

Unveiling chronic spontaneous urticaria pathophysiology through systems biology



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Background: Chronic spontaneous urticaria (CSU) is a rare, heterogeneous, severely debilitating, and often poorly controlled skin disease resulting in an itchy eruption that can be persistent. Antihistamines and omalizumab, an anti-IgE mAb, are the only licensed therapies. Although CSU pathogenesis is not yet fully understood, mast cell activation through the IgE:high-affinity IgE receptor (FcεRI) axis appears central to the disease process. **Objective:** We sought to model CSU pathophysiology and identify *in silico* the mechanism of action of different CSU therapeutic strategies currently in use or under development. **Methods:** Therapeutic performance mapping system technology, based on systems biology and machine learning, was used to create a CSU interactome validated with gene expression data from patients with CSU and a CSU model that was used to evaluate CSU pathophysiology and the mechanism of action of different therapeutic strategies.

Results: Our models reflect the known role of mast cell activation as a central process of CSU pathophysiology, as well as recognized roles for different therapeutic strategies in this and other innate and adaptive immune processes. They also allow determining similarities and differences between them;

anti-IgE and Bruton tyrosine kinase inhibitors play a more direct role in mast cell biology through abrogation of FcεRI signaling activity, whereas anti-interleukins and anti-Siglec-8 have a role in adaptive immunity modulation.

Conclusion: *In silico* CSU models reproduced known CSU and therapeutic strategies features. Our results could help advance understanding of therapeutic mechanisms of action and further advance treatment research by patient profile. (J Allergy Clin Immunol 2023;151:1005-14.)

Key words: Machine learning, chronic spontaneous urticaria, system biology, artificial intelligence, mast cells

Chronic urticaria is a common skin disorder with heterogenous presentation and complex aetiopathogenesis. It is characterized by the occurrence of itchy wheals (“hives”), angioedema, or both daily or almost daily for more than 6 weeks.¹ The current international urticaria guideline differentiates 2 subtypes of chronic urticaria: chronic inducible urticaria (CindU), which is triggered by specific factors, and chronic spontaneous urticaria (CSU), which occurs without specific inducing factors.¹⁻⁸ CSU is a disabling condition that causes significant deterioration in quality of life and has a substantial impact on health care systems.⁹ Prevalence is estimated at between 0.5% and 5% in the general population, with an incidence of around 1.4% annually.¹⁰⁻¹²

The pathogenesis of CSU is yet not fully understood.^{13,14} Mast cells and basophils, whose activation and degranulation lead to histamine release, are proved to be involved.^{15,16} Mast cells also release cytokines and chemokines responsible for recruiting the perivascular infiltrate seen around small venules in the skin of patients with CSU.^{4,17-20} Autoimmunity is thought to be a driving factor in CSU, involving IgG and IgE autoantibodies as well as high-affinity IgE receptor (FcεRI).²¹⁻²³ However, only a small subset of patients meet all of the autoimmunity criteria.²⁴

Currently licensed medications for CSU treatment include non-sedating H₁ antihistamines and the anti-IgE omalizumab.^{5,8} Medications with different mechanisms of action are undergoing efficacy evaluation in clinical trials. This includes Bruton tyrosine kinase inhibitors (BTKIs), cytokine blockers (including IL-4 and IL-5), and immunomodulation through sialic acid-binding immunoglobulin-like lectin 8 (Siglec-8).^{3-5,8,19,25} Omalizumab, an anti-IgE mAb that prevents binding of IgE heavy chain to FcεRI or low-affinity IgE receptor ([CD23] FcεRII), has proved to be effective in CSU,^{19,26} although around 15% to 20% of patients are nonresponders,^{8,27-29} and some are slow to respond.^{3,4,10,29,30} Although the response rate in the pivotal and clinical trials is less than 50%,³¹ real-world evidence reports

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Abbreviations used

ANN:	Artificial neural network
BTKI:	Bruton tyrosine kinase inhibitor
CindU:	Chronic inducible urticaria
CSU:	Chronic spontaneous urticaria
FcεRI:	High-affinity IgE receptor
FcεRII (CD23):	Low-affinity IgE receptor
IL4R:	IL-4 receptor
IL5RA:	IL-5 receptor
Siglec-8:	Sialic acid-binding immunoglobulin-like lectin 8
TPMS:	Therapeutic performance mapping system

that the response rate is much higher.³² New treatment options are therefore needed to account for the diversity of patient response in this disease. Moreover, the exact mechanism by which omalizumab provides relief remains unclear.^{13,18}

The development of new treatments for CSU requires the use of clinical and biologic markers with which to assess their efficacy.⁴ Such markers are not yet available.²² In their place, *in silico* and systems biology-based tools may be useful to elucidate the specific mechanisms underlying CSU pathophysiology and the role of the different CSU therapeutic approaches. The utility of this approach has been demonstrated by several artificial intelligence-based studies in a wide range of complex clinical settings.³³⁻³⁸

The overarching objective of this work was to build an *in silico* model of CSU pathophysiology. The therapeutic performance mapping system (TPMS) technology^{34,37,39} is a validated *in silico* approach^{33,40-42} that allows exploration of the disease central processes. It also provides a framework with which to compare the molecular and cellular mechanisms of action of different CSU therapeutic approaches. Our findings might be useful in generating hypotheses that correlate molecular mechanisms with clinical responses, as well as in understanding how different therapeutic strategies might be suitable for different disease phenotypes.

