

Journal Pre-proof



Unveiling chronic spontaneous urticaria pathophysiology through systems biology

Cristina Segú-Vergés, MSc, Jessica Gómez, MSc, Pau Terradas, MSc., Laura Artigas, MSc, Serge Smeets, PhD, Marta Ferrer, MD, PhD, Sinisa Savic, MD, PhD

PII: S0091-6749(22)02576-3

DOI: <https://doi.org/10.1016/j.jaci.2022.12.809>

Reference: YMAI 15809

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 15 July 2022

Revised Date: 6 December 2022

Accepted Date: 20 December 2022

Please cite this article as: Segú-Vergés C, Gómez J, Terradas P, Artigas L, Smeets S, Ferrer M, Savic S, Unveiling chronic spontaneous urticaria pathophysiology through systems biology, *Journal of Allergy and Clinical Immunology* (2023), doi: <https://doi.org/10.1016/j.jaci.2022.12.809>.

This is a PDF file of an article that has undergone enhancements after acceptance, such as the addition of a cover page and metadata, and formatting for readability, but it is not yet the definitive version of record. This version will undergo additional copyediting, typesetting and review before it is published in its final form, but we are providing this version to give early visibility of the article. Please note that, during the production process, errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

© 2022 Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology.

Unveiling chronic spontaneous urticaria pathophysiology through systems biology

Cristina Segú-Vergés, MSc,^{a,b}, Jessica Gómez, MSc,^a, Pau Terradas, MSc.^c, Laura
Artigas, MSc,^a, Serge Smeets, PhD,^d, Marta Ferrer, MD, PhD,^{e,f*}, Sinisa Savic, MD,
PhD,^{g*}

^a Anaxomics Biotech, Carrer Diputació 237, 1^o, 1^a, 08007, Barcelona, Spain.

^b Research Programme on Biomedical Informatics (GRIB), Departament de Ciències
Experimentals i de la Salut, Universitat Pompeu Fabra, Barcelona, Spain.

^c Novartis, Gran Via de les Corts Catalanes, 764, 08013, Barcelona, Spain.

^d Novartis, Haaksbergweg, 1101 BX, Amsterdam, The Netherlands.

^e Department of Allergy and Clinical Immunology, Clínica Universidad de Navarra, Pio XII, 36,
31008, Pamplona, Spain

^f Instituto de investigación sanitaria de Navarra (IdISNA) 3Cooperative Research Network
Health Oriented (RICORS)

^g Department of Clinical Immunology and Allergy, Leeds Teaching Hospital NHS Trust, Leeds,
GB

* These authors contributed equally

Corresponding author:

Sinisa Savic

e-mail: S.Savic@leeds.ac.uk

Conflict of interest

MF has received honoraria (advisory board, speaker) from Genentech, Menarini, Uriach, FAES, and MSD; has received a Research Grant from GSK and Novartis; and advisory and speaker fees from Novartis. Non-Financial Interests: fellow of the AAAAI.

SiSa has received grants/research funding from Novartis, SOBI and CSL Behring; has served as speaker and/or on scientific advisory boards for Novartis, SOBI, and Takeda; has received honoraria for educational activities/consultancy/advisory role from Novartis, SOBI, and Takeda

Non-Financial Interests: none

SeSm and PT are full time employees at Novartis.

CSV, LA, JG and CSV and JG are full time employees at Anaxomics.

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; or in the drafting of the manuscript.

Funding

Both this research and medical writing, and the article processing fee (APC) was funded by Novartis

Abstract

Background: Chronic spontaneous urticaria (CSU) is a rare, heterogeneous, severely debilitating, and often poorly controlled skin disease resulting in an itchy eruption which can be persistent. Antihistamines and omalizumab, an anti-IgE monoclonal antibody, are the only licenced therapies. Although CSU pathogenesis is not yet fully understood, mast cell activation through the IgE:FcεRI (high affinity IgE receptor) 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 (TPMS) technology, based on systems biology and machine learning, was used to create a CSU interactome, validated with gene expression data from CSU patients, and a CSU model, which was used to evaluate CSU pathophysiology and the mechanism of action of different therapeutic strategies.

Results: Our models reflect the known role of mast cells activation as central process of CSU pathophysiology, as well as recognized roles for different therapeutic strategies over this and other innate and adaptive immune processes. They also allow determining similarities and differences between them: anti-IgE and Bruton tyrosine kinase (BTK) inhibitors present a more direct role on mast cell biology through abrogation of FcεRI signalling activity, while anti-interleukins and anti-Siglec-8 have a role on 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.

Capsule summary

In silico evaluation of the CSU pathophysiology highlights the role of mast cells and that targeting the IgE:FcεRI:BTK axis is the preferred strategy to address it; patient profile could determine benefit by other strategies.

Key words

machine learning, chronic spontaneous urticaria, system biology, artificial intelligence, mast cells

Key Messages

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

Abbreviations used

ANN: artificial neural networks
 BTKi: Bruton tyrosine kinase inhibitor
 CindU: chronic inducible urticaria
 CSU: chronic spontaneous urticaria
 FcεRI: high-affinity IgE receptor
 FcεRII (CD23): low-affinity IgE receptor
 IFNγ: interferon gamma
 IgE: immunoglobulin E
 IgG: immunoglobulin G
 IL-4: interleukin 4

- 94 IL4R: IL-4 receptor
- 95 IL-5: interleukin 5
- 96 IL-13: interleukin 13
- 97 ILR5A: IL-5 receptor
- 98 Siglec-8: sialic acid-binding immunoglobulin-like lectin 8
- 99 TLR: toll-like receptors
- 100 TPMS: therapeutic performance mapping system.
- 101

Introduction

Chronic urticaria is a common skin disorder with heterogeneous 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 two 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 which causes significant deterioration in quality of life and has a substantial impact on health care systems.⁹ Prevalence is estimated at between 0.5 to 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 proven 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 CSU patients.^{4,17-20} Autoimmunity is thought to be a driving factor in CSU, involving IgG and IgE autoantibodies as well as FcεRI (high-affinity IgE receptor).²¹⁻²³ However, only a small subset of patients meets all 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 (BTKi), 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 monoclonal antibody which prevents binding of IgE heavy chain to FcεRI or FcεRII (CD23, low-affinity IgE receptor), has proven to be effective in CSU^{19,26} although around 15-20% of patients are non-responders,^{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 the response rate is much higher.³² New treatment options are therefore needed to account for the diversity of patient response in

128 this disease. Moreover, the exact mechanism by which omalizumab provides relief remains
129 unclear.^{13,18}

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

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

Methods

Gene expression data compilation and treatment

CSU gene expression data from human patients was identified in Gene Expression Omnibus (GEO) public repository,⁴³ 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])”, on August 26th, 2020. The data was filtered by organism (*Homo Sapiens*), entry type (Series), experiment type (*Expression profiling by array*, *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, using GEO2R software⁴⁶ with default settings. Significance criteria were set at false discovery rate (FDR) < 0.05 and absolute value of logarithm of fold change ($|\log FC|$) > 1. Genes were considered as differentially expressed when at least one 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. Seventy results were retrieved using these criteria (Supplementary Table 1 in File S1).

