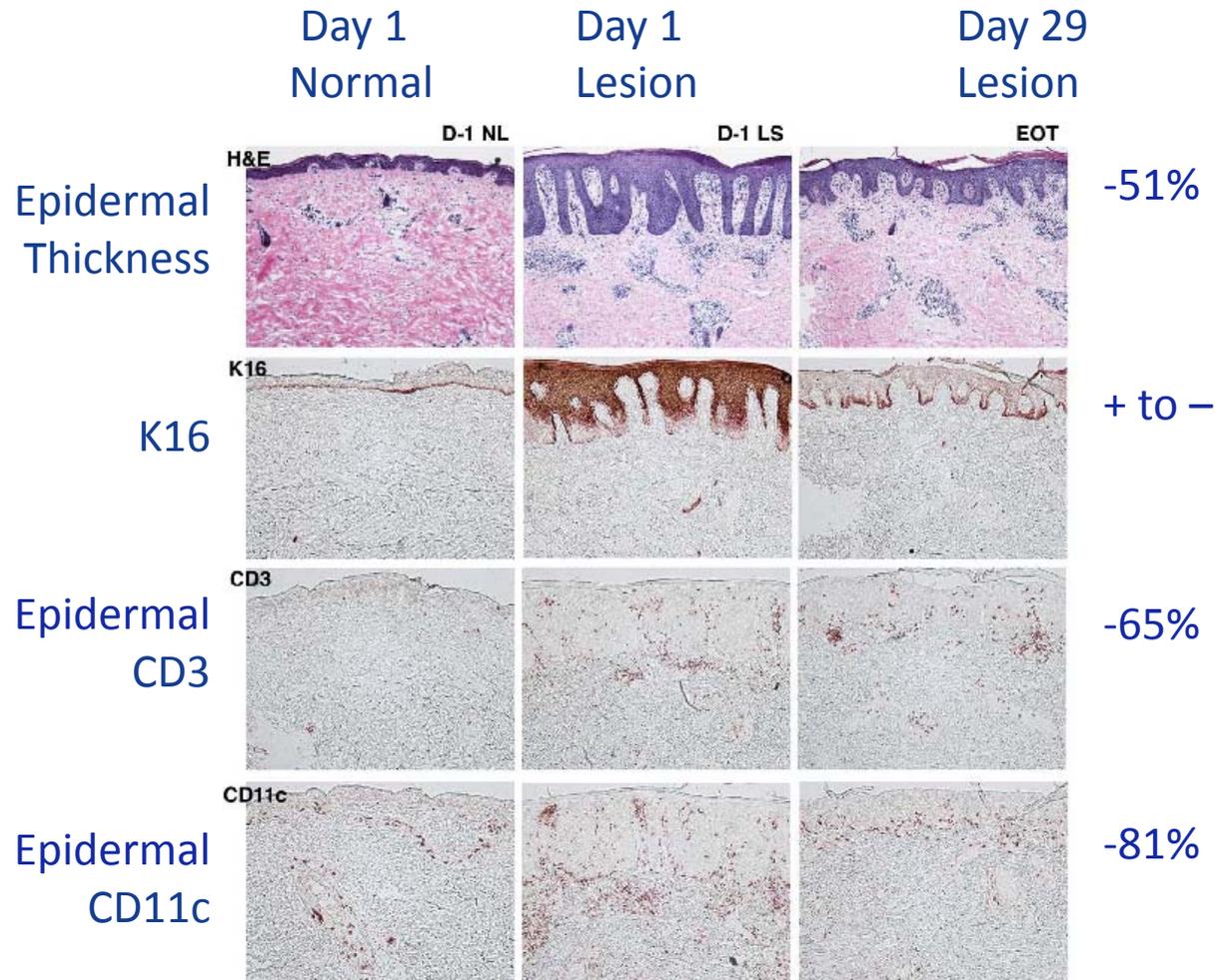


\*  $p=0.04$ , repeated measures analysis;  
 $p<0.02$  for plaque induration and lower limb regional PASI score