METHODS**Gene expression data compilation and treatment**

On August 26th, 2020, CSU gene expression data from human patients were identified in the Gene Expression Omnibus (GEO) public repository⁴³ by using the query (Chronic spontaneous urticaria[All Fields] OR (spontaneous[All Fields] AND ("urticaria"[MeSH Terms] OR urticaria[All Fields])) OR Chronic idiopathic urticaria[All Fields]). The data were filtered by organism (*Homo sapiens*), entry type (series), experiment type (expression profiling by array and protein profiling by protein array), sample type (tissue), and tissue (skin). Two results were obtained: GSE72540⁴⁴ and GSE57178.⁴⁵ Gene expression analysis was performed with lesional skin versus healthy skin samples by using GEO2R software⁴⁶ with the default settings. Significance criteria were set at a false discovery rate less than 0.05 and an absolute value of the logarithm of fold change ($|\log FC|$) greater than 1. Genes were considered to be differentially expressed when at least 1 transcript of the gene complied with the significance criteria in both experiments and did not show any contradiction ($\log FC$ values with opposite signs) between the experiments or with other significantly differentially expressed transcripts of the same gene. Finally, transcripts were mapped to UniProtKB codes for subsequent analyses. In all, 70 results were retrieved by using these criteria (see Table E1 in the Online Repository at www.jacionline.org).

CSU bibliography-based molecular characterization

The molecular characterization of CSU was performed as previously described.^{35,37} A structured search was performed to obtain CSU-related reviews

published between July 2000 and July 2020 from PubMed by using the following search terms: ("chronic spontaneous urticaria"[Title/abstract] OR "CSU "[title/abstract] OR "chronic idiopathic urticaria"[title/abstract] OR "autoimmune urticaria"[title/abstract]) AND ("pathophysiology"[title/abstract] OR "pathogenesis"[title/abstract] OR "molecular"[title/abstract]). A total of 86 articles were retrieved and reviewed at the abstract level or at the full-length level if they contained molecular information on CSU pathophysiology. We performed hand-searching of reference lists from the articles identified within the structured search to further expand the search. The information found was manually reviewed and used to identify the predominant pathophysiologic processes (motives) and the proteins involved in disease pathophysiology (effectors) with consideration for only those proteins for which functional involvement in the disease was found. The characterization was used as a base to build the protein network, or interactome, around the disease.

Bibliography-based molecular characterization of the therapeutic strategies

Afterward, CSU therapeutic strategies (used or under investigation) targeting these proteins were characterized as specific drugs: omalizumab (anti-IgE), dupilumab (anti-IL-4 receptor IL4R), benralizumab (anti-IL-5 receptor IL5RA), remibrutinib (BTKI), and lirenitelimab (anti-Siglec-8). The drug molecular characterization comprised identification of drug targets according to official and publicly available documents (European Medicines Agency and US Food and Drug Administration), specialized databases (DrugBank,^{47,48} Stitch,⁴⁹ and Supertarget⁵⁰), and available literature in PubMed up to August 31, 2020, as previously reported.^{34,37,39} The list of therapeutic strategies evaluated is shown in Table E2 (available in the Online Repository at www.jacionline.org).

TPMS modeling

TPMS technology is based on systems biology, machine learning, and pattern recognition techniques that integrate all available biologic, pharmacologic, and medical knowledge (training set [see Table E3]) to create mathematical models simulating human pathophysiology *in silico*.^{37,39} The predictive and descriptive capacity of the models are validated against the training set, with model accuracy defined as the percentage compliance of this information. Two modeling approaches were used (Fig 1): artificial neural networks (ANNs)^{34,37} and sampling-based methods.^{37,39} The database-based human protein network used for TPMS model construction³³ was applied to expand the CSU characterization and to create the CSU interactome. The models based on sampling methods allow exploration of the predicted activity for each protein (ranging between -1 and 1) within the models; they identify the most frequent pathways occurring between a stimulus and the biologic process definition. When a biologic process is defined (ie, a disease or motive), the impact of a stimulus can be assessed through the T-signal, defined as the average signal arriving at the protein effectors involved in the biologic process.³⁹ Skin-derived gene expression information (see Table E1) was included in the model alongside the TPMS default training information (see Table E3 in the Online Repository at www.jacionline.org).³⁹

Statistical analyses and results visualization

Statistical analyses were carried out using model-derived protein predicted activity obtained from models based on sampling methods, applying parametric and nonparametric comparison tests, and hypergeometric enrichment analysis. Multidimensional scaling and Cytoscape software⁵¹ were used for visualization of results. For further details, see the Supplementary Methods (available in the Online Repository at www.jacionline.org).

RESULTS**Bibliography- and database-based CSU interactome reflects gene expression changes in skin samples from patients with CSU**

Disease characterization enabled the identification of 129 unique CSU effector proteins categorized into 5 motives:

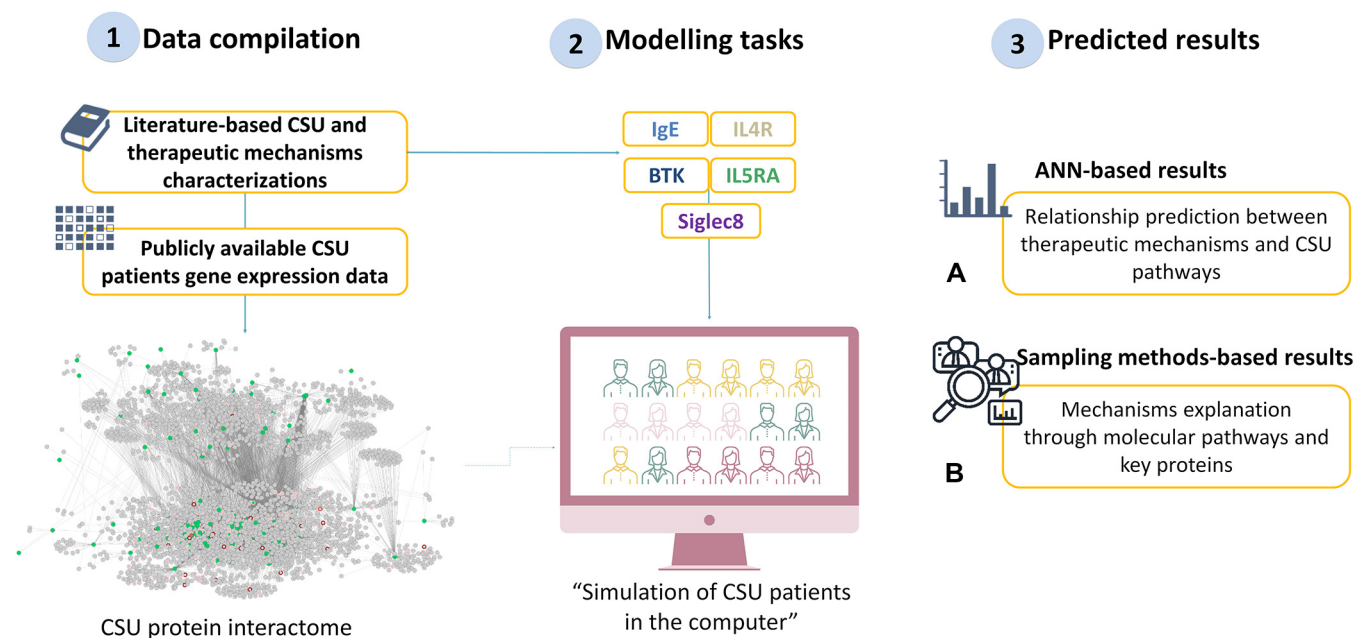


FIG 1. Overview of the analysis methodology. Data compilation and *in silico* modeling were used to characterize the molecular pathways involved in CSU therapies. Two modeling approaches were used (step 3): ANNs,^{34,37} an algorithm able to detect biologic relationships between the drug or drug targets and CSU-related processes through topologic measures, providing a score (from 0 to 100%) associated with a probability (*P* value) of positive functional relationship between the sets of proteins, with an accuracy against the training set greater than 80% (A), and sampling-based methods^{37,39} that generate models similar to a multi-layer perceptron of an ANN using the human protein network as a base and are able to explain biologic relationships by generating a universe of plausible solutions, prioritizing those that are more probable from a mathematical and biologic point of view (B).

activation of the coagulation and complement system (25 proteins); autoallergic and autoimmune triggering (7 proteins); mast cell activation and degranulation (33 proteins); granulocyte homing to skin and activation (43 proteins); and humoral mediators of inflammation and endothelial changes (43 proteins) (see Table E4 in the Online Repository at www.jacionline.org).

The CSU interactome (see Fig E1 in the Online Repository at www.jacionline.org), which contains CSU effectors and directly related proteins according to the human protein network, included 41.43% of the genes with altered expression in skin samples from patients with CSU (see Table E1) and 73.41% of the enriched processes in the expression data (see Table E5 in the Online Repository at www.jacionline.org). These enriched processes represent 60.68% of the interactome and include 127 of 129 CSU protein effectors. These data suggest that to a great extent, the molecular definition of CSU used reflects the functional aspects observed in CSU skin-derived expression data, providing grounds for its use in modeling.

Systems biology-based CSU models show the centrality of mast cell activation and degranulation in CSU pathophysiology and the importance of the IgE:FcεRI axis

Mathematical models simulating CSU were built around the CSU interactome by applying TPMS technology. According to our sampling methods, mast cell activation and degranulation motives hold a central role in CSU definition as a whole, as measured by the T-signal induced by each of the motives over the others (Fig 2 [network obtained by applying the default setting of

the *prefuse force-directed* layout in Cytoscape software with T-signal values]).

Triggering analysis of the CSU effectors in the models also showed that the high-affinity receptor FcεRI and its downstream signaling molecular cascade (including BTK) has a role in inducing mast cell activation and degranulation motives (Fig 3, A). According to this analysis, IgE itself had a more modest role.

The list of CSU effectors evaluated includes proteins suggested as therapeutic targets for CSU. Among them, IL5RA has a remarkably high score, and together with IL4R, it falls in the top 20 of evaluated CSU-related proteins. IL13R and Siglec-8 also demonstrated some triggering potential (Fig 3, A). Interleukin receptors seem to have a more relevant role than the ligands themselves, in a manner similar to the IgE:FcεRI results. We found a modest role for H₁ receptor mast cell activation in CSU. Finally, CD23 was not found to have triggering potential for mast cell activation. Accordingly, we focused our subsequent study on drugs targeting the main CSU effectors (examining only those drugs already approved for use or with proven efficacy in clinical trials as of October 2020), focusing on mechanisms behind the modulation of FcεRI, Btk, IL5RA, IL4R, and Siglec8. All of these targets are modeled to have a measurable, although apparently different, impact on mast cell degranulation (Fig 3, B-E).

Different therapeutic approaches demonstrate modulation of different processes within CSU

To identify the specific processes of CSU in which the therapeutic strategies under study may be involved, the

