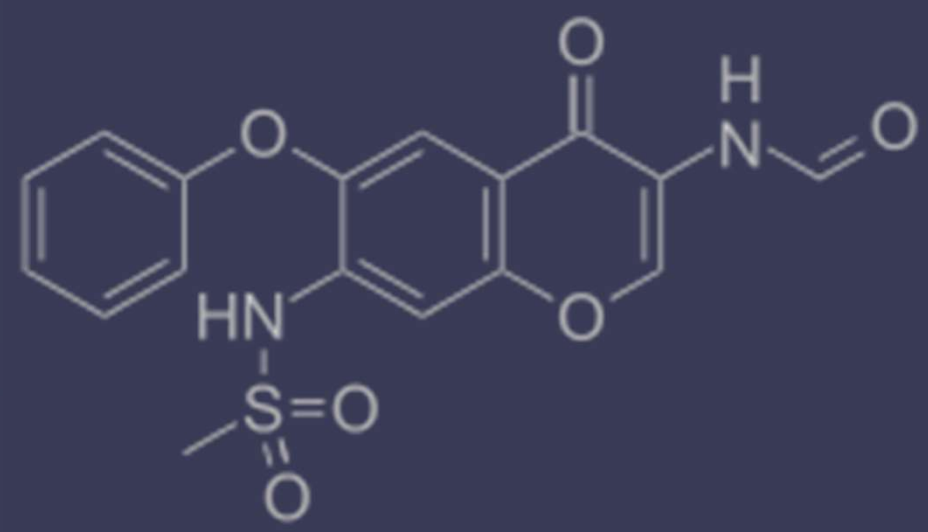
Delta4
digital drug discovery & development

Computational Drug Repurposing of IGURATIMOD

2023-08

STRICTLY CONFIDENTIAL



Final Project Report
Iguratimod Case Study

Contents

1. Project aim

Delta4 will use its computational drug repurposing platform Hyper-C to (i) generate a molecular MoA model for the anti-inflammatory compound iguratimod, (ii) use the constructed molecular MoA model to computationally screen for (novel) diseases / phenotypes / indications[a] modulated by iguratimod, and (iii) mechanistically evaluate selected top-ranked indications in order to predict the detailed mechanistic impact of iguratimod.

No restrictions regarding the therapeutic area will be applied and the complete library of phenotype molecular models within Delta4's computational drug repositioning platform Hyper-C will be considered in the in silico phenotype screen.

2. Project plan

The full project will be conducted within a time frame of 5 months. Project step 1, focusing on the generation of the iguratimod molecular MoA model to assess the modulated signaling pathways, will be completed within 1.5 months. Project step 2, focusing on the identification of diseases / phenotypes / indications suggested to be significantly impacted by iguratimod based on network interference, will be completed within another 1.5 months. Project step 3, focusing on the detailed mechanistic evaluation of some selected indications from project step 2 will last for two months. See figure 1 below.

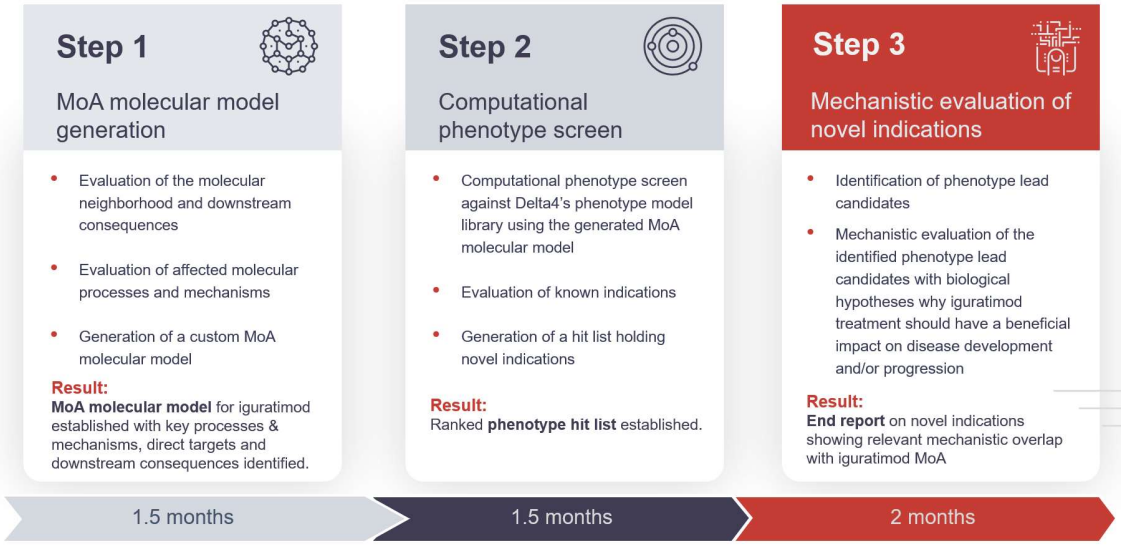


Figure 1: *Iguratimod indication expansion plan.*

[a] The terms "disease", "indication" and "phenotype" are often used as synonyms, whereas the term phenotype in reality can be considered broader also including terms such as fibrosis or inflammation which are not diseases per se but rather pathological conditions or patho-mechanisms. In our phenotype library there is also a specific category called "Pathological Conditions, Signs and Symptoms" holding these specific phenotype terms.

3. Executive summary

In project step 1, Delta4's software platform Hyper-C was used to generate a molecular mechanism of action (MoA) model for the anti-inflammatory compound iguratimod. The MoA model was built around the four primary drug targets of iguratimod, namely the macrophage migration inhibitory factor (MIF)[1], TRAF3 interacting-protein 2 (TRAF3IP2)[2], NF-κB (NFKB1)[3–5], and cyclooxygenase 2 (COX2/PTGS2)[6], plus additional relevant genes regulated by iguratimod. The final constructed molecular MoA model held 130 proteins covering key mechanisms including inflammation process, T-cell activation, oxidative stress, or extracellular matrix organization.

We identified 262 diseases / phenotypes / indications holding direct annotation with iguratimod based on information in scientific literature, patents, or clinical trials as stored in Delta4's Hyper-C platform. This set of indications was considered the "known" disease space.

In project step 2, the generated iguratimod molecular MoA models was used to computationally screen against Delta4's phenotype library (holding more than 3400 unique disease phenotypes) in order to identify diseases modulated by iguratimod. The prediction of this disease-modifying impact by iguratimod was based on mechanistic overlaps (interference) between drug MoA and disease pathophysiology. Delta4's weighted network alignment was used for drug-disease interference scoring and disease ranking. Next to the network alignment scores we considered the relevance of iguratimod's primary drug targets in the pathophysiology of each disease in the final disease ranking.

In project step 3, selected indications were analyzed in high molecular detail in order to predict the expected impact of iguratimod on these diseases and subsequently the associated clinical potential.

Rheumatoid arthritis, an approved indication for iguratimod, served as positive control and was among the top-scoring indications. In addition, we identified 59 "novel" indications seeing significant overlap with iguratimod's MoA on a molecular level. The predicted five most promising indications were a respiratory tract disease, urogenital disease, eye disease, stomatognathic disease and infectious inflammatory disease. Next to these undisclosed indications, endometriosis also ranked among the top-scoring novel indications and was selected to demonstrate our analysis process.

4. Background

4.1 Iguratimod

Iguratimod (T-614) is a non-steroidal anti-inflammatory drug (NSAID) / disease-modifying anti-rheumatic drug (DMARD) approved for the treatment of rheumatoid arthritis (RA) together with methotrexate and is currently approved in Japan (since 2012) and China (since 2011) [7,8]. Iguratimod is currently neither marketed in Europe nor in the United States. While iguratimod can exert pleiotropic actions against inflammation and inflammatory pain, the direct targets are not fully elusive. Initial reports describe iguratimod as inhibitor of PGE2 (prostaglandin E2) signaling [9], bradykinin signaling [10], IL-1β signaling 1289500 and COX-2 (cyclooxygenase 2, PTGS2) [11]. Inhibition of IFN-γ, IL-6, TNF and NF-κB signaling was also demonstrated early [12,13]. Manual curation of more than two decades of research according to ChEMBL suggests MIF (Macrophage migration inhibitory factor), the E3 ubiquitin ligase TRAF3IP2 and COX-2 as targets (CHEMBL2107455) – but the modulated signaling pathways are much more complex, making repositioning of iguratimod an ideal example for drug repurposing based on network interference. Scientific attention for iguratimod has significantly increased in recent years (Fig. 2) due to its potential broader anti-inflammatory and anti-fibrotic effects [6,8] and has led to the evaluation of the impact of iguratimod on many different diseases (see below).