**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<sup>2</sup>)

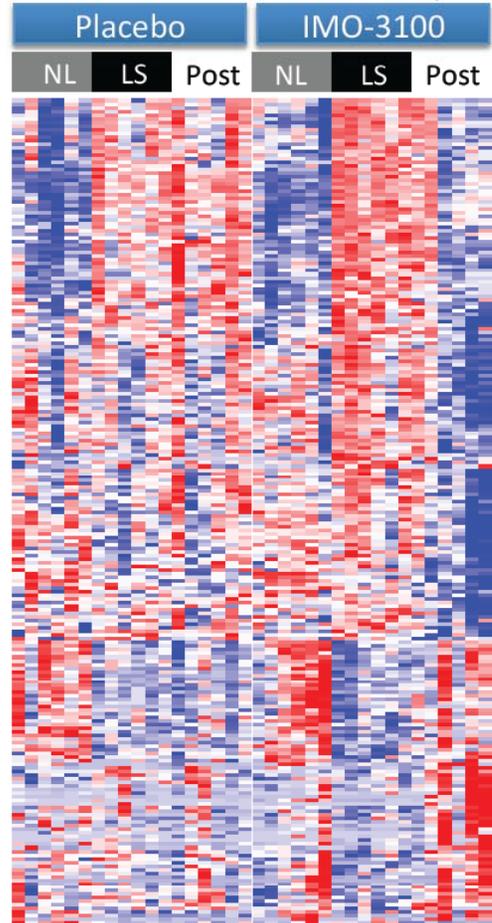
<sup>2</sup>Kimball, A.B. et al., IID 2013  
Presented at ACR 2013, October 28, 2013

# 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<sup>2</sup>)

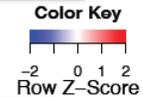
**A) DEG in IMO-3100 and in Any Psoriasis Study**



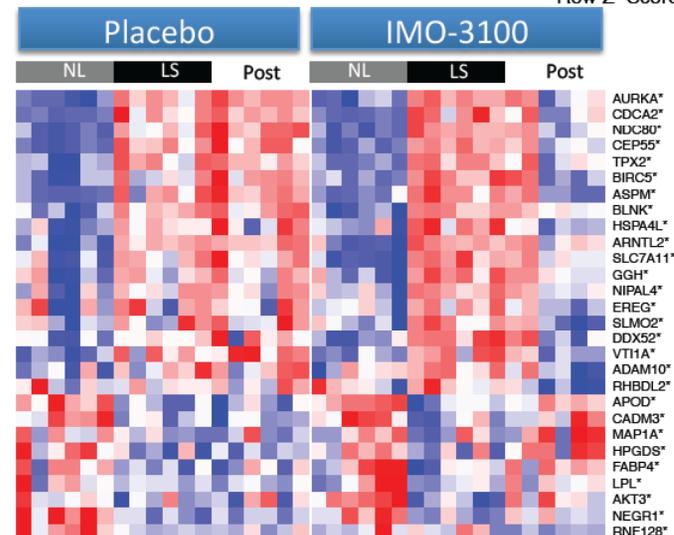
**Differentially Expressed Genes (DEG)**

