Biologic Pathways For Chronic Spontaneous Urticaria And Their Regulation By Benralizumab

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To the Editor,

We previously reported the efficacy of benralizumab (anti–IL5Rα biologic) in controlling CSU refractory to second generation H1-antihistamines (SGHA).[1,2] Herein, we sought to identify CSU-relevant genes and pathways and their regulation by benralizumab.

CSU patients (N=10; unresponsive to up to 4x FDA-approved doses of SGHA) were enrolled in a single-blind, uniform-dose, multi-treatment trial (benralizumab 30mg × 3 treatments; Figure s1). Pre- and post-benralizumab skin and blood samples were analyzed by RNAseq. We identified a CSU signature using machine learning (ML) on an available CSU dataset (GSE72540) which was validated using our samples.[3] Pre-, post-Rx symptom scores, and corresponding blood eosinophil counts are summarized in Table-S1. Comparison between lesional *vs* non-lesional CSU skin indicated upregulation of multiple interleukins (IL-6, IL-20, IL-1β), chemokines (CCL2, CCL3, CCL8, CCL13, CCL18 and CCL21) and adhesion molecules (CD209, ICAM, CD69), while keratin variants (KRT25, KRT26, KRT27, KRT85) were downregulated. Multiple enriched networks were identified with IL1β, IL6, CCL2 and CCL3 as major hub genes (Figure-1A). Machine learning and hierarchical clustering showed that a minimum of 9 genes (OSM, PTGS2, UTP14A, ZFP36, STAT3, CCL2, RPF2, IL6 and LILRB3) were required to stratify lesional from non-lesional samples with $> 90\%$ predictive efficacy (Figure-1B). Compared to healthy control skin, lesional CSU skin showed

upregulation of adhesion molecules (SELE, ICAM1, CD69), low-affinity IgE-receptor (FcER2) and CC-chemokines (CCL2, CCL3, CCL4). Methods and further results are summarized in the on-line supplement.

To determine the effect of benralizumab, the transcripts (Visit 2 pre-treatment *vs* Visit 5 end of study) were arranged by significance (p-values) and the top 500 genes (fold-change value > 2.0; ˜ top 2% of all detected transcripts) were used for downstream analysis.[4,5] A significantly enriched treatment-relevant network showed IL6, TNF and IL1A as major hub-genes. Other relevant transcripts in the network were CD44, mir-24, IFNg, IL4, S100A10 and S100A12. Relevant differentially expressed genes (DEGs) were related to chemokeines/cytokines (CCR1, CCL2, IL6; log2FC -2.04, -2.24, -2.94 respectively), inflammation (PLA2G2A, PTGS2; $\log_2$FC -3.19, -1.02), adhesion (SELE; $\log_2$FC = -3.7) and barrier function (AQP4; Log_2FC 4.27). Focusing on the three complete treatment responders, the same set of hub genes (IL6, TNF and IL1A) in addition to IL22, IL13, collagen-related genes (COL1A1, COL4A2) and NFkB gene members were identified (Figure-1C, D). In addition, PLA2G2A (Phospholipase A2, Group IIA), THBS1 (Thrombospondin-1) and SOCS3 (Suppressor Of Cytokine Signaling 3) were identified as significant DEGs (FDR-adjusted p-values < 0.05; $\log_2$FC values -3.3, -1.9 and -2.5 respectively). The functional significance of these genes in regulating mast cell function, inflammation and cytokine function have previusly been described.[6-8] Moreover, benralizumab responders also demonstrated a significant downregulation of PTGDS2 ($\log_2$FC = -1.59), a major driver of cutaneous inflammation. Baseline transcriptomic differences between CSU responder and non-responder skin indicated that the major significantly upregulated genes ($\log_2$ FC in perentheses) in benralizumab-resistant CSU might be FOS family members of proto-oncogenes FOSB (6.3), Activating Transcription Factor 3 ATF3 (4.5), and nuclear factor subfamily 4 members A1 and A2 (4.4 and 3.92, respectively). Enrichment analysis identified platelet activities in addition to glucocorticoid and phosphatase activities in the non-responsive versus responsive CSU lesional skin. Finally, blood transcriptomes pre *vs* post-benralizumab treatment demonstrated downregulation of transcripts associated with IL5 signaling (e.g., IL5RA and SIGLEC8 $\log_2$FC -2.79 and SIGLEC8 -5.47 respectively; FDR-adjusted p-value < 0.01). When this analysis focused on complete responders to benralizumab, a similar group of transcripts (IL5RA, IL1RL1, RPL34, ALOX15, SIGLEC8 and S100A8) related to IL5 signaling and inflammatory pathways were downregulated. Collectively, benralizumab was associated with blood IL5 downregulation and reversal of the cutaneous CSU transcriptomic signature. Benralizumab-refractory CSU could represent a distinct endotype warranting further investigation.