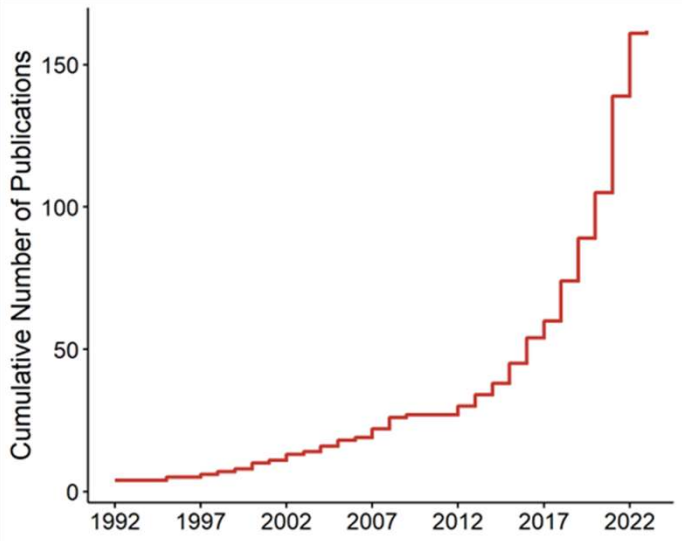


Figure 2: *Cumulative number of publications regarding iguratimod.*

4.2 Iguratimod in (pre-)clinical development

Next to clinical trials in the context of its approved indication RA, iguratimod is currently in clinical development for several inflammatory and autoimmune diseases such as Sjogren's Syndrome, Idiopathic Thrombocytopenic Purpura (ITP), Lupus Nephritis, Immunoglobulin G4-related Disease, Osteoarthritis, and Systemic Scleroderma. Tested indications span a broad spectrum of therapeutic areas such as skin and connective tissue diseases, musculoskeletal diseases, lymphatic disease, as well as eye diseases as indicated in figure 3. Phase IV clinical trials are currently also ongoing for Sjogren's Syndrome and Immunoglobin G4-Related Disease, however information regarding commercial application remains unknown.

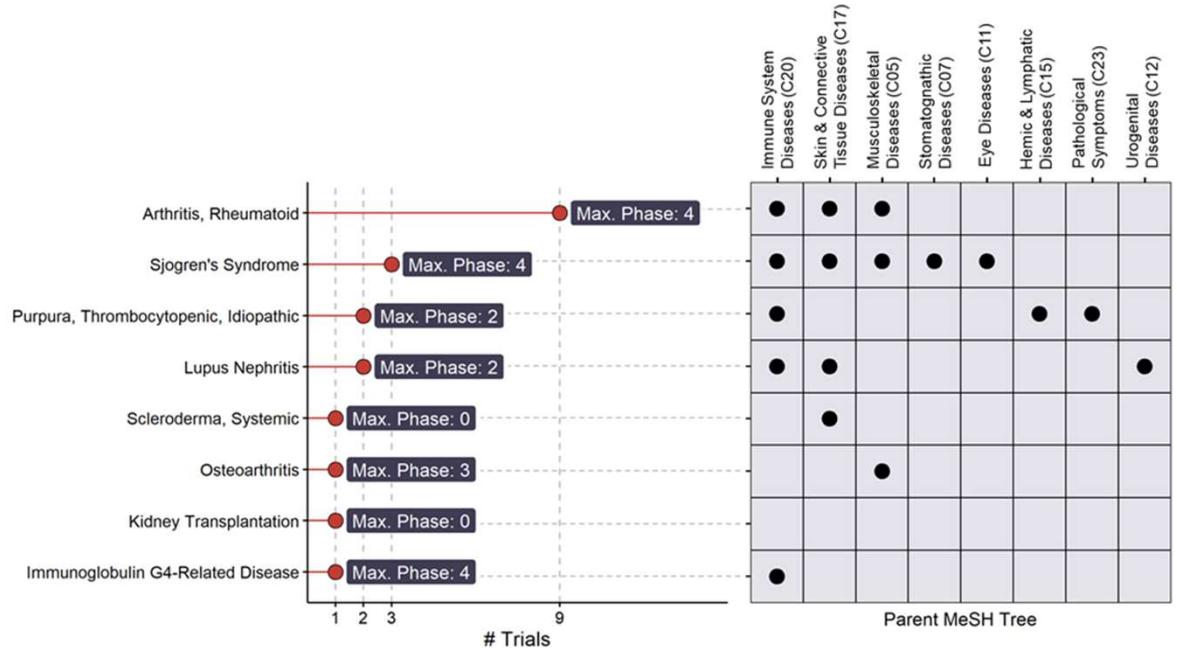


Figure 3: *Clinical development of iguratimod.*

5. Project step 1: Iguratimod molecular MoA model generation

5.1 Defining the iguratimod molecular MoA model input set

5.1.1 Reported primary drug targets of iguratimod

Based on consolidated information from scientific literature and dedicated drug target databases within Delta4's Hyper-C platform, the following iguratimod targets have been considered primary: macrophage migration inhibitory factor (MIF), TRAF3 interacting-protein 2 (TRAF3IP2), NF-kB (NFKB1), and cyclooxygenase 2 (COX2) encoded by the PTGS2 gene. These genes are embedded in signaling cascades that promote disease processes like inflammation, fibrosis, angiogenesis, or tumor cell proliferation and osteoclast differentiation driving bone erosion. By inhibiting these targets iguratimod can ameliorate the respective pathological processes (Fig. 4).

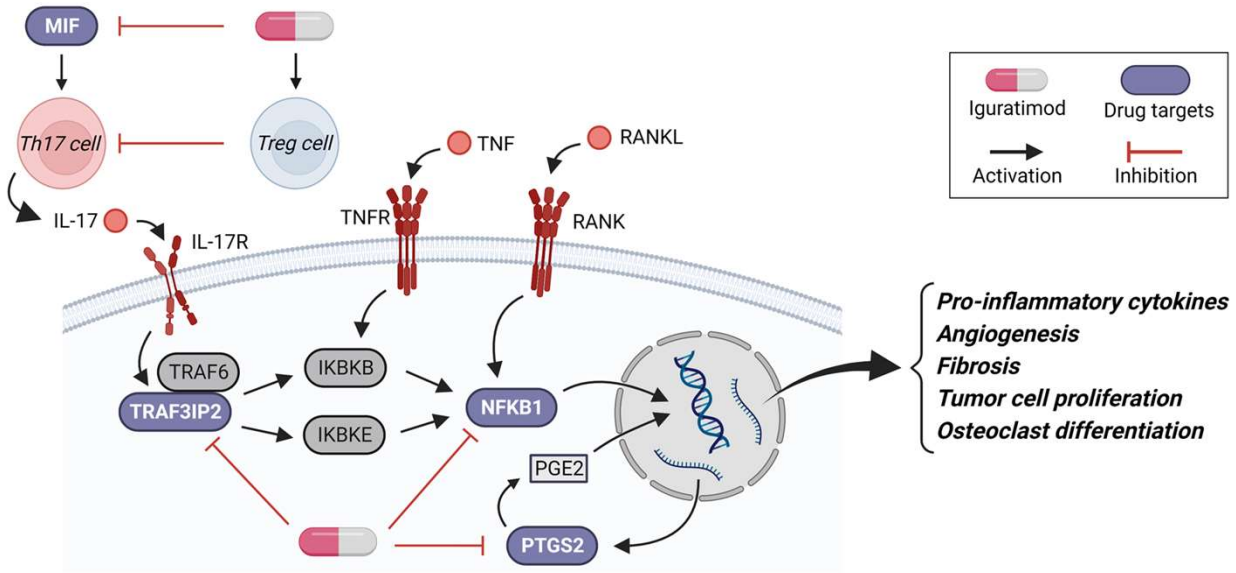


Figure 4: *Iguratimod core MoA centered around the primary drug targets[b].*

[b] Delta4 is also capable of building a molecular model when starting with a drug target and not a drug per se. In that case the molecular context (neighborhood) of the drug target will be considered and modeled, i.e. taking into account direct protein interaction partners of the drug target as well as upstream and downstream regulators.

