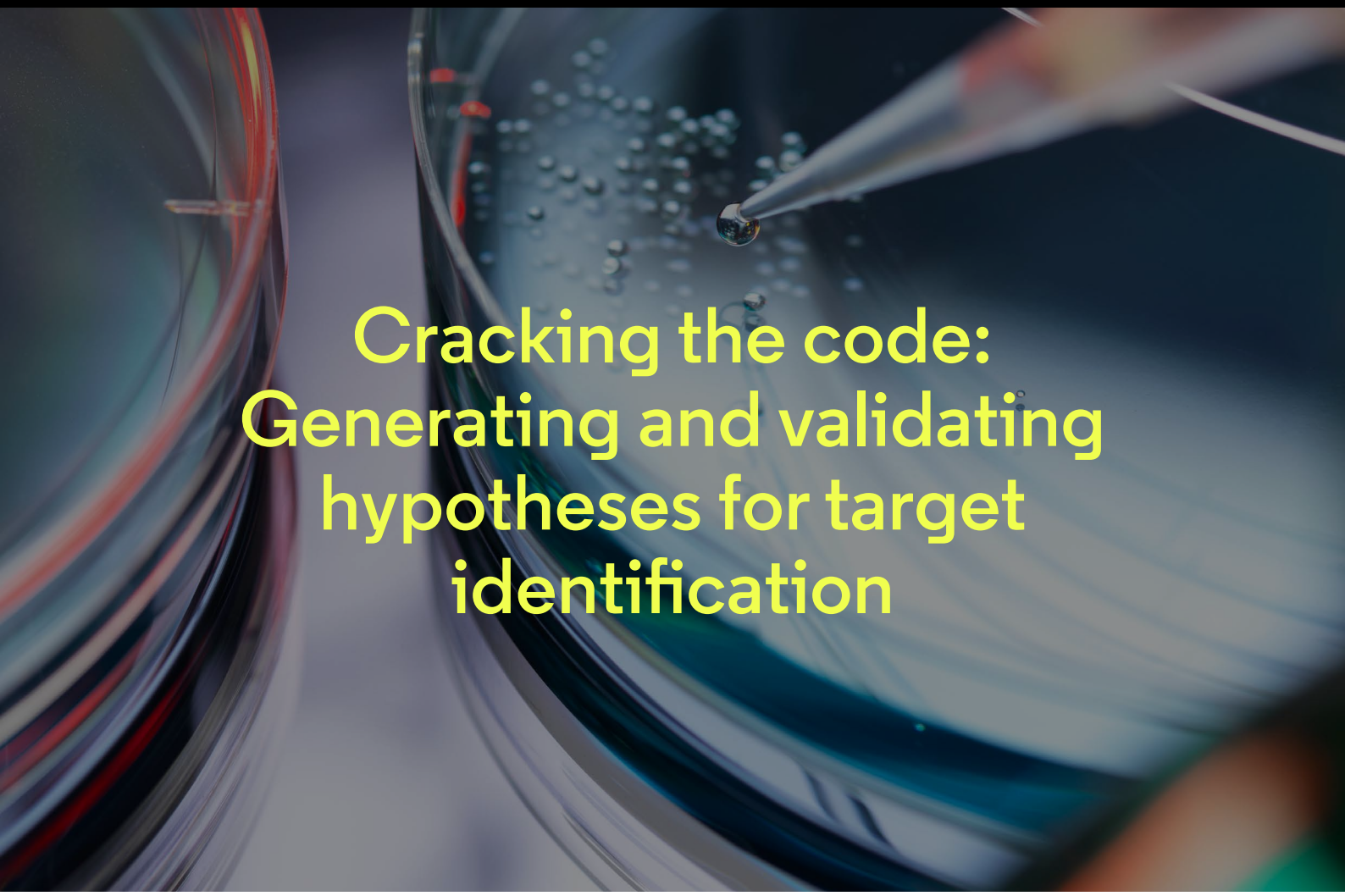


Cracking the code: Generating and validating hypotheses for target identification

Developing a shortlist of potential targets to improve the effect of multisystem inflammatory syndrome in children after acute COVID-19 infection

Selecting the right target is the most important investment decision in drug discovery; 40% of clinical trial failures are due to no clear link being made between target and disease.[1] Clarivate discovery and pre-clinical solutions can empower R&D teams to make faster, more informed decisions to increase the chances of successfully translating research findings into human applications and achieving success in clinical trials.