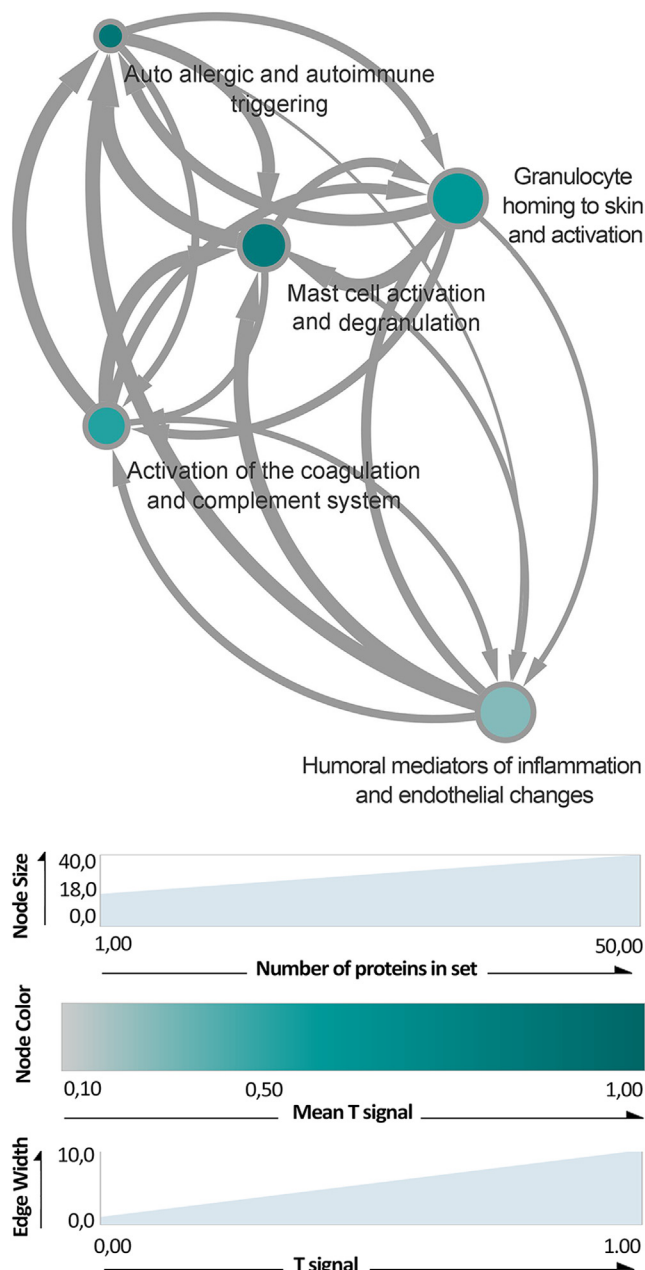


FIG 2. Network displaying a T-signal induced to each CSU motive by each other. *Prefuse force-directed* layout shows mast cell activation centrality within the motive network. Node size indicates the number of proteins involved. Arrow size indicates the value of T-signal obtained in each motive-motive relationship. Node color indicates the mean T-signal obtained for each motive with consideration for all motive-motive relationships (the darker the color, the more stimulated the motive by the rest of the motives).

relationship between the therapeutic approaches and each of the CSU motives was evaluated by using ANN analyses. This analysis provides a predictive score that quantifies the probability of the existence of a functional relationship between protein sets or regions inside the Anaxomics network. All of the evaluated drugs were predicted to be related to CSU, although through different mechanisms (Table I).

According to the ANN analysis, all of the therapeutic strategies show a high probability of relationship ($P < .05$) to at least 1

motive. Anti-IgE and BTKIs present the most probable relationship ($P < .05$) with mast cell activation and degranulation. Anti-Siglec-8 ($P < .05$), anti-IL4R ($P < .05$), and anti-IL5RA ($P < .01$) present the most probable relationship with granulocyte homing to skin and granulocyte activation. Anti-IL5RA and anti-IL4R, both through the IL-4 and IL-13 axes, present the most probable relationship with the motive involving humoral mediators of inflammation and endothelial changes. Among the studied therapeutic strategies, those demonstrating the greatest role in autoallergic and autoimmune triggering are anti-IL4R and anti-IL5RA, although the relationship does not achieve statistical significance ($P < .1$).

Sampling-based methods detail the role of each therapeutic approach

Sampling-based mechanistic techniques for each therapeutic strategy were built, with 250 mechanisms of action solutions each and a mean accuracy of 94%. The most modulated proteins for each mechanism of action ($|\text{predicted protein activity}| > .5$) were analyzed by hypergeometric enrichment analysis to identify enriched pathways within the proteins most modulated by each drug (Fig 4, A and see Table E6 in the Online Repository at www.jacionline.org). According to our models, all of the therapeutic strategies were found to modulate proteins enriched in mast cell activation (Fig 4, B), as well as other CSU-related processes. Anti-IL4R, anti-IL5RA, and anti-Siglec-8 might have a wider role in controlling the adaptive immune system (Fig 4, B). However, anti-IgE and BTKIs appear to have a greater effect on mast cell biology, and they appear to modulate antigen processing and presentation, a key link between the innate and adaptive immune systems (Fig 4, B).

In summary, all of the drugs modulate similar processes involved in controlling CSU pathology. However, they show differences that might be key to understanding their effects.

To further explore the mechanistic differences of each therapeutic strategy and whether these explain the different clinical effects seen, statistical differences in predicted protein activity were evaluated among each of the therapeutic target models (see Table E7 in the Online Repository at www.jacionline.org). Whereas Fig 5 contextualizes the differential mechanisms detected for each therapeutic strategy in CSU, Figs E2 to E6 (available in the Online Repository at www.jacionline.org) detail the mechanistic results obtained from the model applying sampling-based methods. In addition to inhibition of IgE-induced mast cell activation, anti-IgE mechanisms also lead to modulation of FcεRI-mediated expression of IL-17, CD244, or vascular endothelial growth factor (see Fig E2). BTKIs not only inhibit mast cells activation cascade but also prevent Toll-like receptor signaling mechanisms, which are involved in B-cell activation, and modulation of other immune mediators such as IFN-γ (see Fig E3). Anti-IL5RA therapeutics have specific additional effects on eosinophil death, preventing their role in the pathogenesis of CSU and reducing their contribution to the proinflammatory milieu (see Fig E4); in fact, key proinflammatory interleukins (IL-6 or IL-1β) and adhesion molecules (ICAM1) are highlighted in its mechanism, supporting a role for this therapeutic strategy in reducing immune cell recruitment to skin and exacerbation of inflammation. Anti-IL4R differential mechanisms are predicted to involve different pathologic processes: reduction of IgE