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AUTHOR CONTRIBUTIONS

DG and JB designed the study. DG, RK, XZ, US conducted the study, JB supervised the work. DG and JB wrote the manuscript. All authors contributed to the manuscript.

ETHICS STATEMENT

The study protocol was approved by the University of Cincinnati Internal Review Board.

CONFLICTS OF INTEREST

Dr. Bernstein reports grants and consultant fees from AstraZeneca, Sanofi-Regeneron, Novartis, Genentech, Amgen, Celldex, Allakos during the conduct of the study. In addition, he has been a speaker for AstraZeneca, Sanofi-Regeneron, Novartis and Genentech. Outside this work he has received grants and consultant fees from Shire/Takeda, CSL Behring, Pharming, Biocryst, Kalvista, Ionis, Escient, ONO, Incyte, Cycle, Biomarin and Blueprint Medicine. He is a member of the Joint Task Force for the AAAAI and ACAAI, a member of the CU UCARE guideline committee and a UCARE and ACARE center of excellence, and a member of the MAB of the HAEA organization. All other authors have nothing to disclose.

References

1. Bernstein JA, Singh U, Rao MB, Berendts K, Zhang X, Mutasim D. Treatment of chronic spontaneous urticaria with benralizumab: Report of primary endpoint per-protocol analysis and exploratory endpoints. *Allergy.*2021;76(4):1277-1280.

2. Bernstein JA, Singh U, Rao MB, Berendts K, Zhang X, Mutasim D. Benralizumab for Chronic Spontaneous Urticaria. *N Engl J Med.*2020;383(14):1389-1391.

3. Gimenez-Arnau A, Curto-Barredo L, Nonell L, et al. Transcriptome analysis of severely active chronic spontaneous urticaria shows an overall immunological skin involvement. *Allergy.*2017;72(11):1778-1790.

4. Ghosh D, Ding L, Sivaprasad U, et al. Multiple Transcriptome Data Analysis Reveals Biologically Relevant Atopic Dermatitis Signature Genes and Pathways. *PLoS One.* 2015;10(12):e0144316.

5. Ghosh D, Ding L, Bernstein JA, Mersha TB. The Utility of Resolving Asthma Molecular Signatures Using Tissue-Specific Transcriptome Data.*G3 (Bethesda).* 2020;10(11):4049-4062.

6. Murakami M, Taketomi Y. Secreted phospholipase A2 and mast cells.*Allergol Int.* 2015;64(1):4-10.

7. Lopez-Dee Z, Pidcock K, Gutierrez LS. Thrombospondin-1: multiple paths to inflammation. *Mediators Inflamm.* 2011;2011:296069.

8. Chaves de Souza JA, Nogueira AV, Chaves de Souza PP, et al. SOCS3 expression correlates with severity of inflammation, expression of proinflammatory cytokines, and activation of STAT3 and p38 MAPK in LPS-induced inflammation in vivo. *Mediators Inflamm.*2013;2013:650812.

Table-1. Significantly enriched disease-relevant and treatment-relevant biological processes. (A) Processes associated with CSU lesional skin (compared to non-lesional skin) identified by enrichment analysis. Gene Ontology identifiers are shown within parentheses. Top significant processes have been shown with eosinophil chemotaxis (GO:0048245) and eosinophil migration (GO:0072677) were the two significant (adj. p-value = 2.016e-7 for both) biological processes associated with CSU that showed highest combined scores in enrichment analysis. (B) Processes associated with skin transcriptomes before vs. after benralizumab therapy.