This case study demonstrates how researchers can use Clarivate solutions MetaCore™, Cortellis Drug Discovery Intelligence™ and OFF-X to generate and validate hypotheses for target identification, thereby saving time and money before proceeding to further lab-based validation.

[1] Cook, D., Brown, D., Alexander, R. et al. Lessons learned from the fate of AstraZeneca's drug pipeline: a five-dimensional framework. Nat Rev Drug Discov 13, 419–431 (2014). https://doi.org/10.1038/nrd4309

Define hypotheses for putative targets by uncovering the molecular mechanisms underlying various biological phenomena.

↓

Further evaluate these hypotheses with additional evidence of the link between the target and the disease.

↓

Develop a comprehensive understanding of what is known about these targets, before embarking on lab experiments.

Clarivate™

Exploring neutrophil responses in distinct SARS-CoV-2 disease states

Some children develop a severe, life threatening hyperinflammatory illness called multi-system inflammatory syndrome in children (MIS-C) weeks to months after the resolution of the acute COVID-19 infection. Neutrophil activation is a principal component of the immune response against SARS-CoV-2 infection; however, excessive neutrophil hyperactivation can contribute to severe COVID-19 in adults. In the following study,[2] neutrophils are isolated from children with acute COVID-19, children with MIS-C and healthy controls (HC) to define neutrophil responses that might drive these distinct SARS-CoV-2 disease states in children.

In this study the investigators observed increased NETosis formation. NETosis is a process by which certain immune cells, particularly neutrophils, release structures called neutrophil extracellular traps (NETs) to combat pathogens. NETosis is an important mechanism of the innate immune response. However, excessive or dysregulated NETosis has also been implicated in the pathogenesis of various inflammatory and autoimmune diseases.

We will use the three sample groups used in this study for this case study.

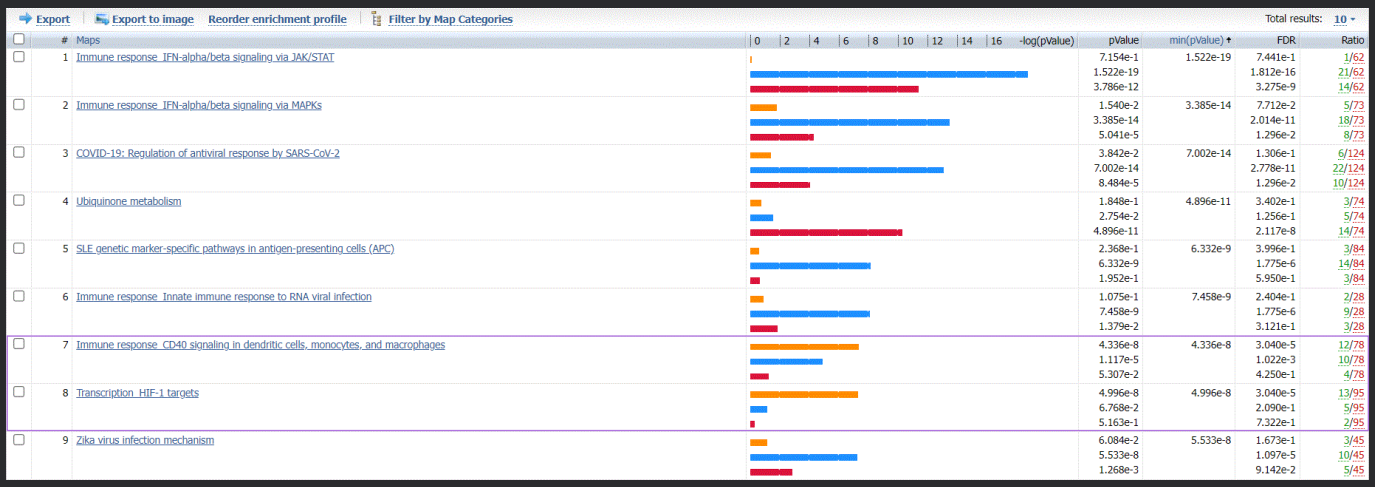
[2] Boribong, P, et al. Neutrophil profiles of pediatric COVID-19 and multisystem inflammatory syndrome in children, Cell Reports Medicine, Volume 3, Issue 12,2022, https://doi.org/10.1016/j.xcrm.2022.100848.

Uncovering key pathways associated with specific biological conditions or experimental treatments

Pathway enrichment analysis is a valuable tool in systems biology to uncover the molecular mechanisms underlying various biological phenomena, such as disease development and drug response. This downstream analysis aims to interpret the consequences of the expression changes defining which biological and cellular processes are affected. Using the one-click analysis on MetaCore, we run a pathway map enrichment analysis selecting p-value ≤ 0.05 for both up- and down-regulated genes.