5.1.2 Reported primary drug targets of iguratimod

We followed a stepwise process to build the iguratimod molecular MoA model. In the first step, an initial gene list containing the four primary drug targets was expanded by genes that were associated with iguratimod in Delta4's Hyper-C platform based on literature co-annotation and represented direct or indirect interaction partners of the primary drug targets within the protein-protein interaction network (see Supplementary data file 1 for more details). We further complemented this gene set with additional genes that showed strong literature co-annotations with either of the four drug targets (see Supplementary data file 1 for more details). To ensure that only highly relevant genes were selected, we manually curated this process and exclusively considered genes that satisfied all of the following criteria:

1) the co-annotation count for a drug target-gene pair was > 2

2) the co-annotation count for a given gene with either of the four drug targets ranked in the top 5% of all genes co-annotated with either drug target

3) the co-annotation count of either of the four primary drug targets ranked in the top 5% of all genes co-annotated with a given gene

4) strong statistical support for co-annotation enrichment, i.e. a drug target and a given gene were co-mentioned more frequently in literature than expected by chance based on the number of documents linked to either of the two genes within the entire pool of biomolecular/genetic literature (odds ratio > 1 and Bayes factor ≥ 10 derived from contingency table analysis assuming hypergeometric sampling).

(criteria 2 and 4 were slightly relaxed for genes co-annotated with TRAF3IP2 due to the overall comparatively low number of publications linked to TRAF3IP2 in general).

5.1.3 Omics-derived genes regulated by iguratimod

Based on thorough literature research[c], we extended our MoA model with genes representing expression changes in response to iguratimod treatment in various relevant disease contexts reported in animal or human studies (Fig. 5). This set of genes displaying experimentally reported expression changes in response to iguratimod that had not been covered in the previous steps outlined in Section 6.1.2 was additionally included in the MoA model input set (see Supplementary data file 1 for more details).

Iguratimod reduces expression of PTGS2, NFKB1, MIF, TRAF3IP2 and proinflammatory cytokines in rheumatoid arthritis, while TGFB1, IL10, SP7 and DLX5 were found to be upregulated.

[c] In our workflows we take a flexible approach to including Omics data in the model building steps, based on data availability, added value for the project at hand, and our clients' requirements. Due to scarcity of available data related to iguratimod, we did not make use of Omics data in this project. Instead, we extracted differentially expressed genes in response to iguratimod treatment as described in literature.

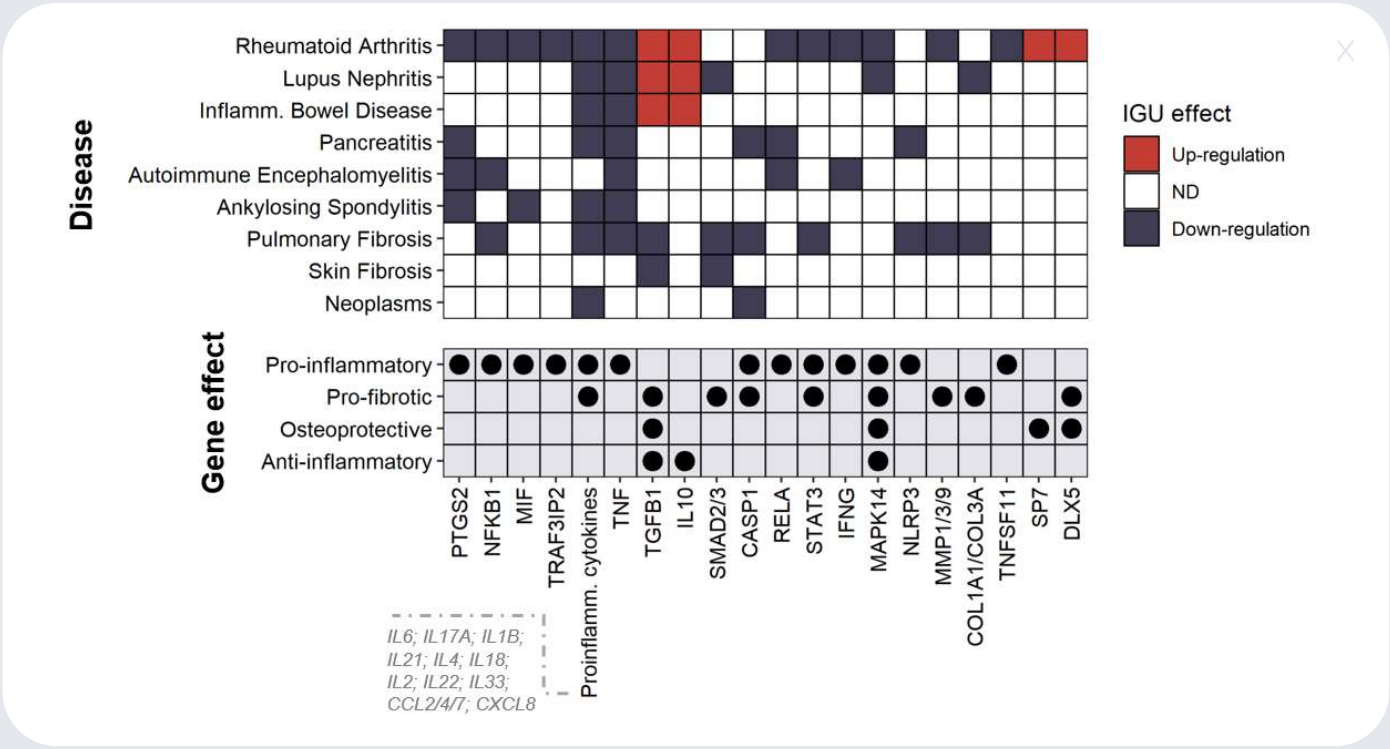


Figure 5: *Regulation of primary drug targets and associated molecules in selected diseases.*

5.2 Constructing the iguratimod molecular MoA model

The 130 proteins linked to iguratimod were used as input for the iguratimod molecular MoA model construction workflow. Delta4's proprietary protein-protein dependency network was used as underlying biological network holding experimentally determined protein-protein interaction data complemented by computationally inferred protein-protein dependencies based on a set of pre-defined data sources. The 130 proteins were mapped onto the proprietary network and protein-protein interactions as well as computationally inferred dependencies were extracted for the set of iguratimod associated proteins.

The resulting network consisted of 130 proteins and 649 protein-protein dependencies of which 413 were experimental protein-protein interactions and 236 were computationally inferred. The largest connected subnetwork held >94% of proteins, while five proteins (DLX5, BGLAP, MRC1, MOG, CP, TNNI3, PTGES) were not connected to the core network.

The average node degree of the resulting network model was 10 [ranging from 0 to 54]. A schematic representation of the iguratimod molecular MoA model is given in figure 6.