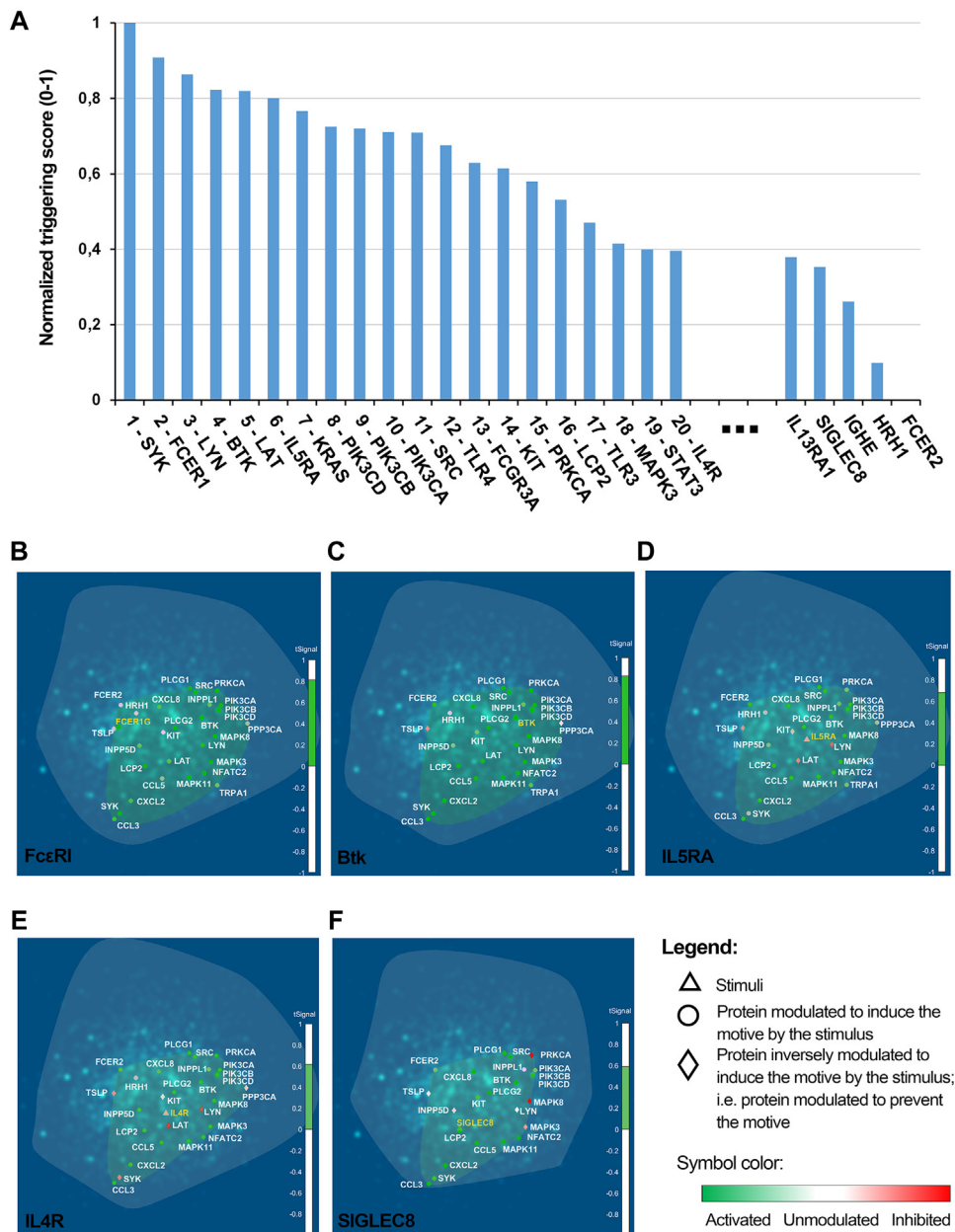


FIG 3. Evaluation of mast cell activation triggering potential of CSU-related proteins. **A**, The top 20 proteins; results for other proteins used and suggested as therapeutic targets for CSU are also displayed for informative purposes. **B-F**, Multidimensional scaling (MDS) representation of a mast cell activation protein network and modulation induced by different targets. The representation shows the area of action of each target, in general (*bluish shadow*) and with consideration for mast cell activation and degranulation (*greenish shadow*) and the strength of the impact (*T-signal scale on the right*), as well as where the target falls in the representation along with the mast cell activation and degranulation effectors modulated by each target.

production from B cells and reduction of the expression of molecules that promote immune cell recruitment, inflammation, and mast cell activation, including the interleukins IL-2 and IL-24, chemokine CXCL9, proinflammatory factors (thrombospondin and leukotriene receptor), and IFN- γ -mediated signaling (probably via IL-13) (see Fig E5). Given the presence of IL4R in different cell types, these effects are hypothesized to be mediated by different cell types. Finally, anti-Siglec-8 differential

mechanisms involve the prevention of an intracellular IgE- or IL-33-induced mast cell activation cascade, modulation of the proinflammatory and/or anti-inflammatory equilibrium (IL-12, IL-18, and IL-1 α), and triggering of eosinophil apoptosis via oxidative stress induction (reduced nicotinamide adenine dinucleotide phosphate [NADPH] oxidase, p38) (see Fig E6). In conclusion, although all of the therapeutic strategies lead to a reduction of mast cell activation, each does so through a different

TABLE I. ANN evaluation of the relationship between drug targets and CSU processes

Drug target	Motives					
	1	2	3	4	5	
Anti-IgE	+++ (86%)	+ (68%)	- (6%)	- (12%)	- (9%)	
BTKI	+++ (90%)	- (34%)	+ (55%)	- (31%)	- (35%)	
Anti-IL4	IL13:IL13R	- (15%)	++ (73%)	- (26%)	+ (38%)	+++ (89%)
	IL4:IL4R	- (24%)	++ (71%)	- (4%)	+++ (85%)	+++ (83%)
Anti-IL5	FCGR3	+ (65%)	++ (72%)	- (9%)	- (35%)	- (11%)
	IL5:IL5RA	+ (47%)	++ (75%)	- (7%)	++++ (92%)	+++ (80%)
Anti-Siglec-8	++ (72%)	- (3%)	- (31%)	+++ (82%)	- (3%)	

The 5 motives as follows: (1) mast cell activation and degranulation, (2) autoallergic and autoimmune triggering, (3) activation of the coagulation and complement system, (4) granulocyte homing to skin and activation, and (5) humoral mediators of inflammation and endothelial changes. ANN scores range from 0% to 100%. Four plus signs indicate $P < .01$; 3 plus signs indicate $P < .05$; 2 plus signs indicate $P < .1$; 1 plus sign indicates $P < .2$; and a minus sign indicates $P > .2$.