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 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]). Eighty-six articles were retrieved, and reviewed at the abstract or full-length level, if containing molecular information on CSU pathophysiology. We performed

handsearching 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 pathophysiological processes (motives) and the proteins involved in disease pathophysiology (effectors), considering only proteins for which functional involvement in the disease was found. The characterisation was used as base to build the protein network, or interactome, around the disease.

Bibliography-based therapeutic strategies molecular characterisation

Afterwards, 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 lirentelimab (anti-Siglec-8). The drug molecular characterization comprised identification of drug targets according to official and publicly available documents (EMA and FDA), specialized databases (DrugBank,^{47,48} Stitch⁴⁹ and Supertarget⁵⁰) and available literature in PubMed up to August 31st, 2020, as previously reported.^{34,37,39} The list of therapeutic strategies evaluated is shown in Supplementary Table 2 in File S1.

Therapeutic performance mapping system modelling

TPMS technology is based on systems biology, machine learning and pattern recognition techniques that integrate all available biological, pharmacological, and medical knowledge (training set, Supplementary Table 2 in File S1) to create mathematical models that simulate 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 modelling approaches were used (Figure 1): artificial neural networks^{34,37} and sampling-based methods.^{37,39} The database-based human protein network used for TPMS model construction³³ was used to expand the CSU characterization and to create the CSU interactome. The sampling-based methods models allow exploration of the predicted activity for

each protein (ranging between [-1,1]) within the models, and identify the most frequent pathways occurring between a stimulus and the biological process definition. When a biological process is defined (i.e., 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 biological process.³⁹ Skin-derived gene expression information (Supplementary Table 1 in File S1) was included in the model alongside the TPMS default training information (Supplementary Table 3 in File S1).³⁹

Statistical analyses and results visualization

Statistical analyses were carried out using sampling-based methods, parametric and non-parametric comparison tests model-derived protein predicted activity, and hypergeometric enrichment analysis. Multidimensional scaling and Cytoscape software⁵¹ were used for visualisation of results. See Supplementary Methods in File S1 for further details.

Results

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

Disease characterization enabled the identification of 129 unique CSU effector proteins, categorized into five motives: activation of the coagulation and complement system, 25 proteins; auto allergic and autoimmune triggering, 7 proteins; mast cell activation and degranulation, 33 proteins; granulocyte homing to skin and activation, 43 proteins; humoral mediators of inflammation and endothelial changes, 43 proteins (Supplementary Table 4 in File S1).

The CSU interactome (Supplementary Figure S1 in File S2), containing CSU effectors and directly related proteins according to the human protein network, included 41.43% of the genes with altered expression in CSU patients skin samples (Supplementary Table 1 in File S1) and 73.41% of the enriched processes in the expression data (Supplementary Table 5 in File S1). These enriched processes represent 60.68% of the interactome and include 127 out of 129 CSU protein effectors. These data suggest that the molecular CSU definition used reflects to a great extent the functional aspects observed in CSU skin-derived expression data, providing grounds for its use in modelling.

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

Mathematical models simulating chronic spontaneous urticaria were built around the CSU interactome, 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 (Figure 2, network obtained applying default setting of *prefuse force directed* layout in Cytoscape software with T-Signal values).

Triggering analysis of the CSU effectors over the models also showed that the high affinity receptor FcεRI and its downstream signalling molecular cascade (including BTK) has a role in inducing mast cell activation and degranulation motives (Figure 3A). 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, falls in the top twenty of evaluated CSU-related proteins. IL13R and Siglec-8 also demonstrated some triggering potential (Figure 3A). Interleukin receptors seem to have a more relevant role than the ligands themselves, in a similar way to the IgE:FcεRI results. We found a modest role for H₁ receptor (H₁R) 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), focussing on mechanisms behind the modulation of FcεRI, Btk, IL5RA, IL4R and Siglec8. All these targets are modelled to have a measurable, although apparently different impact over mast cell degranulation (Figure 3B-E).

Different therapeutic approaches demonstrate modulation of different processes within CSU

In order to identify the specific processes of CSU in which the therapeutic strategies under study may be involved, the relationship between the therapeutic approaches and each of the CSU motives was evaluated using artificial neural network analyses. This analysis provides a predictive score that quantifies the probability of the existence of a functional relationship between protein sets or regions inside Anaxomics' network. All evaluated drugs were predicted to be related to CSU, although through different mechanisms (Table 1).

According to the artificial neural network analysis, all therapeutic strategies show a high probability of relationship ($p < 0.05$) to at least one motive. Anti-IgE and BTKi present the most probable relationship ($p < 0.05$) with mast cell activation and degranulation. Anti-Siglec-8 ($p <$

0.05), anti-IL4R ($p < 0.05$) and anti-IL5RA ($p < 0.01$) present the most probable relationship with granulocyte homing to skin and granulocyte activation. Anti-IL4R, both through the IL-4 and the IL-13 axis, and anti-IL5RA ($p < 0.05$) present the most probable relationship with the motive involving humoral mediators of inflammation and endothelial changes. Among the studied therapeutic strategies, the ones demonstrating the greatest role in auto-allergic and autoimmune triggering are anti-IL4R and anti-IL5RA, although the relationship does not achieve significance ($p\text{-value} < 0.1$).

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 ($|\text{predicted protein activity}| > 0.5$) were analysed by hypergeometric enrichment analysis to identify enriched pathways within the proteins most modulated by each drug (Figure 4A, Supplementary Table 6 in File S1). According to our models, all the therapeutic strategies were found to modulate proteins enriched in mast cell activation (Figure 4B), 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 (Figure 4B). However, anti-IgE and BTKi appear to have a greater effect on mast cell biology, and appear to modulate antigen processing and presentation, a key link between the innate and adaptive immune systems (Figure 4B).