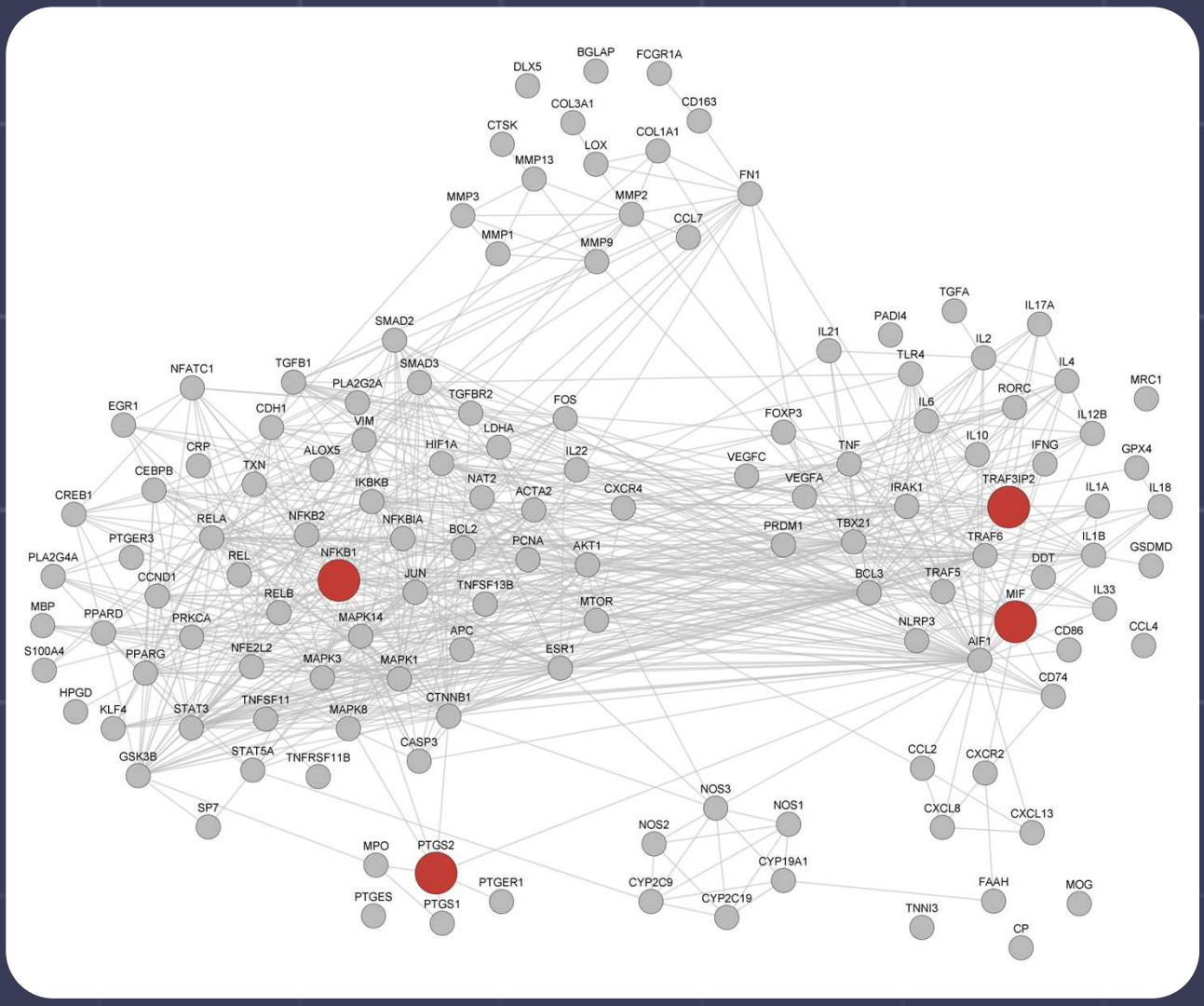


Figure 6: *The constructed iguratimod MoA molecular model with primary drug targets highlighted in red.*

5.3 Characterizing the iguratimod molecular MoA model

Over-representation analysis was performed to identify enriched Gene Ontology (GO) biological processes based on the set of 130 genes in the iguratimod molecular MoA model as well as on the sets of genes of the six network topological clusters as depicted in figure 7 below. Among the most significant enriched biological processes associated with primary targets were regulation of: inflammatory response, cytokine production and leukocyte activation, followed by T-cell activation, epithelial cell differentiation, response to: lipopolysaccharides and oxidative stress (Tab. 1).

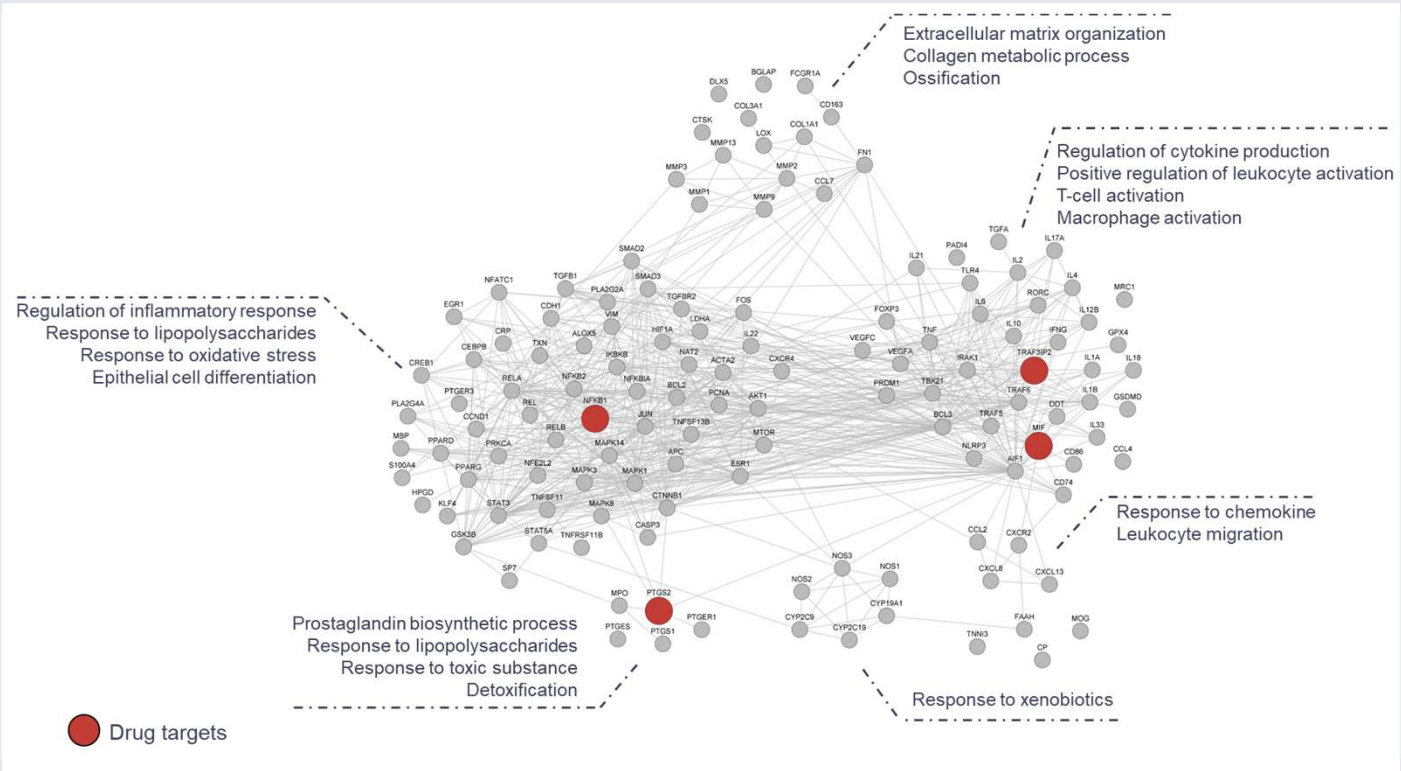


Figure 7: *Significantly enriched biological processes of the different iguratimod MoA modules of the constructed molecular MoA model.*

GO ID	GO Term	Adjusted p-value
GO:0032496	response to lipopolysaccharide	1.42E-14
GO:0006979	response to oxidative stress	6.10E-12
GO:0050673	epithelial cell proliferation	4.77E-12
GO:0050727	regulation of inflammatory response	1.17E-09
GO:0009636	response to toxic substance	6.77E-06
GO:0032496	response to lipopolysaccharide	1.62E-05
GO:0098754	detoxification	1.85E-06
GO:0031394	prostaglandin biosynthetic process	1.00E-02
GO:0009410	response to xenobiotic stimulus	6.76E-05
GO:1990868	response to chemokine	6.91E-08
GO:0002685	leukocyte migration	3.39E-06
GO:0001819	positive regulation of cytokine production	1.51E-28
GO:0042110	T-cell activation	1.45E-21
GO:0042116	Macrophage activation	1.63E-13
GO:0002696	positive regulation of leukocyte activation	1.76E-21
GO:0030198	extracellular matrix organization	5.52E-11
GO:0001503	ossification	1.54E-08
GO:0032963	collagen metabolic process	8.53E-11

Table 1: *Key enriched GO biological process terms based on a set of 130 genes in the iguratimod MoA model.*

We subsequently used co-annotation literature counts with each of the four primary drug targets of iguratimod for node color-coding with darker nodes indicating stronger enrichments in co-annotations with one of the four individual drug targets, respectively (Fig. 8). The most co-annotated genes to the targets were included into the iguratimod MoA model.