FCGR3A, Low-affinity immunoglobulin gamma Fc region receptor III-A.

pathway. This includes regulation of the immune cells involved in mast cell activation and regulation of skin-related changes in keratinocytes or endothelial cells.

DISCUSSION

Our *in silico*, intelligence- and systems biology-based approach enabled us to model the underlying processes of CSU pathophysiology and to provide a comparative analysis of the mechanistic differences of experimental therapies. The pathogenesis of CSU is not yet fully elucidated, but for many patients it involves autoimmune mechanisms driven by both mast cells and basophils, with IgG, IgE, and FcεRI as the main molecular effectors.^{4,13,14,18} Our findings support a central role for mast cell activation in CSU and an important role of FcεRI in disease pathogenesis. The role of H₁ receptor was found to be less important, in keeping with its indirect activation of mast cells⁵² and the clinical observation that many patients are antihistamine unresponsive.⁵³

The models also show that FcεRI has a more important role in CSU-associated mast cell activation than IgE or CD23 do. These results are supported by several pieces of evidence. First, omalizumab is able to directly inhibit basophils and mast cell degranulation via its effects on FcεRI.⁵⁴ Second, some patients do not show benefit from treatment with anti-IgE,⁵⁵ and this is associated with lower IgE levels, suggesting a role for type IIb autoimmunity involving IgG autoantibodies toward FcεRI.^{56,57} Third, FcεRI levels in basophils correlate with response to omalizumab.⁵⁸ Lastly, abnormal basophil function has been described in CSU, including abnormal response to other stimuli through non-FcεRI receptor,¹⁵ abnormally high FcεRI expression,⁵⁹ and desensitization of the FcεRI pathway.⁶⁰ These features are all reversible subsequent to CSU remission.⁶¹ Along with known targets of currently approved treatments,⁶² our model highlighted proteins targeted by molecules that are currently under investigation and that we therefore selected for *in silico* comparison. These included PI3K⁶³ or the topical Syk inhibitor.⁶⁴ Overall, our results support the idea that targeting FcεRI activity in mast cells is likely to achieve therapeutic effects.

The results presented here allow us to propose hypotheses that relate molecular mechanisms of therapeutic strategies to clinical responses in order to assess their strengths and weakness. In light of the direct effect of anti-IgE and BTKIs on mast cell degranulation, it is possible that these drugs can induce more rapid responses in patients with CSU than anti-IL4R and

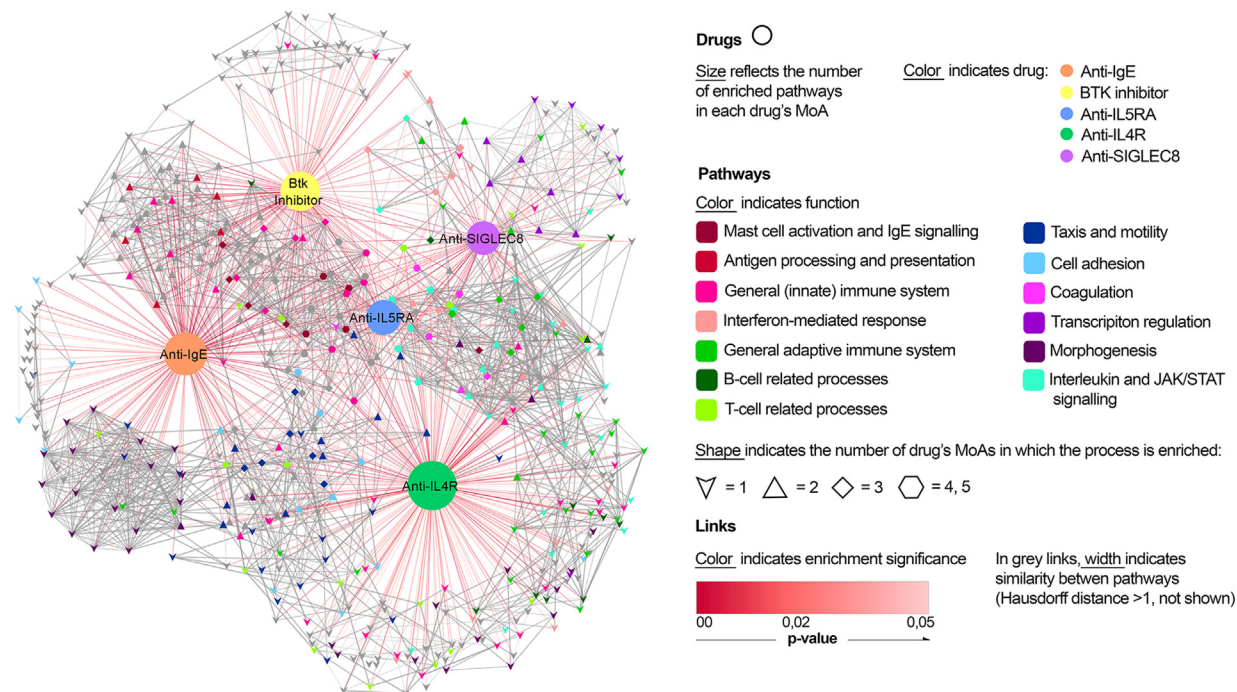
anti-IL5RA can. This hypothesis is supported by the efficacy of agents such as benralizumab, which is an anti-IL5RA,^{65,66} and fenbrutinib⁶⁷ or remibrutinib,⁶⁸ both of which are BTKIs.