In summary, all drugs modulate similar processes involved in controlling CSU pathology. However, they show differences which 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 on predicted protein activity were evaluated among each of the therapeutic target models (Supplementary Table 7 in File S1). While Figure 5 contextualizes the differential mechanisms detected for each therapeutic strategy

in CSU, Supplementary Figures S2-S6 in File S2 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 VEGF (Supplementary Figure S2 in File S2). BTKi do not only inhibit mast cells activation cascade, but also prevents toll-like receptor (TLR) signalling mechanisms, which are involved in B-cell activation, and modulation of other immune mediators, such as IFN γ (Supplementary Figure S3 in File S2). Anti-IL5RA therapeutics have specific additional effects on eosinophil death, preventing their role in the pathogenesis of CSU, and reducing their contribution to the pro-inflammatory milieu (Supplementary Figure S4 in File S2); in fact, key pro-inflammatory interleukins (IL-6 or IL-1 β) 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 pathological processes: reduction of IgE production from B-cells, reduction of the expression of molecules that promote immune cell recruitment, inflammation, and mast cell activation, including interleukins IL2 and IL24, chemokine CXCL9, pro-inflammatory factors (thrombospondin and leukotriene receptor) and IFN γ -mediated signalling (probably via IL-13) (Supplementary Figure S5 in File S2). 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 intracellular IgE- or IL-33-induced mast cell activation cascade, modulation of the pro/anti-inflammatory equilibrium (IL12, IL18, IL-1 α), and triggering of eosinophil apoptosis via oxidative stress induction (NADPH oxidase, p38) (Supplementary Figure S6 in File S2). In conclusion, although all therapeutic strategies lead to a reduction of mast cell activation, each does so through a different 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 H₁R was found to be less important, in keeping with its indirect activation of mast cells⁵² and the clinical observation that many patients are anti-histamine unresponsive.⁵³

The models also show that FcεRI has a more important role in CSU-associated mast cell activation compared to IgE itself, or to CD23. These results are supported by several pieces of evidence. Firstly, omalizumab is able to directly inhibit basophils and mast cell degranulation via its effects on FcεRI.⁵⁴ Secondly, some patients do not show benefit from treatment with anti-IgE⁵⁵ and this is associated with lower IgE levels, suggesting a role for type IIb autoimmunity involving IgG autoantibodies towards FcεRI.^{56,57} Thirdly, 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,⁵⁹ abnormally high FcεRI expression⁶⁰ and desensitization of the FcεRI pathway.⁶¹ These features are all reversible upon CSU remission.⁶² As well as known targets of currently approved treatments,⁶³ our model highlighted proteins targeted by molecules that are currently under investigation and which we therefore selected for *in silico* comparison. These included PI3K⁶⁴ or the topical Syk inhibitor⁶⁵. 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 BTKi on mast cell degranulation, it is possible that

these drugs can induce more rapid responses in CSU patients compared to anti-IL4R and anti-IL5RA. This hypothesis is supported by the efficacy of agents as benralizumab which is an anti-IL5RA^{66,67}, and fenebrutinib⁶⁸ or remibrutinib⁶⁹, both BTKi.

It is of special interest to highlight the important role of the high affinity receptor FcεRI and its downstream signalling molecular cascade compared with IgE itself that 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 inhibiting and in consequence inhibiting the signalling 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 efficacy⁷⁰, 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 pre-clinical models,²² there is limited utility for this method in the prediction of treatment responses. For example, whilst the results suggested that BTKi are likely to provide fast and sustained therapeutic responses in CSU, our modelling could not predict which population of patients would benefit the most from any individual therapy. Nevertheless, the models are a useful starting point for a deeper understanding of CSU pathophysiology and an optimisation of therapeutic options. For instance, given the number of overlapping treatment strategies between CSU and CindU, including anti-IgE,^{7,71} our modelling 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* modelling analyses are limited to the available scientific data about diseases and drugs/investigational compounds at the time of this study. Our modelling 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 modelling approach is supported by the fact that our findings reflect the functional alterations seen in expression data from the skin biopsies of CSU patients.^{44,45} We also identified that mast cell activation and degranulation is a key role of FcεRI in pathophysiology of the disease, 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.^{59,61} Our model therefore has the potential to be a valid tool in the detailed investigation of disease mechanisms as well as 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 IgE:FcεRI axis within CSU pathophysiology, which is in agreement with the current knowledge of the disease.^{3,7-9} Although all 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 on their therapeutic mechanisms. Anti-IgE and BTKi seemed to have a more direct role on mast cells biology through abrogation of FcεRI signalling activity. On the other hand, the anti-interleukins and anti-Siglec-8 showed a dominant role on other immune responses, while BTKi was highlighted by its regulation of cellular immunity and B-cell biology. Our finding, while needing validation, could condition the design of pre-clinical and even clinical future investigations to achieve optimal treatment for urticaria patients.

Acknowledgements

The authors would like to thank José Luis Ruiz (Anaxomics Biotech, Barcelona) for their assistance in results visualization; José Luis Ramírez (medical writer) and Ada Repiso (Anaxomics Biotech, Barcelona) for providing writing and editorial support; and Hugo Somoza (Anaxomics Biotech, Barcelona) and Enric Ivern (VMLY&Rx, Barcelona) for figure assistance. SiSa, MF and LA conceptualized the study. CSV and JG performed the investigation. SiSa, MF and CSV conducted the data curation and formal analyses. SeSM, PT and JG administered the project. SeSm, PT and LA provided the resources. SeSm, MF, SiSa and LA supervised the project. CSV, JG, SiSa and MF drafted the first version of the manuscript and all authors reviewed and edited its final version.

References

1. Zuberbier T, Abdul Latiff AH, Abuzakouk M, Aquilina S, Asero R, Baker D, et al. The international EAACI/GA²LEN/EuroGuiDerm/APAAACI guideline for the definition, classification, diagnosis, and management of urticaria. *Allergy*. 2022;77(3):734-66.
2. Maurer M, Hawro T, Krause K, Magerl M, Metz M, Siebenhaar F, et al. Diagnosis and treatment of chronic inducible urticaria. *Allergy*. 2019;74(12):2550-3.
3. Kocatürk E, Zuberbier T. New biologics in the treatment of urticaria. *Curr Opin Allergy Clin Immunol*. 2018;18(5):425-31.
4. Maurer M, Eyerich K, Eyerich S, Ferrer M, Guterthum J, Hartmann K, et al. Urticaria: Collegium Internationale Allergologicum (CIA) Update 2020. *Int Arch Allergy Immunol*. 2020;181(5):321-33.
5. Maurer M, Weller K, Bindslev-Jensen C, Giménez-Arnau A, Bousquet PJ, Bousquet J, et al. Unmet clinical needs in chronic spontaneous urticaria. A GA²LEN task force report. *Allergy*. 2011;66(3):317-30.
6. Zhao ZT, Ji CM, Yu WJ, Meng L, Hawro T, Wei JF, et al. Omalizumab for the treatment of chronic spontaneous urticaria: A meta-analysis of randomized clinical trials. *J Allergy Clin Immunol*. 2016;137(6):1742-50.e4.
7. Maurer M, Schütz A, Weller K, Schoepke N, Peveling-Oberhag A, Staubach P, et al. Omalizumab is effective in symptomatic dermographism-results of a randomized placebo-controlled trial. *J Allergy Clin Immunol*. 2017;140(3):870-3.e5.
8. Kaplan AP. Chronic Spontaneous Urticaria: Pathogenesis and Treatment Considerations. *Allergy Asthma Immunol Res*. 2017;9(6):477-82.
9. O'Donnell BF. Urticaria: impact on quality of life and economic cost. *Immunol Allergy Clin North Am*. 2014;34(1):89-104.
10. Gaig P, Olona M, Muñoz Lejarazu D, Caballero MT, Domínguez FJ, Echechipia S, et al. Epidemiology of urticaria in Spain. *J Investig Allergol Clin Immunol*. 2004;14(3):214-20.