The results of this analysis (Fig 1) indicate that CD40-signaling and HIF-1 transcription regulation pathways are significantly enriched with the genes differentially expressed in MIS-C vs healthy controls (orange bar). Interestingly, these pathways are not the main ones when comparing COVID vs control (blue), or MIS-C vs COVID (red), suggesting that those biological and cellular processes could play a role in the progression to a severe COVID and the development of MIS-C.

Figure 1: Pathway map enrichment analysis

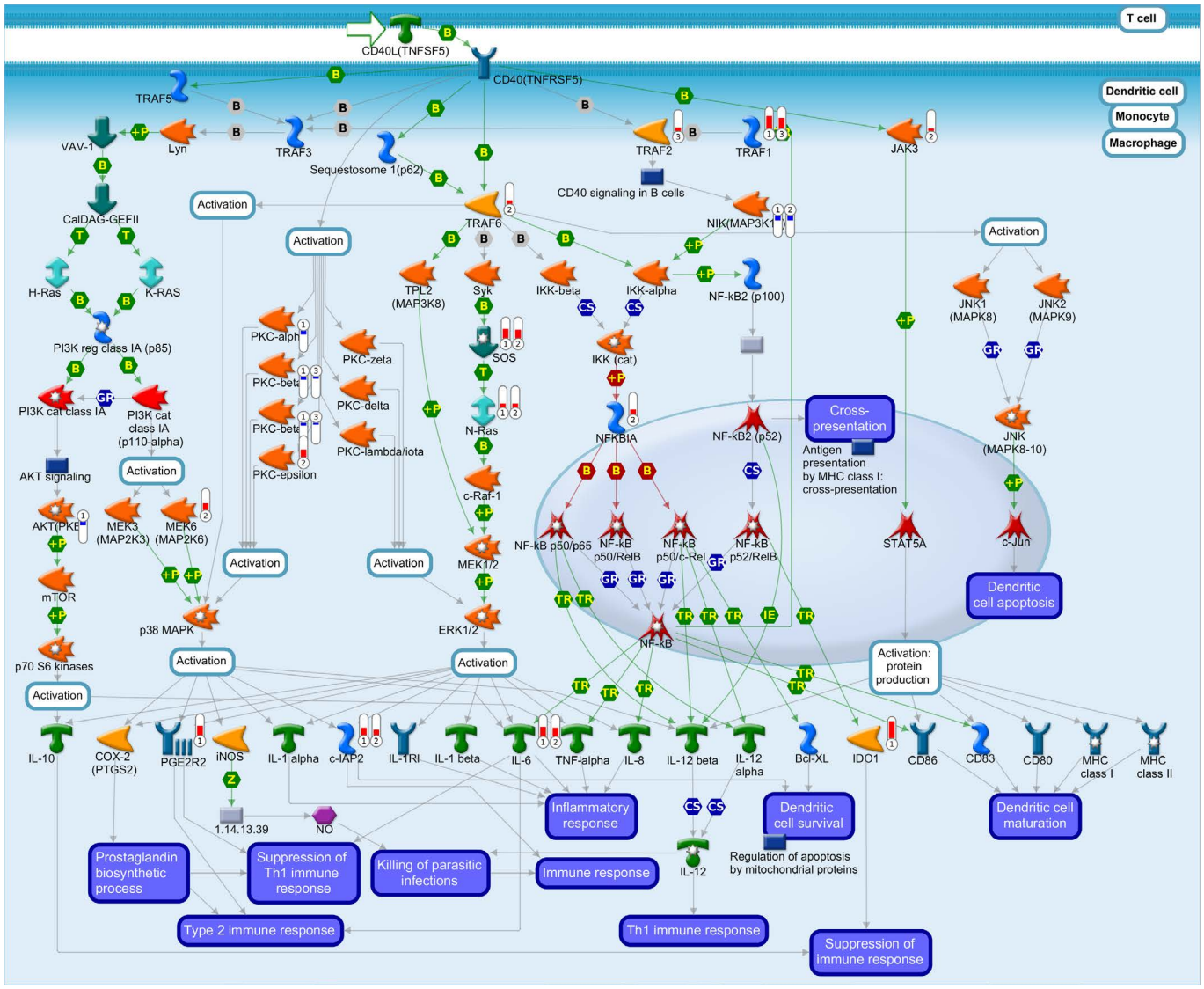


Source: MetaCore

Immune response: CD40-signaling in dendritic cells, monocytes, and macrophages

Focusing on the CD40-signaling in dendritic cells, monocytes, and macrophages pathway map (Fig 2), we can easily see the up- and down-regulated genes in our data sets overlapped in the map for our three sample groups. IL-6 is equally up-regulated in MISC vs controls (1) and COVID vs controls (2), but IDO1 is significantly upregulated only in MISC. IDO1 exerts immunoregulatory effects that can either dampen or exacerbate inflammatory responses, depending on the context.