It is of special interest to highlight the important role of the high-affinity receptor FcεRI and its downstream signaling molecular cascade compared with that of IgE itself, which in our study has a more modest role. The efficacy of omalizumab might be derived from its ability to remove IgE bound to the receptor and in consequence inhibiting the signaling cascade beside sequestration of free IgE.

In our study, we were unable to distinguish between different CSU pathotypes based on the predominant autoantibody mechanisms, which can include anti-IgE autoantibodies of IgE and IgG serotypes, as well as IgG autoantibodies directed against FcεRI. Although our method has the potential for predicting treatment efficacy,⁶⁹ this function would depend largely on knowing what the predominant disease pathotype is for each patient. At present, given the lack of suitable biomarkers or preclinical models,²² there is limited utility for this method in the prediction of treatment responses. For example, although the results suggested that BTKIs are likely to provide fast and sustained therapeutic responses in CSU, our modeling could not predict which population of patients would benefit the most from any individual therapy. Nevertheless, the models are a useful starting point for deeper understanding of CSU pathophysiology and optimization of therapeutic options. For instance, given the number of overlapping treatment strategies between CSU and CindU, including anti-IgE,^{7,70} our modeling approach could be used to study therapeutic strategies in CindU. This was beyond the scope of our present study.

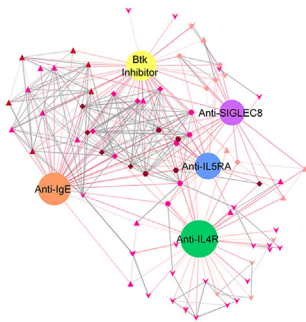
There are additional limitations of our study. The *in silico* modeling analyses are limited to the available scientific data about diseases and drugs/investigational compounds at the time of this study. Our modeling is based on the disease's protein interactome and literature-based protein effector description, which is a simplification of the inherently more complex pathophysiology of CSU.^{4,8,18} For instance, bias within the literature might lead to our models being predominantly non-histamine-dependent. However, most current work is devoted to finding therapeutic solutions for patients refractory to antihistamines, and thus, our models may still offer an interesting platform for further investigations. The validity of our modeling approach is supported by the fact that our findings reflect the functional alterations seen in expression data from skin biopsy samples from patients with CSU.^{44,45} We also determined that mast cell activation and

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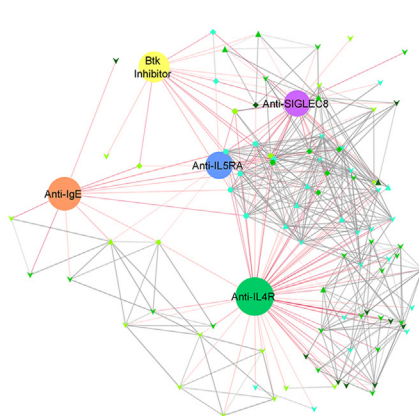


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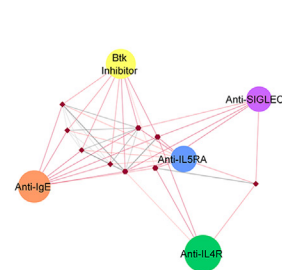
Innate Immune System



