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Comparing cutaneous molecular improvement with different treatments in atopic dermatitis patients

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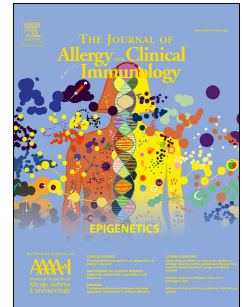
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Title: Comparing cutaneous molecular improvement with different treatments in atopic dermatitis patients

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Capsule summary: This is the first study that objectively compares the molecular cutaneous improvement with different treatments in AD and may facilitate personalized medicine by identifying the best treatments for individual phenotypes, based on immune/barrier profiles.

Keywords: Atopic dermatitis, precision medicine, ASN002, cyclosporine, crisaborole, dupilumab, fezakinumab, ustekinumab, narrow-band ultraviolet type B, corticosteroids

44 **Abbreviations:**

45	AD	atopic dermatitis
46	BID	bis in die (twice a day)
47	CE	cornified envelope
48	CsA	cyclosporine
49	EASI	Eczema Area and Severity Index
50	EDC	epidermal differential complex
51	JAK	Janus kinase
52	LS	lesional
53	MADAD	meta-analysis-derived atopic dermatitis
54	NB	narrow band
55	NL	nonlesional
56	OR	online repository
57	SYK	spleen tyrosine kinase
58	T _H	T helper cell type
59	UVB	type B ultraviolet

To the Editor:

Atopic dermatitis/AD is a common inflammatory skin disease that affects up to 10% of adults worldwide.¹ AD is a heterogeneous disease with primary T_H2/T_H22 -skewing, and variable T_H1/T_H17 contribution. Due to its multi-cytokine nature, many broad-acting and specific systemic and topical treatments were developed targeting certain cytokine pathways, with variable efficacy.¹ Recently, broader agents (i.e cyclosporine/CsA, narrow-band UVB/NB-UVB, Janus kinase/JAK inhibitors/ASN002, corticosteroids), and more specific antagonists targeting $T_H2/IL-4R$ (dupilumab), $T_H22/IL-22$ (fezakinumab), $T_H17/T_H22/T_H2$ (crisaborole), and $IL-12/IL-23$ (ustekinumab) have shown significant changes in lesional AD molecular profiles compared to placebo.¹⁻⁸ Nevertheless, a comprehensive, objective means to compare cutaneous effects of various drugs and placebos is lacking. To better understand how different pathways contribute to AD pathogenesis, it is important to objectively compare relative cutaneous changes in immune and barrier mechanisms with different treatments.

We used a meta-analysis approach similar to a recent psoriasis study,⁹ to evaluate molecular changes in skin with different AD therapeutics. We only included clinical trials in which Affymetrix U133Plus 2.0 lesional and nonlesional skin gene-arrays were available from AD patients treated with broad (CsA, NB-UVB, JAK/SYK inhibitor/ASN002, topical corticosteroids) and specific (dupilumab, fezakinumab, ustekinumab, crisaborole) treatments (Table E1). Seven publicly available microarray datasets (Six^{1-3, 5-7} in moderate-to-severe AD and one⁴ in mild-to-moderate AD patients) were found in Gene Expression Omnibus/GEO (GSE133385/GSE133477/GSE58558/GSE130588/GSE99802/GSE27887/GSE140684; Table E1).¹⁻⁷ The main outcome of interest was percent improvement by gene-signatures (see Methods section in this article's Online Repository/OR at www.jacionline.org). The R *limma*

package was used to model expression values by mixed-effect models from all mechanistic skin studies derived from microarray trial data. Drug improvement percentages were then calculated at available timepoints for each gene, and grouped by previously reported AD-related gene-subsets.¹ These signatures include upregulated/downregulated meta-analysis derived AD/MADAD transcriptome^{1,2}, immune and barrier gene-subsets^{1,2}, negative regulators^{1,2}, and T_H1/T_H2/T_H17/IL-22/IL-34-specific genes. Due to the inherent normalization of percent drug improvement, grouped AD-related gene signatures could be directly compared across treatments by Wilcoxon test.

Overall, CsA showed the highest improvements in AD-related gene signatures (Figs 1, 2), significantly outperforming all other drugs/placebos in most gene-subsets (i.e up- and down-regulated MADAD transcriptome, immune genes, epidermal differentiation and lipid genes, negative regulators and T_H1/T_H17/T_H22/IL-22-regulated genes; $P < .01$, Figs 1, 2; Tables E2,E3); however, dupilumab shows the highest overall improvement in T_H2-specific genes (median=205%), and significantly outperforms other drug and placebo arms with available evaluations at Wk16, across the MADAD, immune genes, negative regulators, and T_H2 genes ($P < .05$, Figs 1, 2; Table E2). Although dupilumab significantly outperforms placebo and ustekinumab at Wk16 for T_H1/T_H17 genes ($P < .05$, Fig 2; Table E2), it shows higher median improvement for T_H17 (125%) compared to T_H1 genes (74%). At Wk4, ASN002-40mg significantly improved the upregulated MADAD profile and T_H22/IL-22-specific genes more than ASN002-20mg/ASN002-80mg/fezakinumab/placebo with median differences greater than 36% ($P < .05$, Fig 1; Table E2). While IL-22-targeting with fezakinumab showed modest improvements on immune pathways, it showed comparable or higher improvement in MADAD down-regulated and barrier genes to dupilumab, highlighting the role of IL-22 in the AD barrier