Figure 2: CD40-signaling in dendritic cells, monocytes, and macrophages pathway map displays up-regulated genes



Source: MetaCore

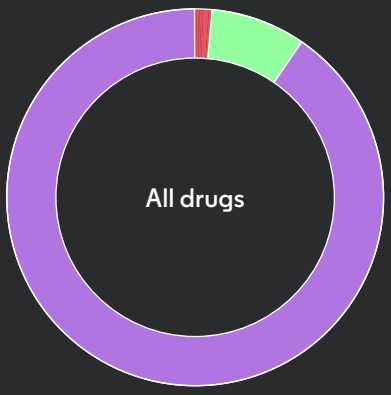
Hypothesis 1: IDO1 as a potential target for MIS-C

Given IDO1's role in modulating the immune response and its role in key pathways associated with MIS-C, IDO1 could be a potential therapeutic target for modulating the immune response that leads to MIS-C.

Exploring the target landscape for IDO1

Our next step is to examine the target landscape on Cortellis Drug Discovery Intelligence. Our search reveals that IDO1 is a well-known target with two launched drugs (non-specific, antifungal) and 16 drugs under active development for oncology conditions.

Figure 3: Drugs acting on IDO1



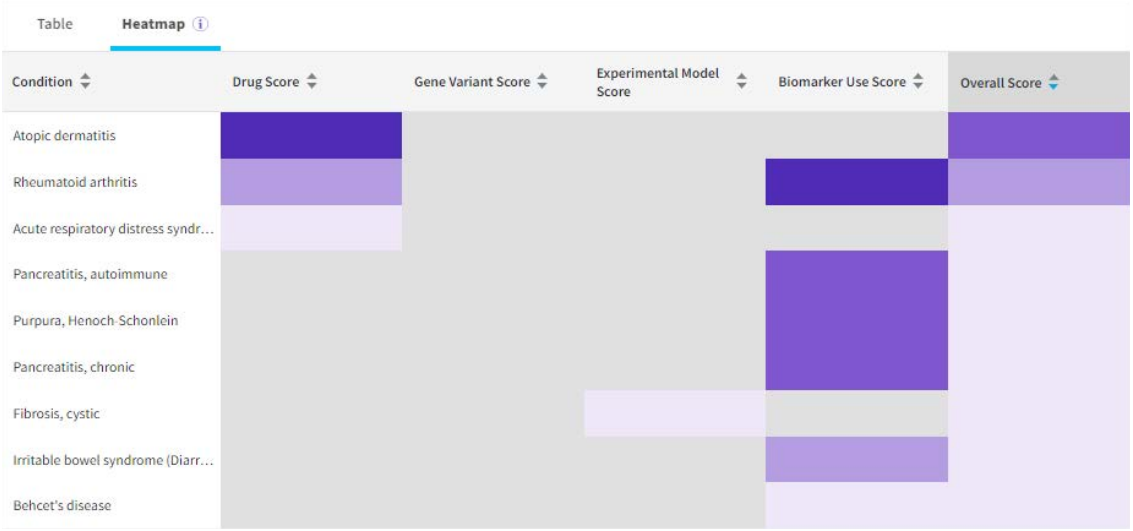
Biological testing	**1700**
Preclinical	**153**
Other	**28**
Phase I	(9)
Discontinued	(4)
Phase III	(4)
Phase I/II	(3)
Phase II	(3)
Launched	(2)
Phase II/III	(1)
Clinical	(1)
Not determined	(1)

Preclinical	**5**
Phase I	**5**
Phase I/II	**2**
Phase III	**2**
Phase II	**1**
Phase II/III	**1**

Source: Cortellis Drug Discovery Intelligence

The target-conditions heatmap (Fig 4) shows that IDO1 is related to 434 conditions, including many diseases with an inflammatory component in which neutrophil activation plays an important role.

Figure 4: Ranking the relationship between target and condition



Source: Cortellis Drug Discovery Intelligence

The heat map (Fig 5) also informs us that research studies using biomarkers indicate a relationship between IDO1 and multiple infections, among them COVID-19.