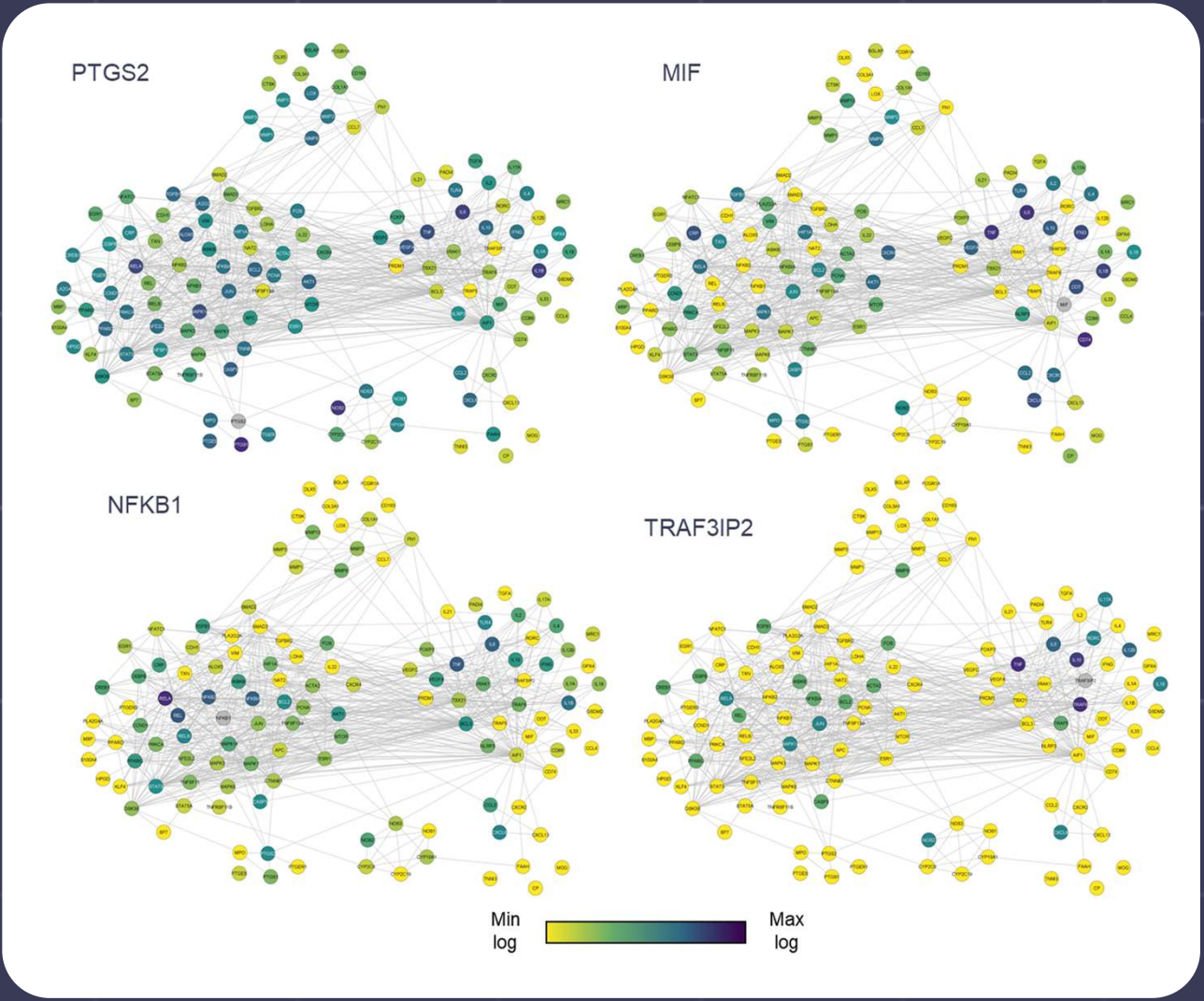


Figure 8: *The iguratimod MoA model with nodes highlighted based on co-annotation with one of the four primary drug targets respectively.*

6. Project step 2: Computational phenotype screening

In project step 2, a ranked list of indications showing significant overlap with the iguratimod MoA model was generated based on the computational network interference phenotype screen.

6.1 Delta4's phenotype search space

Delta4's phenotype library currently holds more than 3400[d] entries categorized into 19 broad categories as listed in the table below. Delta4's phenotype library is based on the Medical Subject Headings (MeSH) ontology (Tab. 2). It is manually curated to only hold entries of human diseases that can in principle be addressed by therapeutic intervention with drugs. Individual phenotype entries may be assigned to more than one category; e.g. the term "Liver neoplasms" that is assigned to "C01 Infections" as well as to the category "C06 Digestive System Diseases".

MeSH Tree	Disease Category	Description	# Models
C01	**Infections**	Invasion of the host organism by microorganisms or their toxins or by parasites that can cause pathological conditions or diseases.	582
C04	**Neoplasms**	New abnormal growth of tissue, tumorigenesis and metastasis.	575
C05	**Musculoskeletal Diseases**	Diseases of the muscles and their associated ligaments and other connective tissue and of the bones and cartilage.	243
C06	**Digestive System Diseases**	Diseases in any part of the gastrointestinal tract or the accessory organs (liver; biliary tract; pancreas).	251
C07	**Stomatognathic Diseases**	General or unspecified diseases of the stomatognathic system, comprising the mouth, teeth, jaws, and pharynx.	133
C08	**Respiratory Tract Diseases**	Diseases involving the respiratory system.	177
C09	**Otorhinolaryngologic Diseases**	Pathological processes of the ear, the nose, and the throat, also known as ENT diseases.	76
C10	**Nervous System Diseases**	Diseases of the central and peripheral nervous system.	644
C11	**Eye Diseases**	Diseases affecting the eye.	201

[d] The phenotype library in Delta4's platform is constantly evolving and the number of entries with each data-mining iteration is expanded.

MeSH Tree	Disease Category	Description	# Models
C12	**Urogenital Diseases**	Pathological processes of the urinary tract and the reproductive system.	289
C14	**Cardiovascular Diseases**	Pathological conditions involving the cardiovascular system.	397
C15	**Hemic and Lymphatic Diseases**	Hematologic diseases and diseases of the lymphatic system collectively.	265
C16	**Congenital, Hereditary, and Neonatal Diseases and Abnormalities**	Diseases existing at birth and often before birth, or that develop during the first month of life, regardless of causation.	568
C17	**Skin and Connective Tissue Diseases**	A collective term for diseases of the skin and its appendages and of connective tissue.	354
C18	**Nutritional and Metabolic Diseases**	A collective term for nutritional disorders resulting from poor absorption or nutritional imbalance, and metabolic disorders resulting from defects in biosynthesis or breakdown of endogenous substances.	324
C19	**Endocrine System Diseases**	Pathological processes of the endocrine glands, and diseases resulting from abnormal level of available hormones.	154
C20	**Immune System Disorders**	Disorders caused by abnormal or absent immunologic mechanisms, whether humoral, cell-mediated, or both.	215
C23	**Pathological Conditions, Signs and Symptoms**	Abnormal anatomical or physiological conditions and objective or subjective manifestations of disease, not classified as disease or syndrome.	501
F03	**Mental Disorders**	Psychiatric illness or diseases manifested by breakdowns in the adaptational process expressed primarily as abnormalities of thought, feeling, and behavior producing either distress or impairment of function.	159

Table 2: *List of categories in Delta4's phenotype library.*

The full library of more than 3400 phenotype terms was used in the ranking, but we excluded a specific set of phenotype categories in the final disease selection.

6.2 Molecular model interference analysis

The constructed drug molecular model served as input for the computational phenotype screening against Delta4's phenotype model library. Network-alignment algorithms were applied to generate a ranked list of phenotypes showing significant molecular model interference between the constructed drug molecular MoA model and the respective phenotype model.

The alignment of drug and phenotype networks was evaluated based on an array of parameters that were aggregated into a composite rank score:

- ***Node overlap***: number of shared nodes between drug and phenotype model; statistical effect size (odds ratio) and the corresponding p-value were derived from Fisher's exact test.
- ***Weighted network similarity***: node weights were assigned based on probability-adjusted gene-phenotype literature co-annotations. The weighted network similarity was calculated as the fraction of shared node weights between the drug and a given phenotype network relative to the sum of weights present in both. In this process, weights for the four primary iguratimod drug targets were augmented by a factor of two to promote scores for phenotypes that are directly associated with the drug targets relative to phenotypes lacking such a direct association and merely overlapping with non-target components of the drug model. To distinguish strong, significant similarities from coincidental ones, we estimated the statistical effect size and significance using permutation tests. Briefly, the observed similarity between a given drug-phenotype pair was compared to a simulated distribution of similarities that would be expected if node weights were distributed randomly between the shared and unshared nodes of the two networks. The effect size and significance increases with larger distance of the observed similarity to the center of the simulated random distribution.