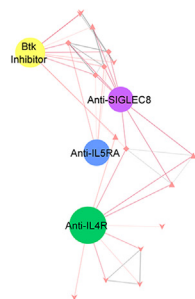
Adaptive Immune System



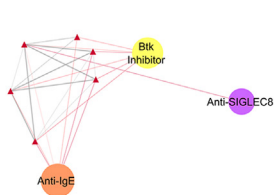
Mast cell activation and IgE signalling



Interferon-mediated response



Antigen processing and presentation



Adhesion and motility

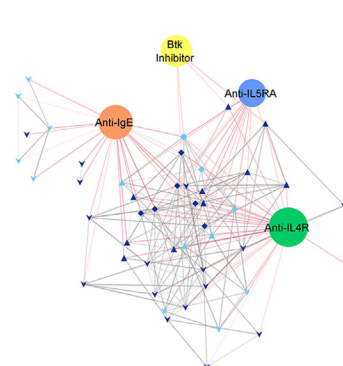
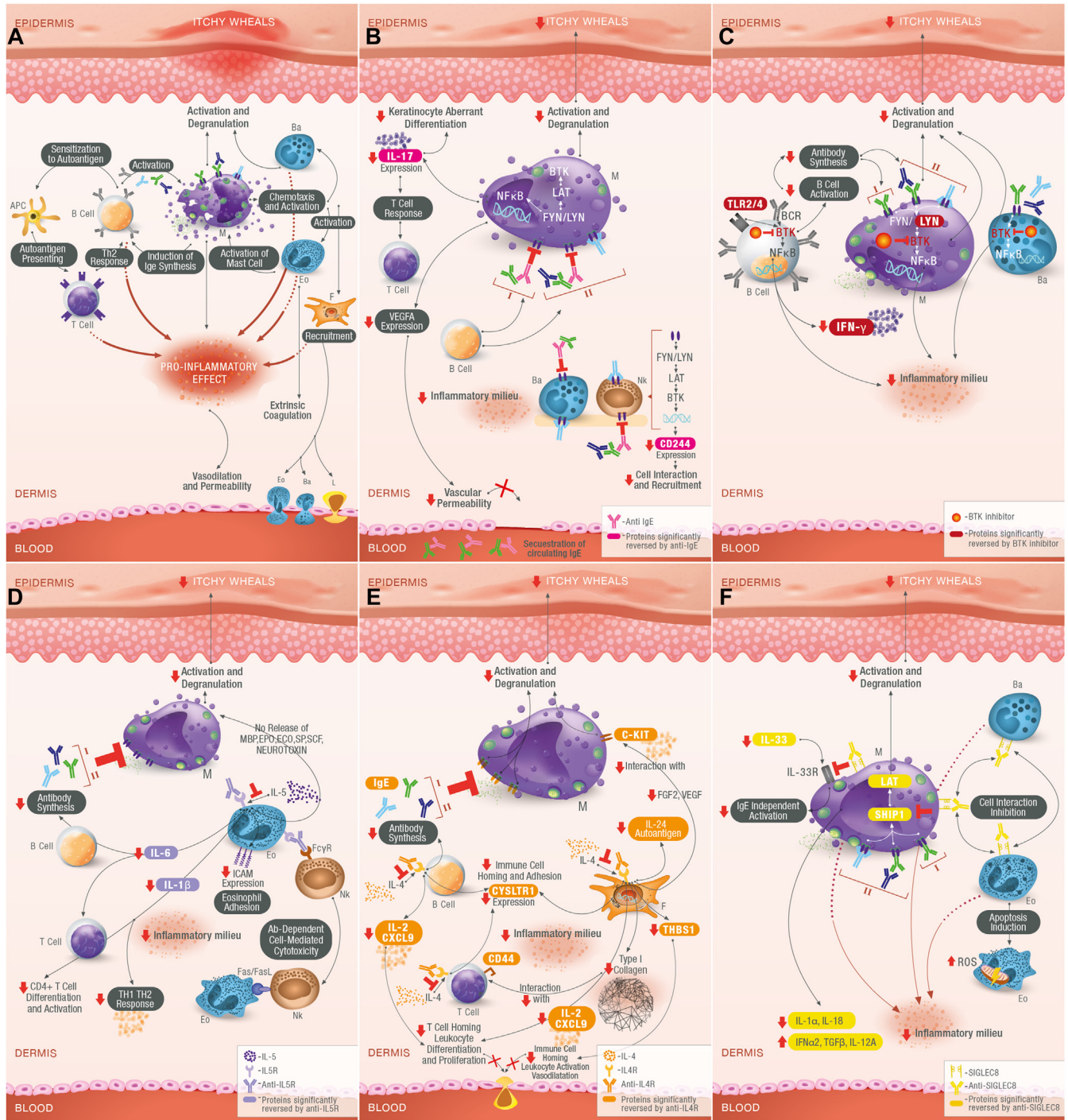


FIG 4. General evaluation of the processes modulated by each therapeutic mechanism. Hypergeometric enrichment analysis of the most modulated proteins ($|\text{predicted protein activity}| > .5$) in each therapeutic mechanism model. **A**, Overview of the mechanisms of the studied drugs representing common and differential signaling pathways of the most modulated processes per each drug mechanism of action. **B**, Networks displaying subsets of processes associated with CSU in the most modulated processes, divided into general (innate) immune system, adaptive immune system, mast cell activation and IgE signalling, antigen processing and presentation, interferon-mediated response, and adhesion and motility.



APC: antigen-presenting cells; **Eo:** eosinophil; **Ba:** basophil; **NK:** natural killer; **M:** mast cell; **L:** lymphocyte; **F:** fibroblast.

Y - IgG anti IgE | Y - IgG anti-FcERI | Y - IgE | O - FcERI | I, II - CSU endotype

FIG 5. Contextualized representation of the most differential mechanisms of action of each therapeutic strategy in the treatment of CSU, according to the evaluation of our models (see Fig E2-E6). **A**, Pathways underlying the pathophysiology of untreated CSU. Schematic representation of the predicted mechanism of action of anti-IgE (**B**), BTKIs (**C**), anti-IL5RA (**D**), anti-IL4R (**E**), and anti-Siglec-8 (**F**) to treat CSU. Details on the step-by-step predicted mechanisms are provided in Figs E2 to E6, and bibliographic information supporting each step can be found in Table E8 (in the Online Repository at www.jacionline.org).

degranulation is a key role of FcεRI in pathophysiology of the disease, which is in keeping with previous literature.^{4,18,19} The key role of FcεRI also explains the typical basophil activation features found in patients with CSU.^{15,60} Our model therefore has the potential to be a valid tool in the detailed investigation of disease mechanisms as well as in the assessment of novel therapies. New clinical data on different CSU therapies may, in turn, strengthen the validity of our model.

Despite its limitations, systems biology combined with new, high-throughput data, would be beneficial in the rapid expansion of our knowledge of CSU biology. Ultimately, this may lead to more effective, or even curative, treatments.

Conclusions

The systems biology-based models presented here successfully simulated the disease, highlighting the central role of mast cell activation and the IgE:FcεRI axis within CSU pathophysiology, which is in agreement with current knowledge of the disease.^{3,7-9} Although all of the evaluated therapies lead to a reduction of mast cell activation, each of them potentiates other downstream or upstream pathways, which allowed us to determine similarities and differences in their therapeutic mechanisms. Anti-IgE and BTKIs seemed to have a more direct role in mast cells' biology through abrogation of FcεRI signaling activity. On the other hand, the anti-interleukins and anti-Siglec-8 showed a dominant role in other immune responses, whereas BTKI was highlighted by its regulation of cellular immunity and B-cell biology. Our finding, although needing validation, could condition the design of preclinical and even clinical future investigations to achieve optimal treatment for patients with urticaria.

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Key messages

- Because CSU is a complex disease that is often poorly controlled, we computationally modeled its pathophysiology and the mechanism of action of different 68 therapeutic approaches.
- Mast cells were modeled to play a central role in the CSU process.
- Targeting the IgE:FcεRI axis and BTK appeared to be the preferred strategies to address this process; other strategies are suggested as being promising depending on the patient profile.

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