- 438 11. Lapi F, Cassano N, Pegoraro V, Cataldo N, Heiman F, Cricelli I, et al. Epidemiology of
 439 chronic spontaneous urticaria: results from a nationwide, population-based study in
 440 Italy. *Br J Dermatol*. 2016;174(5):996-1004.
- 441 12. Zuberbier T, Balke M, Worm M, Edenharter G, Mauer M. Epidemiology of urticaria: a
 442 representative cross-sectional population survey. *Clin Exp Dermatol*. 2010
 443 Dec;35(8):869-73.
- 444 13. Ferrer M. Immunological events in chronic spontaneous urticaria. *Clin Transl Allergy*.
 445 2015;5:30.
- 446 14. Saini SS, Kaplan AP. Chronic Spontaneous Urticaria: The Devil's Itch. *J Allergy Clin*
 447 *Immunol Pract*. 2018 Jul-Aug;6(4):1097-106.
- 448 15. Luquin E, Kaplan AP, Ferrer M. Increased responsiveness of basophils of patients with
 449 chronic urticaria to sera but hypo-responsiveness to other stimuli. *Clin Exp Allergy*.
 450 2005;35(4):456-60.
- 451 16. Niimi N, Francis DM, Kermani F, O'Donnell BF, Hide M, Kobza-Black A, et al.
 452 Dermal mast cell activation by autoantibodies against the high affinity IgE receptor in
 453 chronic urticaria. *J Invest Dermatol*. 1996;106(5):1001-6.
- 454 17. Ying S, Kikuchi Y, Meng Q, Kay AB, Kaplan AP. TH1/TH2 cytokines and
 455 inflammatory cells in skin biopsy specimens from patients with chronic idiopathic
 456 urticaria: comparison with the allergen-induced late-phase cutaneous reaction. *J Allergy*
 457 *Clin Immunol*. 2002;109(4):694-700.
- 458 18. Bracken SJ, Abraham S, MacLeod AS. Autoimmune Theories of Chronic Spontaneous
 459 Urticaria. *Front Immunol*. 2019;10:627.
- 460 19. Kolkhir P, Altrichter S, Munoz M, Hawro T, Mauer M. New treatments for chronic
 461 urticaria. *Ann Allergy Asthma Immunol*. 2020;124(1):2-12.
- 462 20. Giménez-Arnau AM, DeMontojoye L, Asero R, Cugno M, Kulthanan K, Yanase Y, et
 463 al. The Pathogenesis of Chronic Spontaneous Urticaria: The Role of Infiltrating Cells. *J*
 464 *Allergy Clin Immunol Pract*. 2021;9(6):2195-208.

21. Ferrer M, Kin  t JP, Kaplan AP. Comparative studies of functional and binding assays for IgG anti-Fc(epsilon)RIalpha (alpha-subunit) in chronic urticaria. *J Allergy Clin Immunol*. 1998;101(5):672-6.
22. Konstantinou GN, Asero R, Ferrer M, Knol EF, Maurer M, Raap U, et al. EAACI taskforce position paper: evidence for autoimmune urticaria and proposal for defining diagnostic criteria. *Allergy*. 2013;68(1):27-36.
23. Yu L, Buttgereit T, Stahl Skov P, Schmetzer O, Scheffel J, Kocat  r E, et al. Immunological effects and potential mechanisms of action of autologous serum therapy in chronic spontaneous urticaria. *J Eur Acad Dermatol Venereol*. 2019;33(9):1747-54.
24. Schoepke N, Asero R, Ellrich A, Ferrer M, Gimenez-Arnau A, Grattan CEH, et al. Biomarkers and clinical characteristics of autoimmune chronic spontaneous urticaria: Results of the PURIST Study. *Allergy*. 2019;74(12):2427-36.
25. Altrichter S, Staubach P, Pasha M, Singh B, Chang AT, Bernstein JA, et al. An open-label, proof-of-concept study of liletelimab for antihistamine-resistant chronic spontaneous and inducible urticaria. *J Allergy Clin Immunol*. 2022;149(5):1683-90.e7.
26. Maurer M, Ros  n K, Hsieh HJ, Saini S, Grattan C, Gim  nez-Arnau A, et al. Omalizumab for the treatment of chronic idiopathic or spontaneous urticaria. *N Engl J Med*. 2013;368(10):924-35.
27. Kaplan A, Ferrer M, Bernstein JA, Antonova E, Trzaskoma B, Raimundo K, et al. Timing and duration of omalizumab response in patients with chronic idiopathic/spontaneous urticaria. *J Allergy Clin Immunol*. 2016;137(2):474-81.
28. Ferrer M, Boccon-Gibod I, Gon  alo M,   nal  z HS, Knulst A, Lapeere H, et al. Expert opinion: defining response to omalizumab in patients with chronic spontaneous urticaria. *Eur J Dermatol*. 2017;27(5):455-63.
29. Asero R. Omalizumab in severe chronic urticaria: are slow and non-responders different? *Eur Ann Allergy Clin Immunol*. 2021;53(6):263-6.

30. Gericke J, Metz M, Ohanyan T, Weller K, Altrichter S, Skov PS, et al. Serum autoreactivity predicts time to response to omalizumab therapy in chronic spontaneous urticaria. *J Allergy Clin Immunol*. 2017;139(3):1059-61.e1.
31. Casale TB, Bernstein JA, Maurer M, Saini SS, Trzaskoma B, Chen H, et al. Similar Efficacy with Omalizumab in Chronic Idiopathic/Spontaneous Urticaria Despite Different Background Therapy. *J Allergy Clin Immunol Pract*. 2015;3(5):743-50.e1.
32. Tharp MD, Bernstein JA, Kavati A, Ortiz B, MacDonald K, Denhaerynck K, et al. Benefits and Harms of Omalizumab Treatment in Adolescent and Adult Patients With Chronic Idiopathic (Spontaneous) Urticaria: A Meta-analysis of "Real-world" Evidence. *JAMA Dermatol*. 2019;155(1):29-38.
33. Lorén V, Garcia-Jaraquemada A, Naves JE, Carmona X, Mañosa M, Aransay AM, et al. ANP32E, a Protein Involved in Steroid-Refractoriness in Ulcerative Colitis, Identified by a Systems Biology Approach. *J Crohns Colitis*. 2019;13(3):351-61.
34. Artigas L, Coma M, Matos-Filipe P, Aguirre-Plans J, Farrés J, Valls R, et al. In-silico drug repurposing study predicts the combination of pirfenidone and melatonin as a promising candidate therapy to reduce SARS-CoV-2 infection progression and respiratory distress caused by cytokine storm. *PLoS One*. 2020;15(10):e0240149.
35. Carcereny E, Fernández-Nistal A, López A, Montoto C, Naves A, Segú-Vergés C, et al. Head to head evaluation of second generation ALK inhibitors brigatinib and alectinib as first-line treatment for ALK+ NSCLC using an in silico systems biology-based approach. *Oncotarget*. 2021;12(4):316-32.
36. Akil H, Gordon J, Hen R, Javitch J, Mayberg H, McEwen B, et al. Treatment resistant depression: A multi-scale, systems biology approach. *Neurosci Biobehav Rev*. 2018;84:272-88.
37. Segú-Vergés C, Coma M, Kessel C, Smeets S, Foell D, Aldea A. Application of systems biology-based in silico tools to optimize treatment strategy identification in Still's disease. *Arthritis Res Ther*. 2021;23(1):126.