dysregulation. As expected with an IL-22 inhibitor, fezakinumab has a median improvement of 84% on $T_H22/IL-22$ genes, outperforming placebo by a median difference of 49% at Wk12 ($P<.01$); however, it shows no significant difference in improvement compared to NB-UVB at Wk12 for $T_H22/IL-22$ genes ($P>.05$, Fig 1; Table E2).

NB-UVB, a safe and efficacious albeit time-consuming treatment, shows significant improvements on MADAD, negative regulators, T_H1 and immune genes, with lesser efficacy on barrier-associated markers; it outperforms fezakinumab with median differences greater than 20% across the upregulated MADAD transcriptome, negative regulators, T_H1 and immune genes ($P<.01$, Figs 1, 2; Table E2). Crisaborole improves AD-related signatures more than placebo ($P<.05$), with greatest improvements in T_H22/T_H17 -related genes, and shows significantly more improvement than with systemic ASN002 across the upregulated MADAD transcriptome at Wk2 ($P<.001$, Fig 1; Table E2).

While ustekinumab does not have robust improvement for AD-related gene-subsets (Figs 1, 2), mild-to-moderate topical steroids (triamcinolone 0.025%) were used consistently twice daily until Wk16 during the placebo-controlled portion, and published separately detailing continuous improvement beyond Wk4 with topical corticosteroids.⁸ The topical steroid group (“ustekinumab placebo”) significantly outperforms dupilumab’s placebo and ustekinumab at Wk16 by a median difference of at least 13% across upregulated/downregulated MADAD transcriptomes, immune genes, negative regulators and T_H17 ($P<.05$, Figs 1, 2; Table E2). This highlights the relevance of topical corticosteroid use when evaluating placebo and drug responses and for trial design. It also serves as a reminder that caution is needed when comparing drugs to “placebo” allowing topical medications. Finally, we compared improvements in IL-34, a negative regulator of inflammation, that is normally down-regulated in AD, and a biomarker of

response for different therapies.^{2, 4, 6, 8} Interleukin-34 is a cytokine that promotes the differentiation and viability of monocytes and macrophages through the colony-stimulating factor-1 receptor, and has been shown to play important role in autoimmune disorders, inflammation and cancer. Interestingly, IL-34 discriminated molecular response between efficacious drugs and placebo, using a 35% cut-off.

These results confirm CsA as the gold standard of cutaneous improvement in AD, but also show that narrow-targeting with dupilumab achieves broad changes in skin, beyond those expected with specific T_H2 inhibition. These data also shed light onto the relative improvement with different treatments in various immune and barrier mechanisms, highlighting unique properties of targeting a particular axis. For example, targeting the T_H22 axis with fezakinumab showed major improvement on barrier dysfunction, but only modest improvement in inflammatory signatures.

Our approach has the potential to facilitate future development of a personalized medicine approach in AD, and identify the best treatments for individual phenotypes, based on immune and barrier profile. For instance, a patient with T_H2 driven disease may primarily benefit from dupilumab, while for a patient with high T_H2 and T_H1 axes JAK targeting may be preferable.

Our study has several limitations. It compares therapeutics with different modes of action and their counterpart placebos. The actual “placebo” may vary across trials, as in the ustekinumab study. Also, studies differ in their experimental design and patient numbers. For instance, the crisaborole study incorporated an inpatient design, the ASN002 study had 36 patients with 9 patients per arm, and the CsA and NB-UVB studies had no placebo. Further, the timepoints for biopsy evaluations varied between studies, as well as the early and late biopsy

timepoints, thus the data analysis was restricted to available timepoints. Almost all studies included moderate-to-severe AD except the crisaborole study that included mild-to-moderate patients. As a result, drug improvements may have been smaller for milder compared to more severe patients. Finally, the therapeutics were administered disproportionately to white populations making it more difficult to generalize the analysis. Overall, this approach may determine which treatments best benefit various AD characteristics and can promote a precision medicine strategy.

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Figure legends**Figure 1. Drug improvement percentages for AD-related gene signatures:****upregulated/downregulated MADAD transcriptomes, immune genes, EDC-CE and lipids,****T_H2, and T_H22/IL-22 specific genes at weeks 1, 2, 4, 12, and 16.** Stars denote significant

differences in the improvement scores between drug and placebo groups from the same study by

Wilcoxon test. Bars superimposed on each point represent the median (scaled by study size) $\pm$

IQR/2 and are colored by treatment. +P < .1, *P < .05, **P < .01, ***P < .001. EDC-CE,

epidermal differential complex—cornified envelope.