We ranked each phenotype based on the node overlap and weighted network similarity with the iguratimod drug model, and the corresponding statistical effect sizes. Subsequently, we calculated a composite sum-rank aggregating all individual ranks to represent the overall ranking score of the individual phenotypes based on the network alignment screening (Fig. 9).

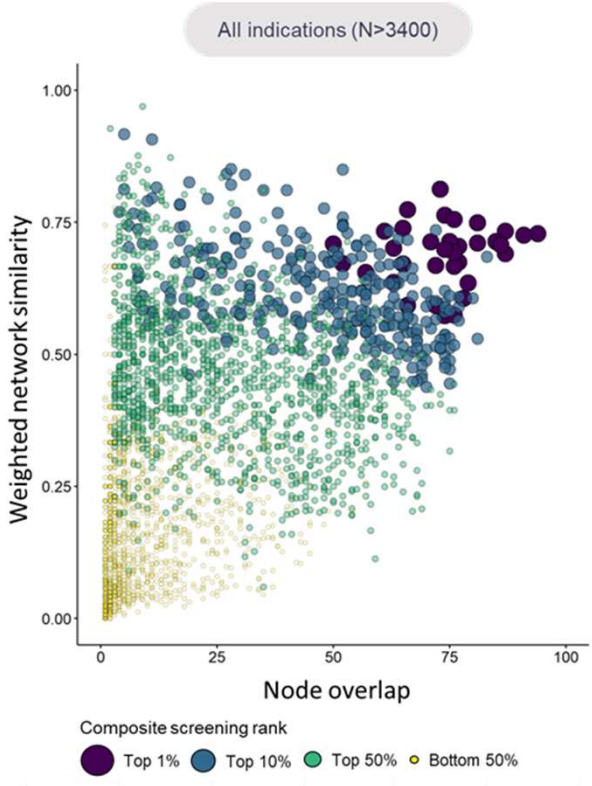


Figure 9: *Results of the weighted network alignment algorithms.*

6.3 Evaluation of the “known” indications

Further indication screening was based on an aggregate score of all the alignment parameters established in the previous step. The set of “known” indications consisted of the approved indications, those in clinical trials as well as the ones showing co-annotation in scientific literature with attention to functional connection. Additionally, to the list of known indications those that were mentioned in scientific or patent literature were added. The figure below shows the disease landscape of indications seeing annotation evidence with iguratimod in scientific literature (Fig. 10). The broadest array of indications that are currently under investigation for iguratimod belong to the following MeSH Tree Numbers: Muscoloskeletal Diseases (C05), Respiratory Tract Diseases (C08), Skin and Connective Tissue Diseases (C17), Immune System Diseases (C20) and Pathological Symptoms (C23).

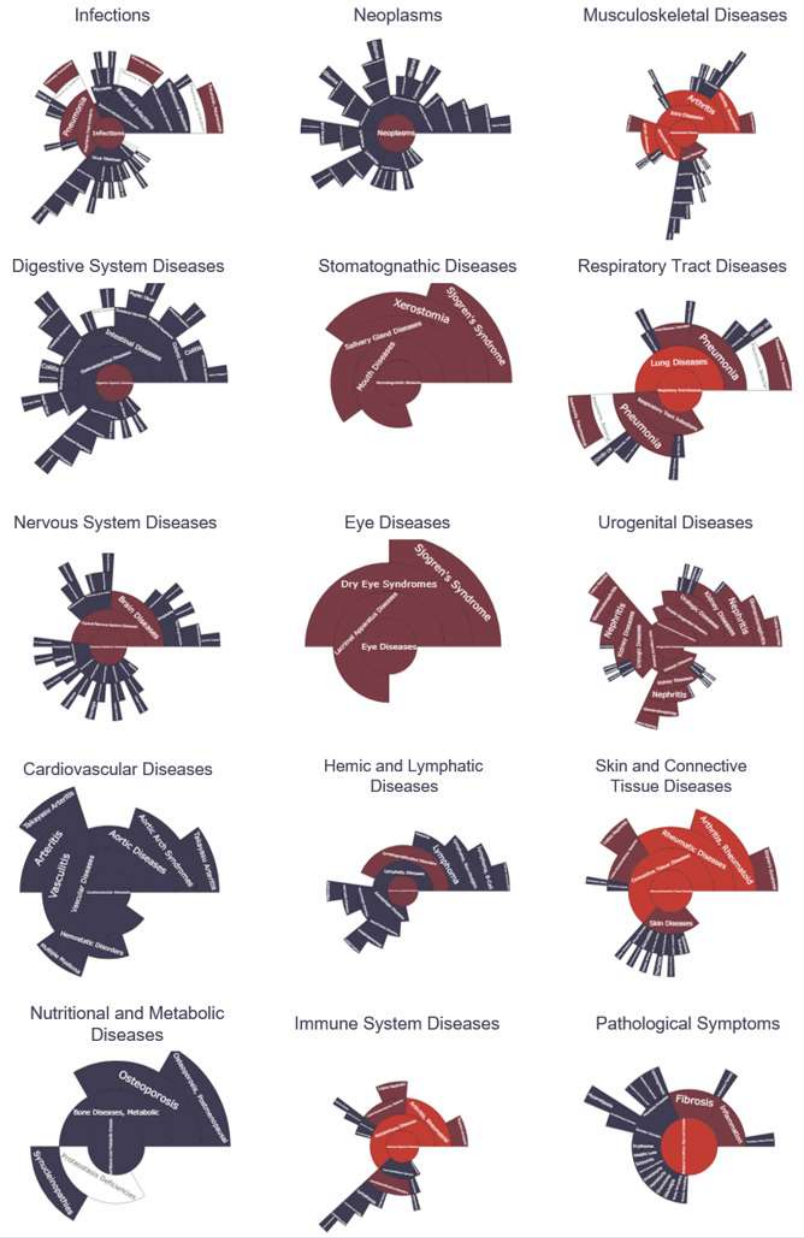


Figure 10: *Phenotype sunburst plot presenting “known” indication space of iguratimod.*

Screening results of “known” indications are graphically displayed in the figure below. Each data point represents a disease/indication, which are plotted along two of the key parameters used to evaluated the network interference with iguratimod drug model, the color intensity of the data points indicates the overall ranking of the indication in the screening based on an aggregate score of all the alignment parameters. Indications that are in clinical trials are situated at least in the top 50%, most of them in the top 10% of screening ranking (Fig. 11).