38. Davis JD, Kumbale CM, Zhang Q, Voit EO. Dynamical systems approaches to personalized medicine. *Curr Opin Biotechnol.* 2019;58:168-74.
39. Jorba G, Aguirre-Plans J, Junet V, Segú-Vergés C, Ruiz JL, Pujol A, et al. In-silico simulated prototype-patients using TPMS technology to study a potential adverse effect of sacubitril and valsartan. *PLoS One.* 2020;15(2):e0228926.
40. Iborra-Egea O, Santiago-Vacas E, Yurista SR, Lupón J, Packer M, Heymans S, et al. Unraveling the Molecular Mechanism of Action of Empagliflozin in Heart Failure With Reduced Ejection Fraction With or Without Diabetes. *JACC Basic Transl Sci.* 2019;4(7):831-40.
41. Villalba A, Rodriguez-Fernandez S, Perna-Barrull D, Ampudia RM, Gomez-Muñoz L, Pujol-Autonell I, et al. Repurposed Analog of GLP-1 Ameliorates Hyperglycemia in Type 1 Diabetic Mice Through Pancreatic Cell Reprogramming. *Front Endocrinol (Lausanne).* 2020;11:258.
42. Herrando-Grabulosa M, Mulet R, Pujol A, Mas JM, Navarro X, Aloy P, et al. Novel Neuroprotective Multicomponent Therapy for Amyotrophic Lateral Sclerosis Designed by Networked Systems. *PLoS One.* 2016;11(1):e0147626.
43. Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. *Nucleic Acids Res.* 2002;30(1):207-10.
44. Giménez-Arnau A, Curto-Barredo L, Nonell L, Puigdecanet E, Yelamos J, Gimeno R, et al. Transcriptome analysis of severely active chronic spontaneous urticaria shows an overall immunological skin involvement. *Allergy.* 2017;72(11):1778-90.
45. Patel OP, Giorno RC, Dibbern DA, Andrews KY, Durairaj S, Dreskin SC. Gene expression profiles in chronic idiopathic (spontaneous) urticaria. *Allergy Rhinol (Providence).* 2015;6(2):101-10.
46. Barrett T, Troup DB, Wilhite SE, Ledoux P, Evangelista C, Kim IF, et al. NCBI GEO: archive for functional genomics data sets--10 years on. *Nucleic Acids Res.* 2011;39(Database issue):D1005-10.

- 545 47. Wishart DS, Knox C, Guo AC, Shrivastava S, Hassanali M, Stothard P, et al.
 546 DrugBank: a comprehensive resource for in silico drug discovery and exploration.
 547 Nucleic Acids Res. 2006;34(Database issue):D668-72.
- 548 48. Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR, et al. DrugBank 5.0: a
 549 major update to the DrugBank database for 2018. Nucleic Acids Res.
 550 2018;46(D1):D1074-82.
- 551 49. Szklarczyk D, Santos A, von Mering C, Jensen LJ, Bork P, Kuhn M. STITCH 5:
 552 augmenting protein-chemical interaction networks with tissue and affinity data. Nucleic
 553 Acids Res. 2016;44(D1):D380-4.
- 554 50. Günther S, Kuhn M, Dunkel M, Campillos M, Senger C, Petsalaki E, et al. SuperTarget
 555 and Matador: resources for exploring drug-target relationships. Nucleic Acids Res.
 556 2008;36(Database issue):D919-22.
- 557 51. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a
 558 software environment for integrated models of biomolecular interaction networks.
 559 Genome Res. 2003;13(11):2498-504.
- 560 52. Carlos D, Sá-Nunes A, de Paula L, Matias-Peres C, Jamur MC, Oliver C, et al.
 561 Histamine modulates mast cell degranulation through an indirect mechanism in a model
 562 IgE-mediated reaction. Eur J Immunol. 2006;36(6):1494-503.
- 563 53. Guillén-Aguinaga S, Jáuregui Presa I, Aguinaga-Ontoso E, Guillén-Grima F, Ferrer M.
 564 Updosing nonsedating antihistamines in patients with chronic spontaneous urticaria: a
 565 systematic review and meta-analysis. Br J Dermatol. 2016;175(6):1153-65.
- 566 54. Serrano-Candelas E, Martinez-Aranguren R, Valero A, Bartra J, Gastaminza G,
 567 Goikoetxea MJ, et al. Comparable actions of omalizumab on mast cells and basophils.
 568 Clin Exp Allergy. 2016;46(1):92-102.
- 569 55. Weller K, Ohanyan T, Hawro T, Ellrich A, Sussman G, Koplowitz J, et al. Total IgE
 570 levels are linked to the response of chronic spontaneous urticaria patients to
 571 omalizumab. Allergy. 2018;73(12):2406-8.