⁺⁺ Subjects treated in the ustekinumab study were given a background of triamcinolone .025%
BID. The placebo group used more triamcinolone .025% than the drug group.⁵**Figure 2. Drug improvement percentages for AD-related gene signatures: T_H1, T_H17,****negative regulators, and IL-34 specific genes at weeks 1, 2, 4, 12, and 16.** Stars denote

significant differences in the improvement scores between drug and placebo groups from the

same study by Wilcoxon test. Bars superimposed on each point represent the median (scaled by

study size) $\pm$ IQR/2 and are colored by treatment. +P < .1, *P < .05, **P < .01, ***P < .001.⁺⁺ Subjects treated in the ustekinumab study were given a background of triamcinolone .025%
BID. The placebo group used more triamcinolone .025% than the drug group.⁵

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Figure 1

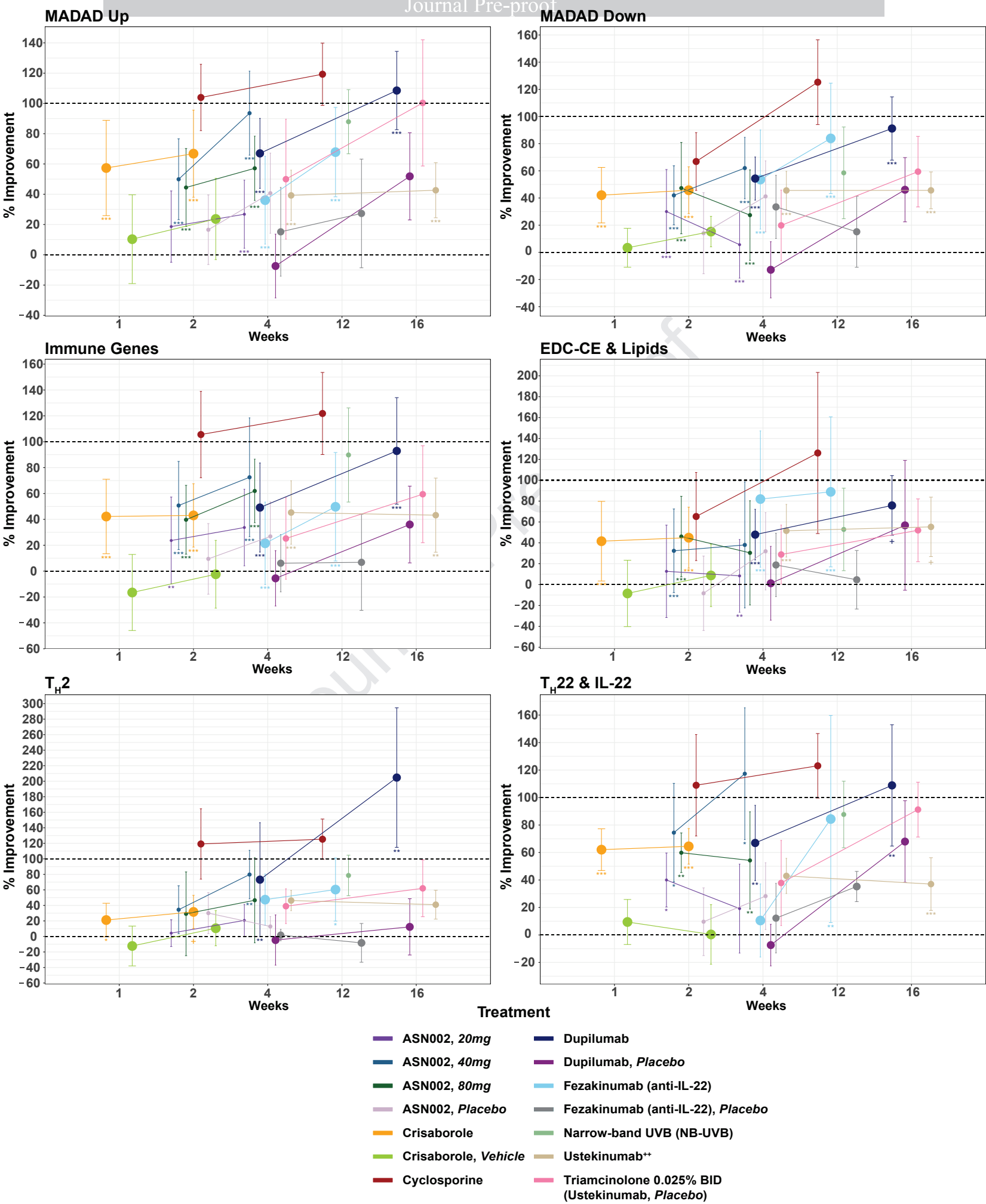


Figure 2