Table-1A

	Name	P-value	Adjusted p-value	Odds Ratio
1	cellular response to cytokine stimulus (GO:0071345)	1.120e-18	1.753e-15	9.58
2	cytokine-mediated signaling pathway (GO:0019221)	2.330e-17	1.824e-14	7.88
3	inflammatory response (GO:0006954)	6.181e-16	3.224e-13	13.23
4	positive regulation of MAPK cascade (GO:0043410)	1.809e-15	7.076e-13	11.55
5	positive regulation of ERK1 and ERK2 cascade (GO:0070374)	1.128e-13	3.530e-11	14.10
6	regulation of ERK1 and ERK2 cascade (GO:0070372)	2.091e-12	5.455e-10	10.56
7	response to interleukin-1 (GO:0070555)	8.527e-12	1.907e-9	20.28
8	monocyte chemotaxis (GO:0002548)	6.560e-11	1.283e-8	33.54
9	lymphocyte chemotaxis (GO:0048247)	1.028e-10	1.788e-8	31.62

	Name	P-value	Adjusted p-value	Odds Ratio
10	positive regulation of response to external stimulus (GO:0032103)	1.961e-10	3.069e-8	12.95
11	eosinophil chemotaxis (GO:0048245)	2.576e-9	2.016e-7	72.51
12	eosinophil migration (GO:0072677)	2.576e-9	2.016e-7	72.51

Table-1B

	Name	P-value	Adjusted p-value	Odds Ratio	Combi
1	C-X-C chemokine binding (GO:0019958)	0.005933	0.1770	26.10	133.82
2	C-X-C chemokine receptor activity (GO:0016494)	0.005933	0.1770	26.10	133.82
3	CCR1 chemokine receptor binding (GO:0031726)	0.005933	0.1770	26.10	133.82
4	complement receptor activity (GO:0004875)	0.002206	0.1770	14.71	89.96
5	CCR chemokine receptor binding (GO:0048020)	0.0005788	0.1036	6.57	48.95
6	ciliary neurotrophic factor receptor binding (GO:0005127)	0.01999	0.2937	11.18	43.76
7	chemokine activity (GO:0008009)	0.005642	0.1770	4.79	24.82
8	C-C chemokine receptor activity (GO:0016493)	0.01897	0.2937	5.88	23.31
9	C-C chemokine binding (GO:0019957)	0.02128	0.2937	5.60	21.56
10	chemokine receptor binding (GO:0042379)	0.008041	0.2056	4.37	21.06

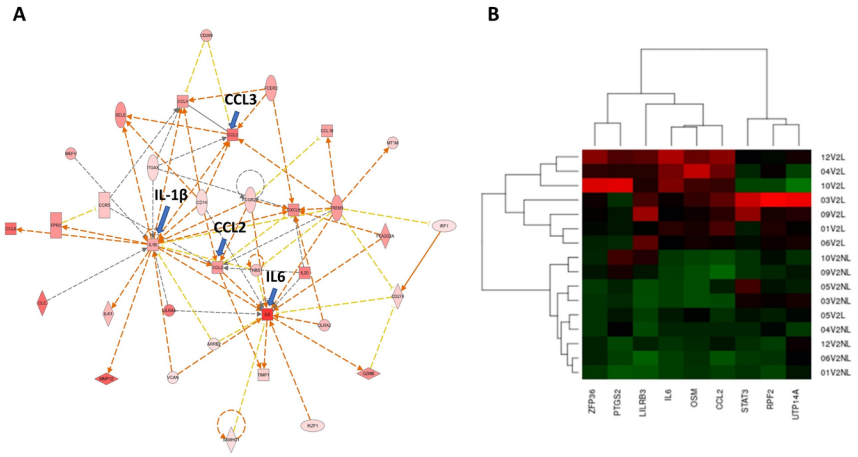

Figure-1