Figure 5: Leveraging biomarkers to understand the target-condition relationship

Source: Cortellis Drug Discovery Intelligence

Zeroing in to biomarkers intel, we observe that IDO1 has been linked to COVID-19 in several high-throughput nucleotide sequencing studies, strengthening the evidence for our hypotheses.

Figure 6: Insights derived from biomarkers intel

Condition	Population	Role	Highest Use Validity	Technique/Substrate	Drugs	Supporting studies
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (COVID-19)	All	Diagnosis	Experimental	High Throughput Nucleotide Sequencing / mRNA	0	1
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (COVID-19)	All	Disease Profiling	Experimental	"High Throughput Nucleotide Sequencing / mRNA High Throughput Nucleotide Sequencing / RNA"	0	2
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (COVID-19)	All	Predicting Treatment Efficacy	Experimental	Real Time RT-PCR / mRNA	1	1
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (COVID-19)	Moderate	Disease Profiling	Experimental	High Throughput Nucleotide Sequencing / mRNA	0	1

Source Cortellis Drug Discovery Intelligence

A review of the biomarker-related research also led us to a study[3] exploring the role of neutrophil functions in patients with severe COVID-19 pneumonia. Although it does not focus on children (our population of interest), it does focus on a population with a more severe form of COVID-19, and elevated plasma levels of NET markers are found. The study suggests that metabolic remodeling is vital for the formation of NET and boosts neutrophil inflammatory response.

As the study finds that glycogen phosphorylase L (PYGL) abolishes the ability of neutrophils to produce NET, and HIF-1alpha stabilizes PYGL, the authors suggest that modulating HIF-1alpha or PYGL could represent a novel therapeutic approach.

This information aligns with the results from our earlier analysis, that indicated that HIF-1A is a regulator of PYGL and the HIF-1 signaling cascade was the other pathway significantly enriched in our content set.

[3] Borella, R. et al, Metabolic reprograming shapes neutrophil functions in severe COVID-19. European Journal of Immunology. Volume 52, Issue3, 484-502 (2022) https://doi.org/10.1002/eji.202149481

Exploring the safety profile of targets in a specific biological context

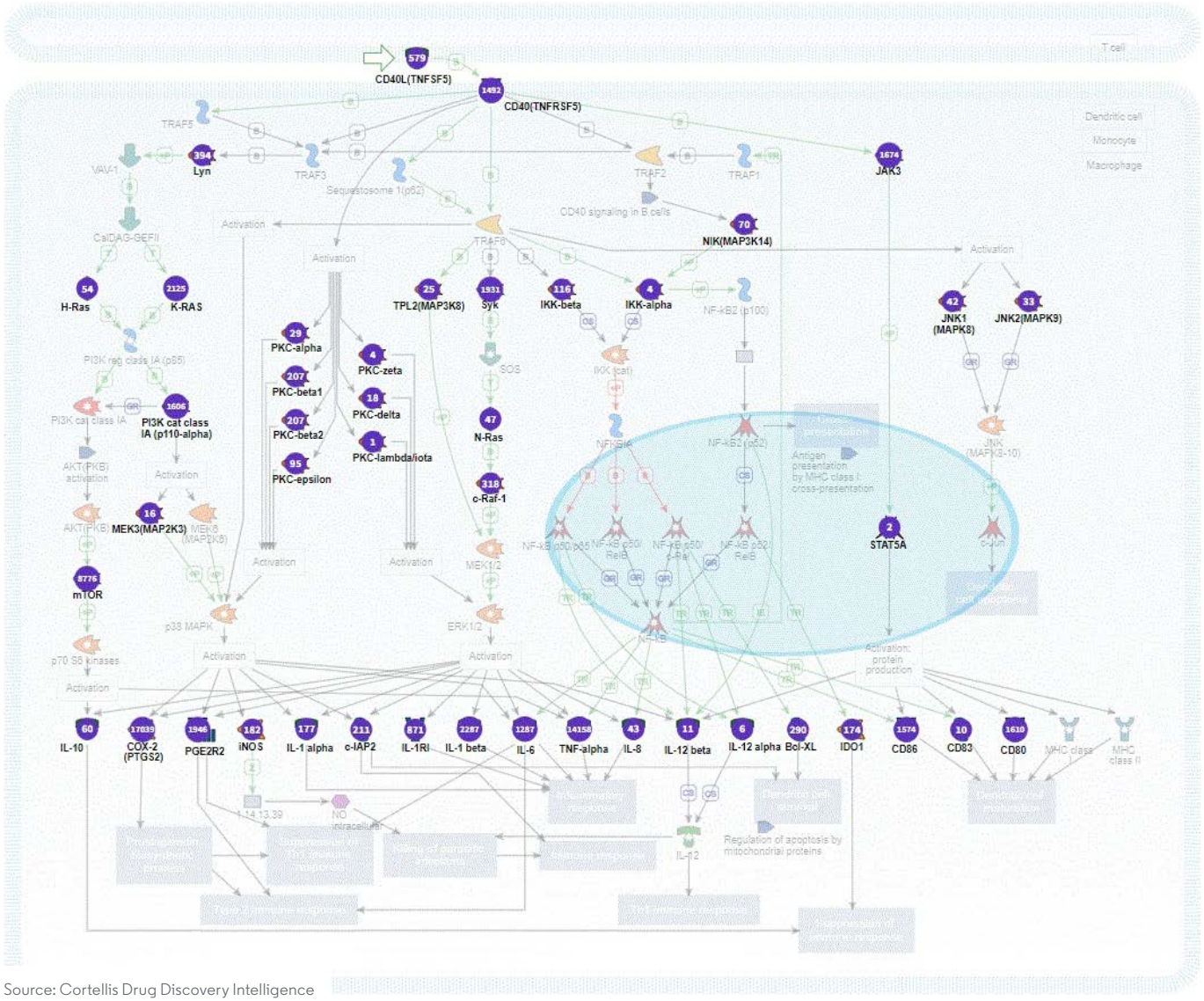
Understanding the safety profile for our shortlist of targets in a particular biological context can help anticipate safety liabilities and prioritize further research. To do this, we refer to the Immune response CD40-signaling in dendritic cells, monocytes, and macrophages pathway map on Cortellis Drug Discovery Intelligence (Fig 7), displaying the count of reported associations between the target and an adverse event.