Post vs Pre

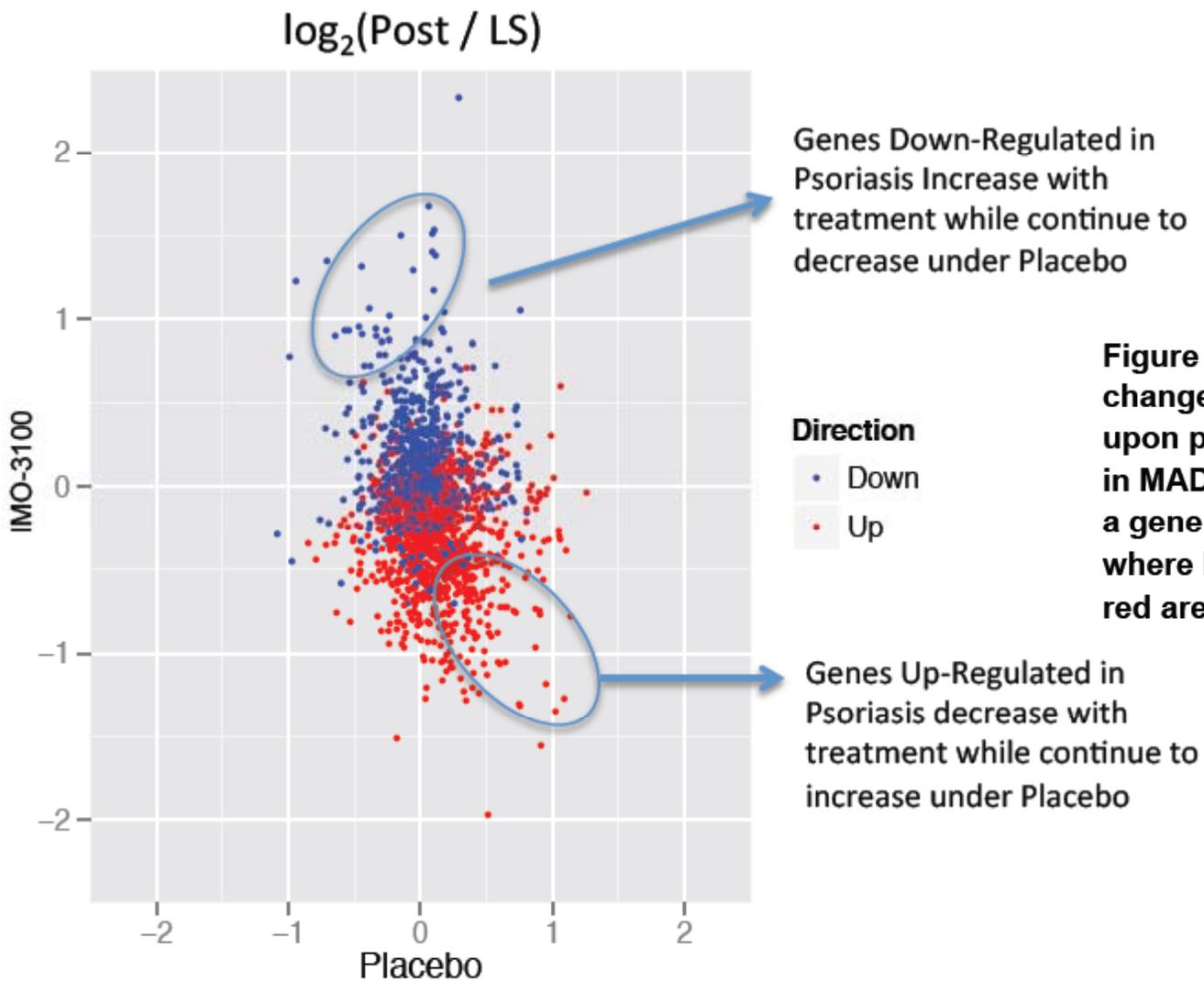
FCH>1.5 $p < 0.01$	Down	Up
Placebo_Post vs LS	29	24
IMO-3100_Post vs LS	177	91
IMO-3100vsPlacebo_PostvsLS	269	104



**B) DEG in IMO-3100 and in MAD3**



**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)<sup>1</sup>. 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).<sup>1</sup>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.**