56. Altrichter S, Fok JS, Jiao Q, Kolkhir P, Pyatlova P, Romero SM, et al. Total IgE as a Marker for Chronic Spontaneous Urticaria. *Allergy Asthma Immunol Res.* 2021;13(2):206-18.
57. Kolkhir P, Church MK, Weller K, Metz M, Schmetzer O, Maurer M. Autoimmune chronic spontaneous urticaria: What we know and what we do not know. *J Allergy Clin Immunol.* 2017;139(6):1772-81.e1.
58. Deza G, March-Rodríguez A, Sánchez S, Ribas-Llauradó C, Soto D, Pujol RM, et al. Relevance of the Basophil High-Affinity IgE Receptor in Chronic Urticaria: Clinical Experience from a Tertiary Care Institution. *J Allergy Clin Immunol Pract.* 2019;7(5):1619-26.e1.
59. Luquin E, Kaplan AP, Ferrer M. Increased responsiveness of basophils of patients with chronic urticaria to sera but hypo-responsiveness to other stimuli. *Clin Exp Allergy.* 2005;35(4):456-60.
60. Lourenço FD, Azor MH, Santos JC, Prearo E, Maruta CW, Rivitti EA, et al. Activated status of basophils in chronic urticaria leads to interleukin-3 hyper-responsiveness and enhancement of histamine release induced by anti-IgE stimulus. *Br J Dermatol.* 2008;158(5):979-86.
61. Sabroe RA, Francis DM, Barr RM, Black AK, Greaves MW. Anti-Fc(epsilon)RI auto antibodies and basophil histamine releasability in chronic idiopathic urticaria. *J Allergy Clin Immunol.* 1998;102(4 Pt 1):651-8.
62. Oliver ET, Sterba PM, Saini SS. Interval shifts in basophil measures correlate with disease activity in chronic spontaneous urticaria. *Allergy.* 2015;70(5):601-3.
63. Johal KJ, Saini SS. Current and emerging treatments for chronic spontaneous urticaria. *Ann Allergy Asthma Immunol.* 2020;125(4):380-7.
64. Vadasz Z, Toubi E. New Biological Treatment Options in CSU. In: Papakonstantinou E, editor. *Urticaria – Diagnosis and Management.* London: IntechOpen; 2021.
65. Dickson MC, Walker A, Grattan C, Perry H, Williams N, Ratia N, et al. Effects of a topical treatment with spleen tyrosine kinase inhibitor in healthy subjects and patients

- with cold urticaria or chronic spontaneous urticaria: Results of a phase 1a/b randomised double-blind placebo-controlled study. *Br J Clin Pharmacol*. 2021;87(12):4797-808.
66. 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):1277-80.
67. Bernstein JA, Singh U, Rao MB, Berendts K, Zhang X, Mutasim D. Benralizumab for Chronic Spontaneous Urticaria. *N Engl J Med*. 2020;383(14):1389-91.
68. Metz M, Sussman G, Gagnon R, Staubach P, Tanus T, Yang WH, et al. Fenebrutinib in H1 antihistamine-refractory chronic spontaneous urticaria: a randomized phase 2 trial. *Nat Med*. 2021;27(11):1961-9.
69. Kaul M, End P, Cabanski M, Schuhler C, Jakab A, Kistowska M, et al. Remibrutinib (LOU064): A selective potent oral BTK inhibitor with promising clinical safety and pharmacodynamics in a randomized phase I trial. *Clin Transl Sci*. 2021;14(5):1756-68.
70. Gutiérrez-Casares JR, Quintero J, Jorba G, Junet V, Martínez V, Pozo-Rubio T, et al. Methods to Develop an in silico Clinical Trial: Computational Head-to-Head Comparison of Lisdexamfetamine and Methylphenidate. *Front Psychiatry*. 2021 Nov 3;12:741170.
71. Chicharro P, Rodríguez P, de Argila D. Omalizumab in the Treatment of Chronic Inducible Urticaria. *Actas Dermosifiliogr*. 2017;108(5):423-31.

Tables

Table 1. Artificial neural network (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%)

Motives: 1) Mast cell activation and degranulation; 2) Auto allergic 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 0-100%).

++++ p-value < 0.01; +++ p-value < 0.05; ++ p-value < 0.1; + p-value < 0.2; - p-value > 0.2

Abbreviations: BTK, Bruton tyrosine kinase; FCGR3A, low affinity immunoglobulin gamma Fc region receptor III-A; IgE, immunoglobulin E; IL, interleukin; IL4R, IL-4 receptor; IL5RA, IL-5 receptor; IL13R, IL-13 receptor; Siglec-8, sialic acid-binding Ig-like lectin 8.

Figure Legends

Figure 1. Overview of analysis methodology. Data compilation and *in silico* modelling were used to characterize the molecular pathways involved in CSU therapies. Two modelling approaches were used (step 3): A) artificial neural networks (ANN),^{34,37} an algorithm able to detect biological relationships between drug or drug targets and CSU-related processes through topological measures, providing a score (from 0 to 100%) associated with a probability (p-value) of positive functional relationship between the sets of proteins, with accuracy against the training set >80%; and, B) sampling-based methods,^{37,39} which generate models similar to a Multilayer Perceptron of an ANN over the human protein network, able to explain biological relationships by generating a universe of plausible solutions, prioritising those more probable form from a mathematical and biological point of view. Abbreviations: ANN, artificial neural

network; BTKi, Bruton tyrosine kinase inhibitor; CSU, chronic spontaneous urticaria; IgE, immunoglobulin E; IL, interleukin; IL4R, IL-4 receptor; IL5RA, IL-5 receptor; Siglec8, sialic acid-binding Ig-like lectin 8.

Figure 2. Network displaying T-Signal induced to each CSU motive by each other. *Prefuse force directed* layout show mast cell activation centrality within the motive network. Node size indicated the number of proteins involved. Arrow size indicates the value of T-Signal obtained in each motive-motive relationship. Node colour indicates the mean T-Signal obtained for each motive considering all motive-motive relationships (the darker, the more stimulated the motive by the rest of the motives).

Figure 3. Evaluation of mast cell activation triggering potential of CSU-related proteins. A) Top20 proteins; results for other proteins used and suggested as therapeutic targets for CSU are also displayed for informative purposes. B-F) Multi-dimensional scaling (MDS) representation of 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 considering 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 and which mast cell activation and degranulation effectors modulates.

Figure 4. General evaluation of the processes modulated by each therapeutic mechanism. Hypergeometric enrichment analysis of the most modulated proteins ($|\text{predicted protein activity}| > 0.5$) in each therapeutic mechanism model. A) Overview of the mechanisms of the studied drugs representing common and differential signalling pathways of most modulated processes per each drug mechanism of action. B) Figures displaying subsets of processes associated to CSU in 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.

Figure 5. Contextualized representation of the most differential mechanisms of action of each therapeutic strategy in the treatment of CSU, according to our models evaluation (Supplementary Figures S2-S6 in File S2). A) Pathways underlying the pathophysiology of untreated CSU. Schematic representation of predicted mechanism of action of B) anti-IgE, C) BTKi, D) anti-IL5RA, E) anti-IL4R and F) anti-Siglec8 over CSU. Detail on the step-by-step predicted mechanisms is provided in Supplementary Figures S2-S6 in File S2, and

680 bibliographical information supporting each step can be found on Supplementary Table 8 in File
681 S1.
682