Those with a lower count would be better candidates, as less safety alerts have been associated to them.

We observe that IDO1 has 174 associated safety alerts, that indicate associations between a drug or target-action and an adverse event. The pathway map links directly to OFF-X safety intelligence solution (Fig 8), offering an analysis of all the adverse events associated to IDO1, grouped by System Organ Class. We can then zero in to granular details about the adverse event, including its source of information.

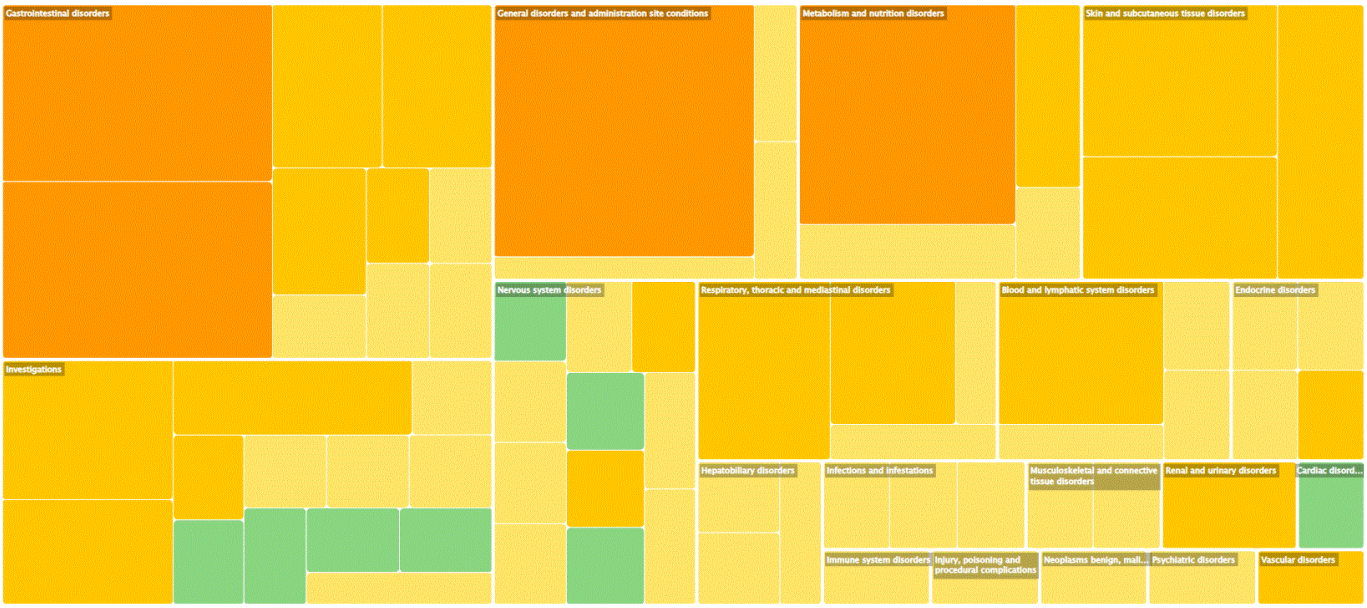
Understanding potential safety issues for these targets can help de-risk preclinical development programs by guiding experimental studies, facilitating dynamic target safety assessments, and informing clinical design.