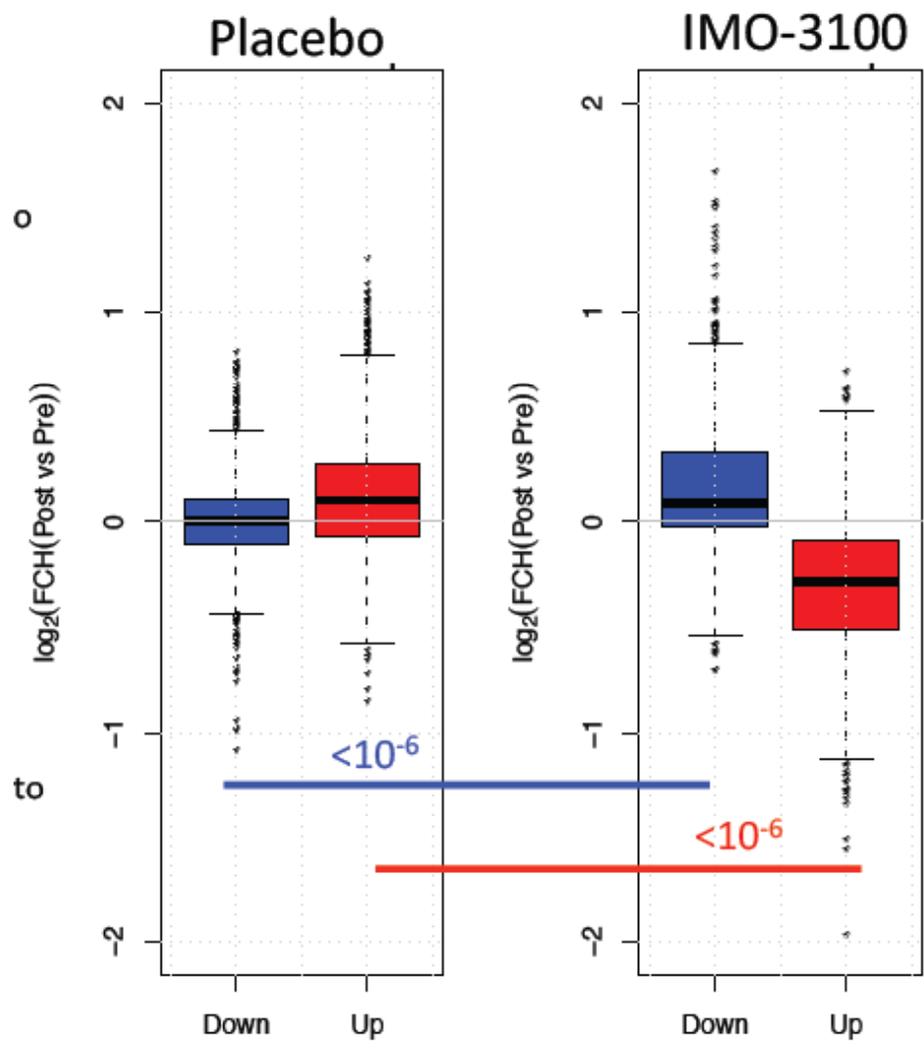


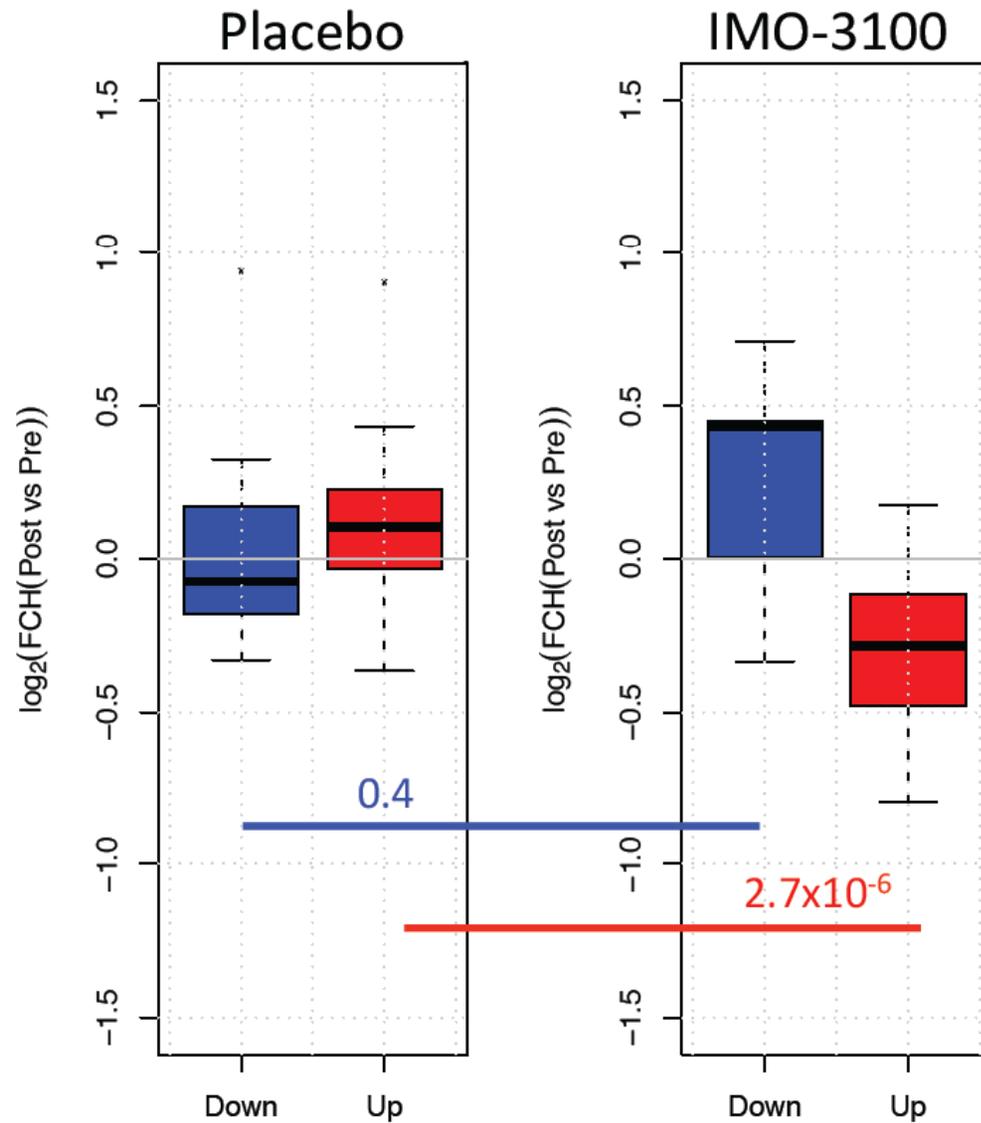
Figure 6. IMO-3100 has a greater treatment response on core pathogenesis psoriasis transcriptome genes that were meta-analysis derived (MAD3) compared to placebo. Blue are down-regulated and red are up-regulated