Journal Pre-proof

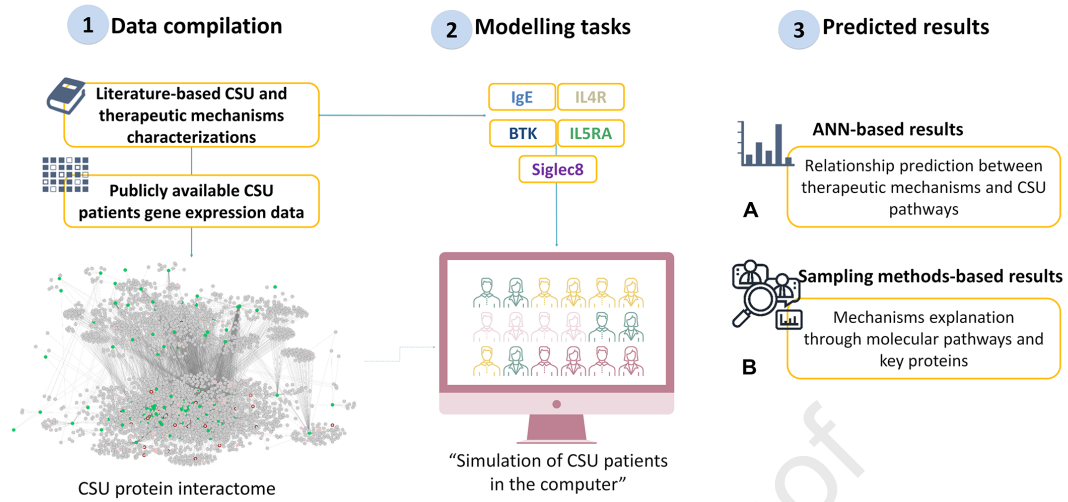


Figure 1_Segú-Vergés C. et al.

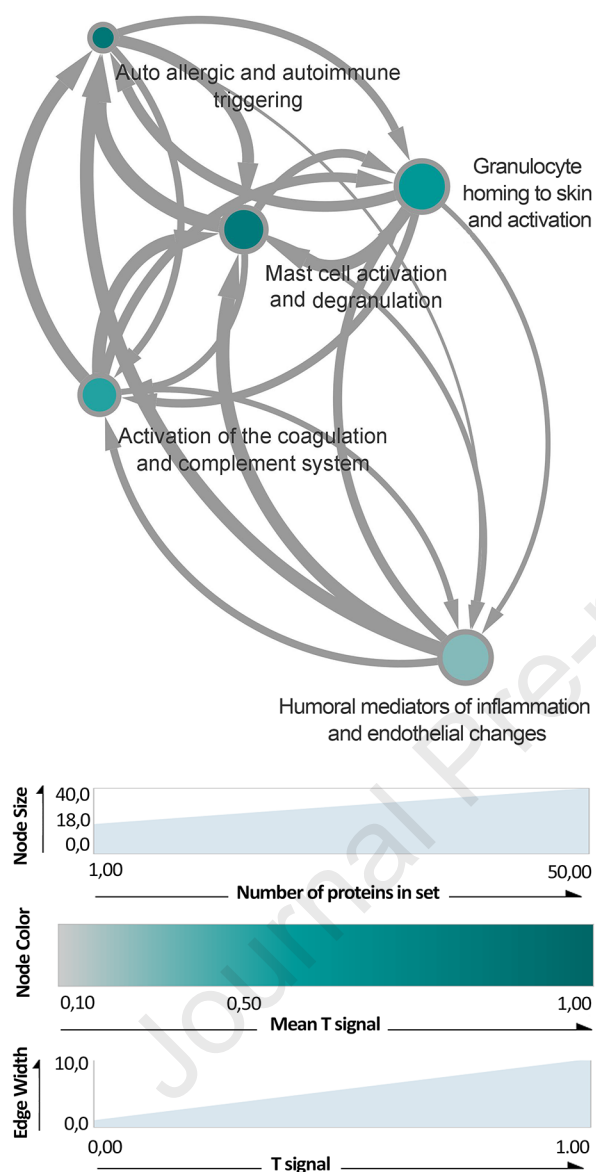


Figure 2_Segú-Vergés C. et al.

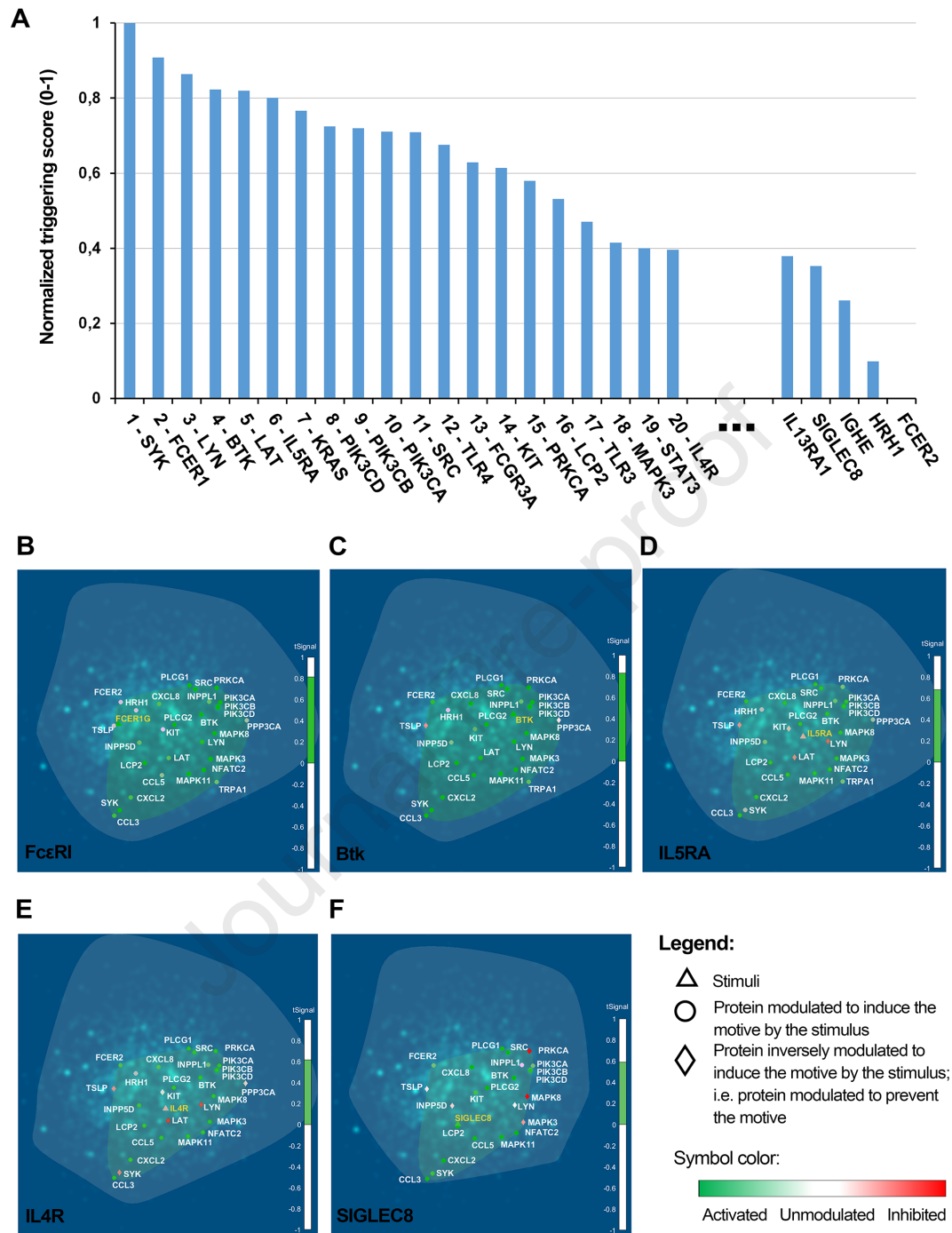


Figure 3_Segú-Vergés C. et al.