Figure 7: Immune response CD40-signaling in dendritic cells, monocytes, and macrophages pathway map showing the count of safety alerts for each target



Source: Cortellis Drug Discovery Intelligence

Figure 8: Rating the associations between IDO1 and adverse events



Source: OFF-X

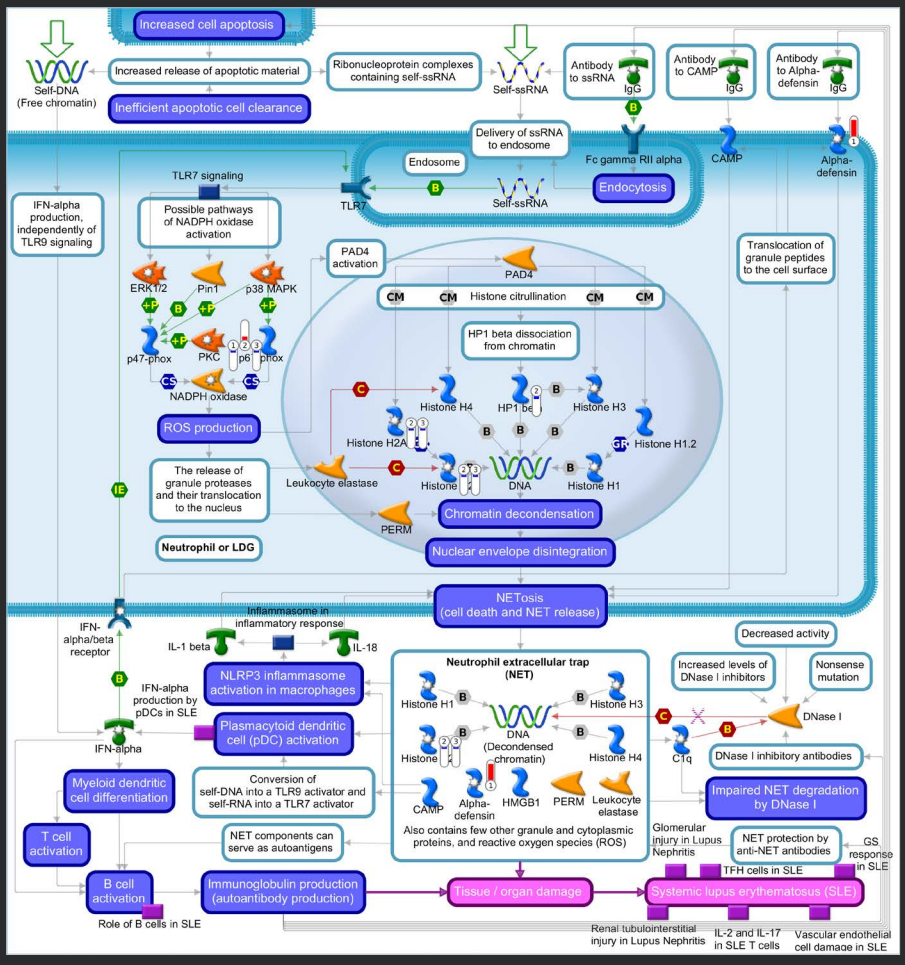
Understanding potential safety issues for these targets can help de-risk preclinical development programs by guiding experimental studies, facilitating dynamic target safety assessments, and informing clinical design.

Hypothesis 2: Alpha-defensin 1 (DEFA1) as a putative target

Another approach we can take is to search for NETosis in MetaCore. We retrieve a map focusing on NETosis in Systemic Lupus Erythematosus (SLE). As SLE has a clear inflammatory and immune component, we explore the map with our experimental results overlaid on it.

The increase of Alpha-defensin in MIS-C vs Controls group is very significant, but not in the other experimental datasets. Alpha-defensins are part of the innate immune system and play a crucial role in host defense against microbial infections. In addition, they have immunomodulatory properties that contribute to the regulation of immune responses and their dysregulation has been implicated in the pathogenesis of inflammatory disorders such as IBD, psoriasis, and CF.