# GSEA Analysis

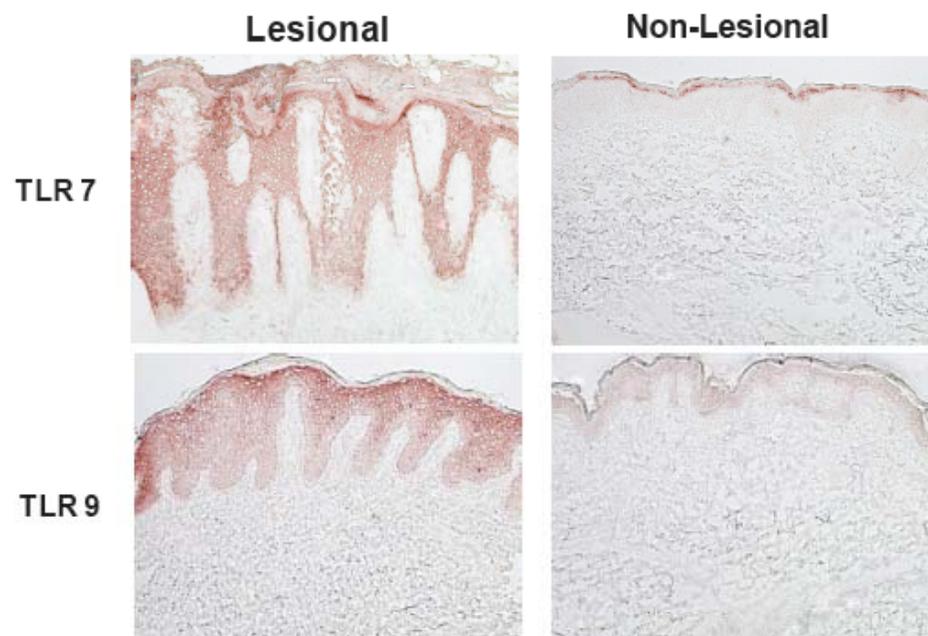
	NAME	Rx16		Etanercept	
		ES	NES	ES	NES
Psoriasis Genes Up-reg	Gudjonsson (2009)	-0.66	-3.11	-0.84	-4.26
	S-F (2010)	-0.66	-3.07	-0.83	-4.33
	MAD-5	-0.63	-2.94	-0.82	-4.47
	S-F <sup>+</sup> (2012)	-0.60	-2.88	-0.74	-4.20
	Zhou (2005)	-0.64	-2.86	-0.78	-3.70
	MAD-3	-0.59	-2.81	-0.79	-4.40
	Yao (2007)	-0.57	-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

## IL 17 genes (unique)



**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 NF $\kappa$ B and links TLR pathways with both the TNF response in keratinocytes and with the IL-17 pathway response, where NF $\kappa$ B 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- $\alpha$  blockers, consistent with both decreasing NF- $\kappa$ B.**
- 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.**