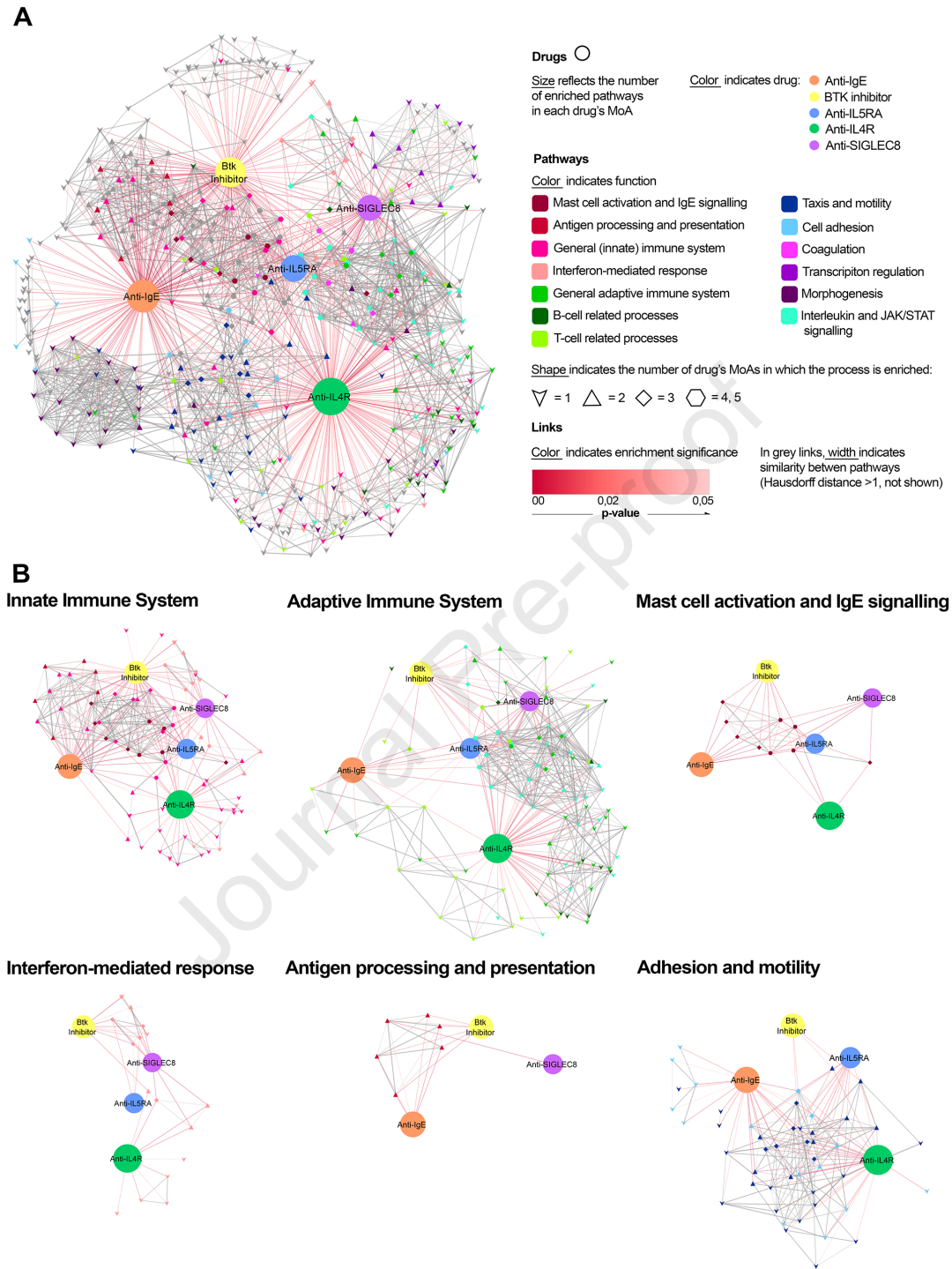


Figure 4_Segú-Vergés C. et al.

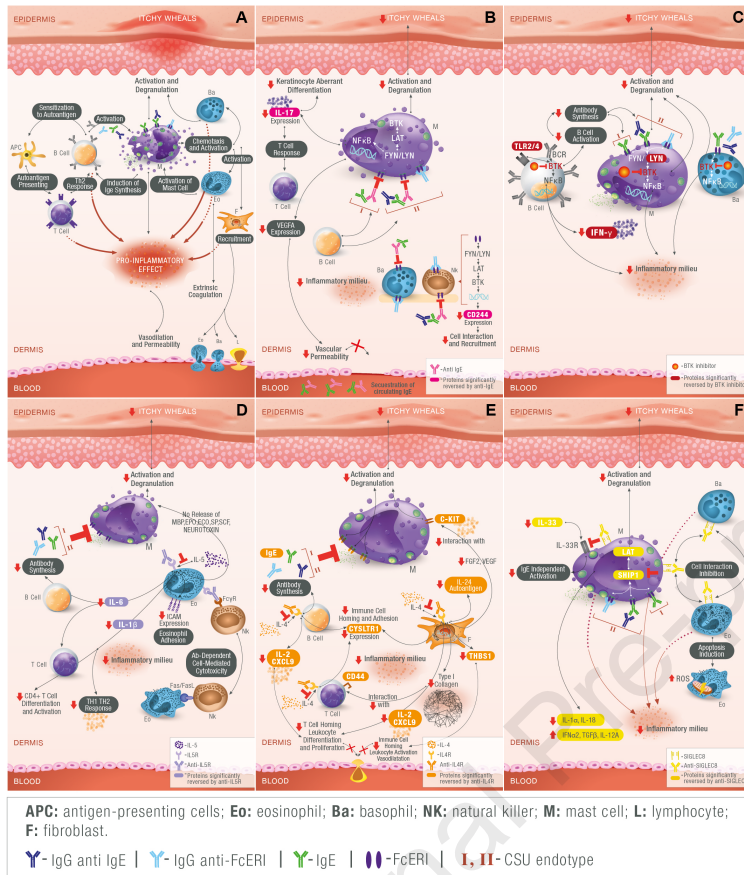


Figure 5_Segú-Vergés C. et al.