Figure 9: A significant increase in Alpha-defensin in MIS-C vs Controls group



Source: MetaCore

Turning to Cortellis Drug Discovery Intelligence to validate this hypothesis, we observe that:

- DEFA1 is a less explored target, with only four drugs in biological testing or preclinical studies for the treatment of inflammation and sepsis, among others.
- DEFA1 is associated to multiple inflammatory diseases via biomarker studies, genetic variants and/or experimental models.
- Biomarker studies link DEFA1 with severe COVID, increasing the confidence in our hypothesis.

Understanding which organizations are working on therapeutic options for a target of interest can help companies identify potential partners for research. In this case, the main organizations working on therapeutic options to target DEFA1 are in the academic space (Fig 11).

Figure 10: Biomarker uses based on published research

Condition	Population	Role	Highest Use Validity	Technique/ Substrate	Supporting study
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (COVID-19)	Severe	Diagnosis	Early Studies in Humans	IA / Plasma	1
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (COVID-19)	All	Staging	Early Studies in Humans	IA / Plasma	1

Source: Cortellis Drug Discovery Intelligence

Figure 11: Organizations developing therapeutic options to target DEFA1

Academisch Ziekenhuis Maastricht
Ludwig-Maximilians-Universitaet Muenchen
Universiteit Maastricht
Zhejiang University
NCE Biomedical
Sichuan University (SCU)

Source Cortellis Drug Discovery Intelligence

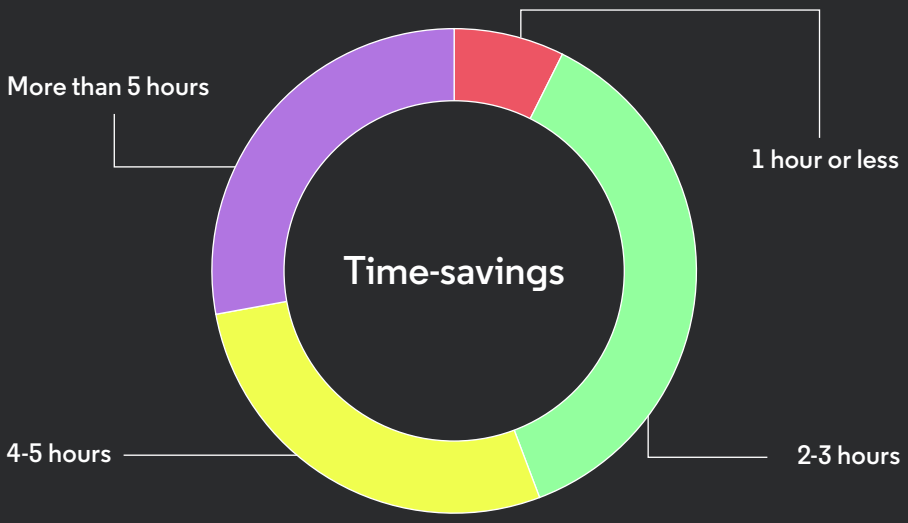
Conclusions

Analysis of our experimental data in MetaCore allowed us to define two hypotheses for putative targets to ameliorate the effect and progression of MIS-C. Cortellis Drug Discovery Intelligence allowed us to analyze further evidence of the link between the target and the disease and develop a robust understanding of what is known about these targets. And understanding potential safety issues for targets can help de-risk preclinical development programs by guiding experimental studies, facilitating dynamic target safety assessments, and informing clinical design.

This example illustrates how quickly researchers were able to uncover potential strategies that can be further validated via lab work; for example, proceeding with a more well-known target that can be repurposed for the condition of interest (e.g. IDO1, HIF1alpha), or if one is seeking novelty, with a lesser-known target (e.g. (DEFA1).

This approach helps accelerate discovery research by narrowing down large lists of putative targets – within minutes - to come up with a shortlist for further validation in the lab.

Weekly time-savings using Clarivate discovery and pre-clinical solutions



Source: Client survey of 122 users

BXD1354735085 / 04

About Clarivate

Clarivate is a leading global information services provider. We connect people and organizations to intelligence they can trust to transform their perspective, their work and our world. Our subscription and technology-based solutions are coupled with deep domain expertise and cover the areas of Academia & Government, Life Sciences & Healthcare and Intellectual Property. For more information, please visit **clarivate.com**.

Learn more about how we can help you optimize drug development :

clarivate.com/products/biopharma/research-development/

Contact our experts today:

clarivate.com