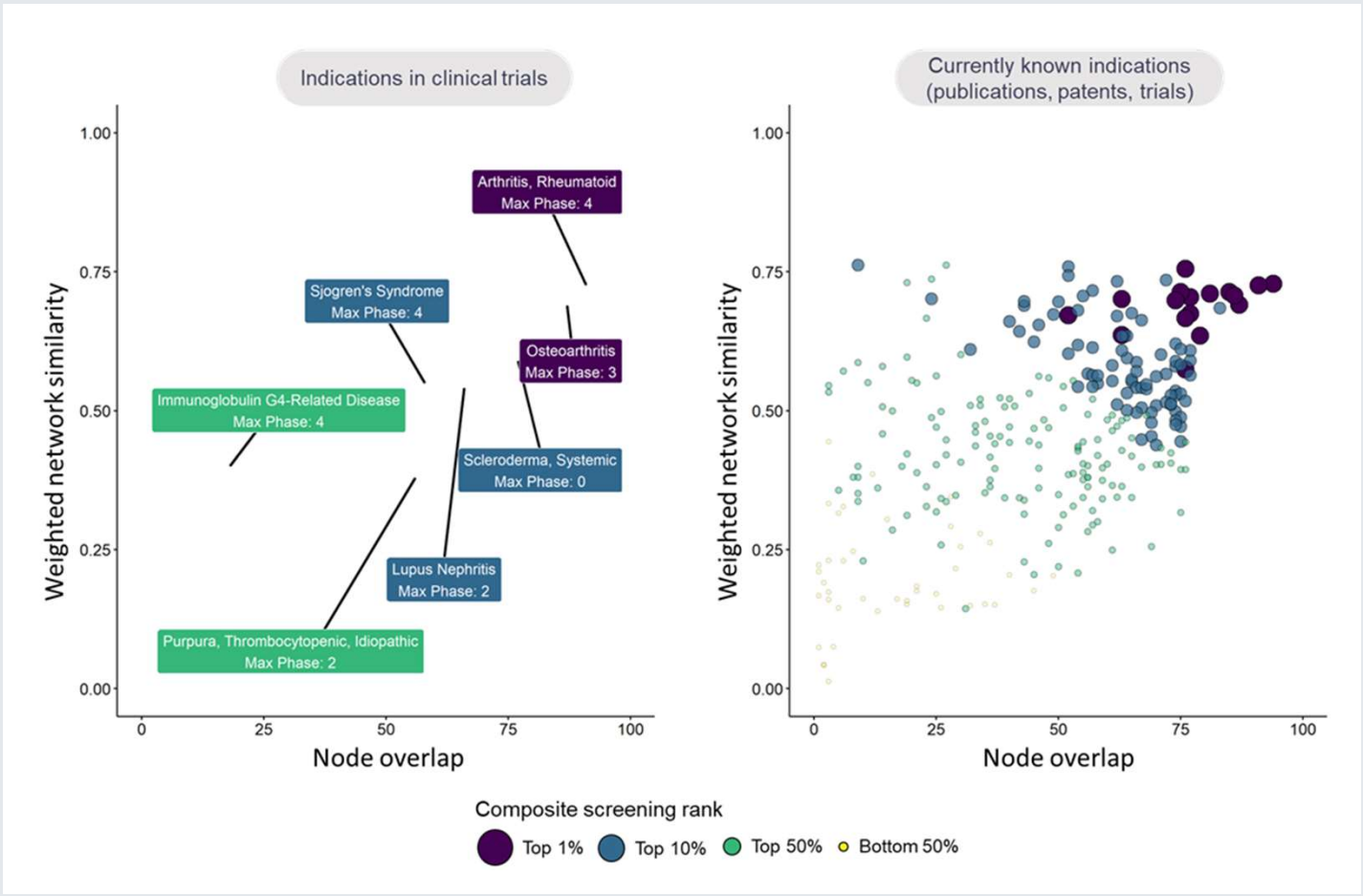


Figure 11: *Screening results of the “known” indications.*

6.4 Generating the phenotype ranking of novel indications

In project step 3, the phenotype ranking was generated, i.e. the set of indications with relevant disease mechanisms and processes being significantly affected by the drug MoA based on interference of the phenotype molecular model and the respective drug molecular MoA model (Fig. 12).

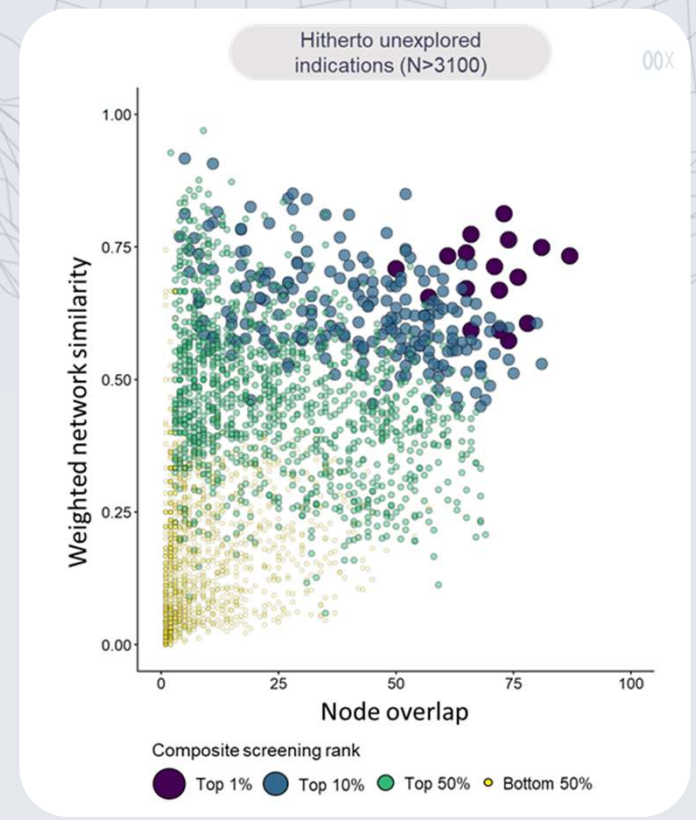


Figure 12: *Phenotype screening results of potentially "novel" indications hits for iguratimod.*

We further narrowed down the top diseases by focusing on the relevance of iguratimod's primary drug targets in the particular diseases. To this end, we initially selected the top 10% of the phenotypes based on the composite screening rank derived from the network alignments. Subsequently, we calculated probability-adjusted literature co-annotations of those phenotypes with the four iguratimod drug targets. The phenotypes were ranked based on the co-annotations of the drug targets with a given phenotype relative to all other co-annotated genes, as well as co-annotations of a phenotype within the drug targets relative to all other co-annotated phenotypes. Based on this two-sided ranking, we selected those phenotypes that ranked in the top 20% in terms of co-annotations with either of the four primary drug targets for further evaluation (Fig. 13).

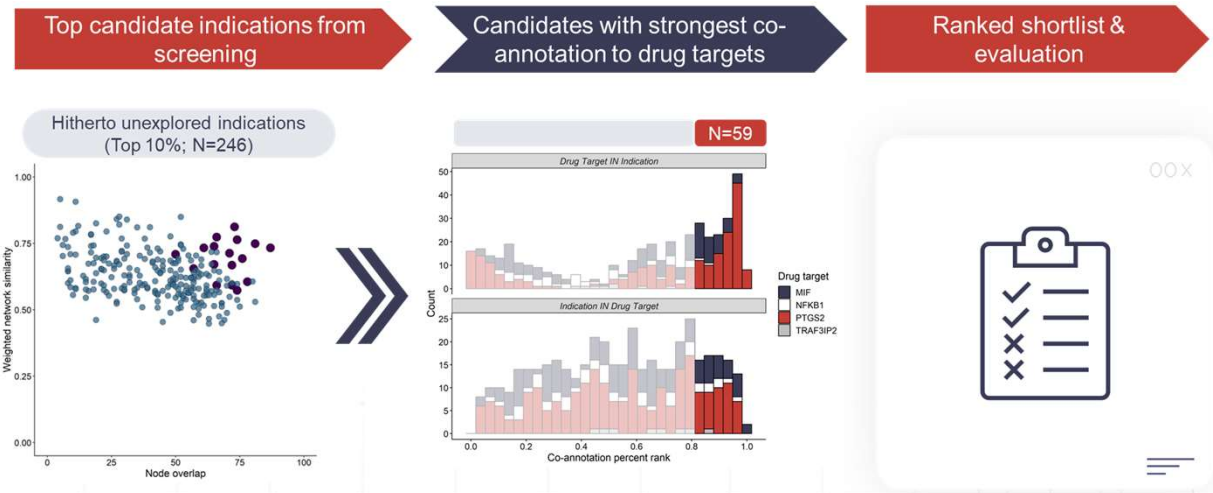


Figure 13: *Phenotype screening process (indication shortlisting).*

The figure below shows an excerpt of the ranked list containing the 59 indications that were selected for further in-depth evaluation (Fig. 14). During this detailed evaluation, additional criteria beyond the network alignment-based screening were taken into consideration. These steps involved:

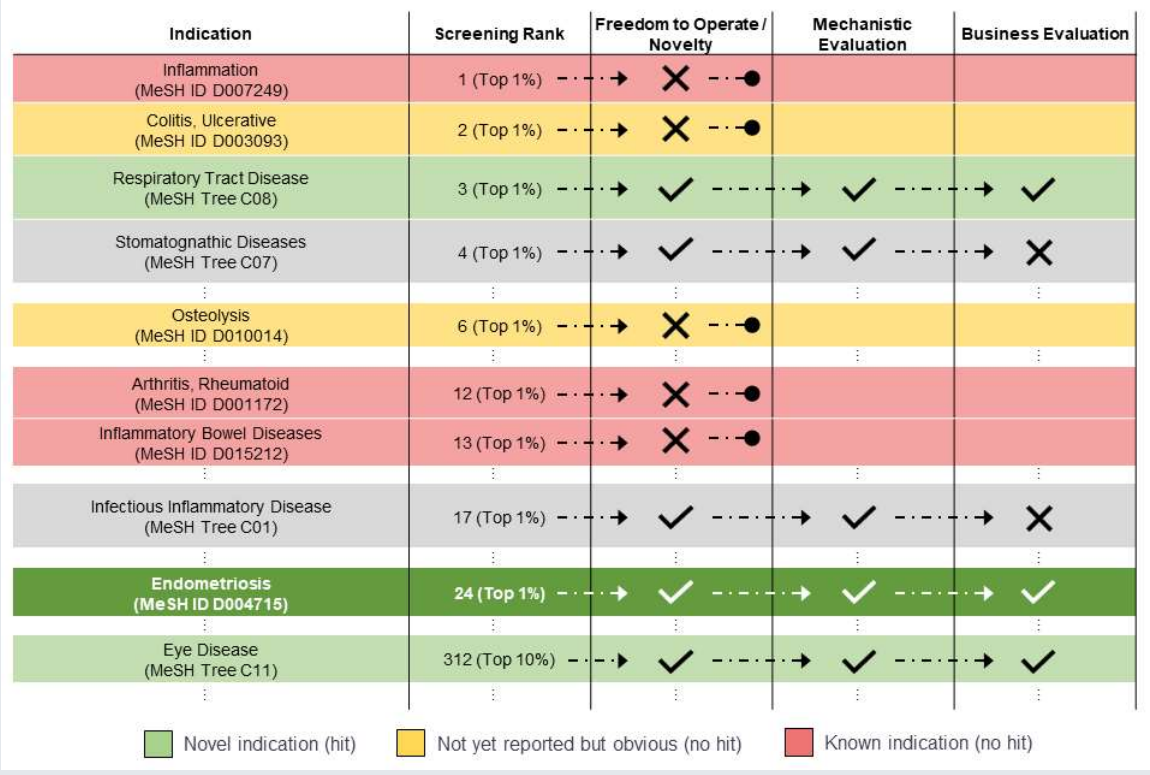
1) assessments of the novelty of the application of iguratimod in a particular indication or disease area[e];

2) a mechanistic evaluation of the hypothesized MoA and efficacy in a disease from a molecular biological perspective; and

3) a business evaluation based on criteria such as medical need, competitor landscape, etc.

Each column in the table below schematically represents the outcome of each of these three evaluation steps following the network-based screening. Indications that were deemed unfavorable at a given evaluation step were not considered further for the next step, as indicated by an "X" in the respective column and a blunt-headed arrow. Approved indications (highlighted in red) as well as indications from a closely related disease area –and hence obvious applications for iguratimod (highlighted in orange) – did not pass the novelty assessment and thus were not considered further. Other indications that passed subsequent evaluations but were deemed unattractive from a business standpoint are marked in grey. The three indications that ultimately passed each evaluation step and were thus considered promising new applications for iguratimod are shown in green, with particular emphasis on endometriosis highlighted in dark green.

Figure 14: *Excerpt of the ranked list containing the 59 indications that were selected for further in-depth evaluation. Novel indications holding potential for further development of iguratimod are shown in green with endometriosis in dark green as selected example to outline project step 3 results.*



[e] The definition of what is considered "novel" by a customer is usually defined in the project kick-off meeting between Delta4 and representatives from the customer as this definition might substantially vary.

We selected endometriosis to showcase our mechanistic analyses within project step 3 as described below. In a typical customer project, up to five indications are usually evaluated in project step 3.

7. Project step 3: Mechanistic evaluation of selected indications

7.1 Endometriosis

Endometriosis is a syndrome characterized by the presence of endometrial-like tissue outside the uterus plus associated symptoms. These symptoms include inflammation, severe pain and fibrosis in the pelvis and reduced fertility. Of note, up to 50% of asymptomatic women might also have endometrial lesions causing no problems[14]. Endometriosis affects 6-10% of women at reproductive age globally and can start with the first menstrual period and last until menopause [15,16]. There is currently no therapeutic cure for endometriosis. Surgery is often used to remove extra-uterine tissue and treatment focuses on the amelioration of pain and inflammation.

While the exact molecular causes of endometriosis and the associated pain are still widely elusive, dysregulation of several molecular pathways impacting endometriosis have been described. These include steroid hormone (particularly GnRH/estrogen) signaling and the VEGF-, Wnt-, PI3K/mTOR-, TGF-β, NF-κB-, COX- pathways [14,17–21].

7.1.1 Mechanistic evaluation

The molecular model for endometriosis in Delta4's phenotype library consists of 420 proteins.

In terms of interference with endometriosis, iguratimod inhibits the NF-κB, and COX signaling pathway[5]. Furthermore, iguratimod interferes with MIF, IL-1β, IL-17 and NLRP3 signaling, which might all contribute to endometriosis[5,22] (Tab. 3).

GENE	PREDICTED EFFECT IN ENDOMETRIOSIS
NF-κB (RELA, NFKB1)	inhibition interferes with ectopic lesion growth, glandular hyperplasia, and interstitial inflammation[23]; is dysregulated by sex hormones in endometrial diseases and promotes proliferation, inflammation and immune modulation[20]
COX2 (PTGS2)	can contribute to proliferation and pain[24]
MIF	can contribute to proliferation and inflammation[25]
IL-1b	promotes inflammatory pain and neuro-angiogenesis[26]
IL-17A	regulates the immune microenvironment, the invasion, and growth of ectopic lesions[27]
NLRP3	inhibition can reduce lesion size and inflammation[28,29]

Table 3: Relevance of key genes in endometriosis.

Furthermore, outside the endometriosis context, targeting TRAF3IP2 has been shown to ameliorate inflammation and angiogenesis and downregulate several genes associated with endometriosis[30–32].

On the process level, the endometriosis model is enriched in key biological processes such as tissue remodeling, macrophage activation, migration and chemotaxis, I-kappaB kinase/NF-κB signaling, regulation of the Wnt signaling pathway, the prostaglandin metabolic process and the stress-activated MAPK cascade (Fig. 15). Several key pathological processes characterizing endometriosis are linked to iguratimod's primary targets, for example NF-κB signaling and prostaglandin metabolic processes. This corroborates the hypothesis that iguratimod might exert beneficial effects on endometriosis.

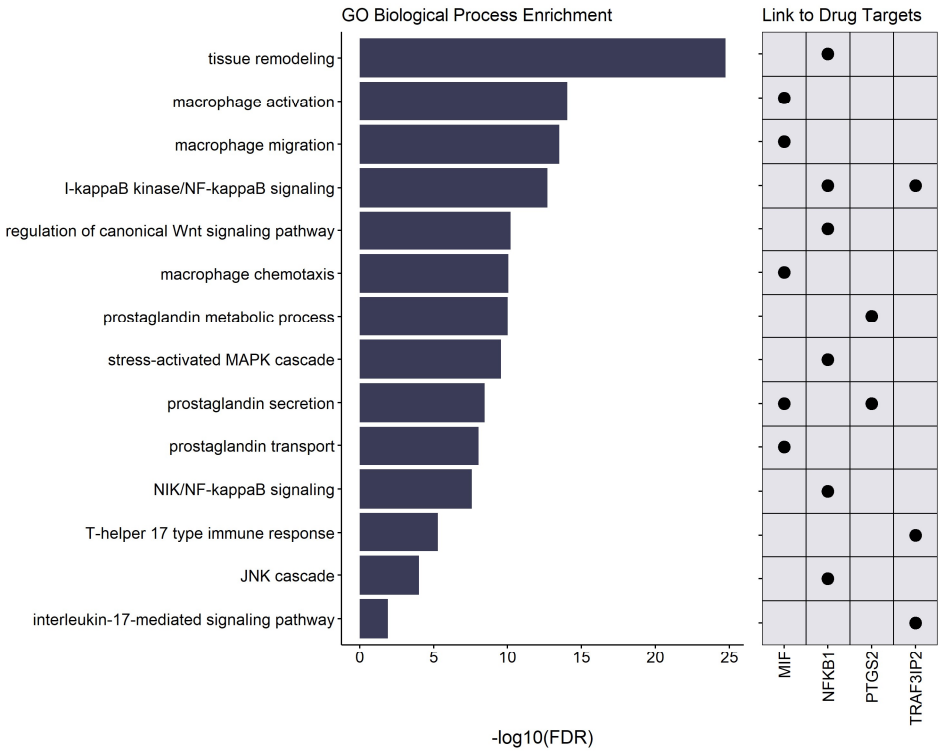


Figure 15: Enriched GO bps based on the endometriosis molecular model feature set and association with iguratimod's direct drug targets.

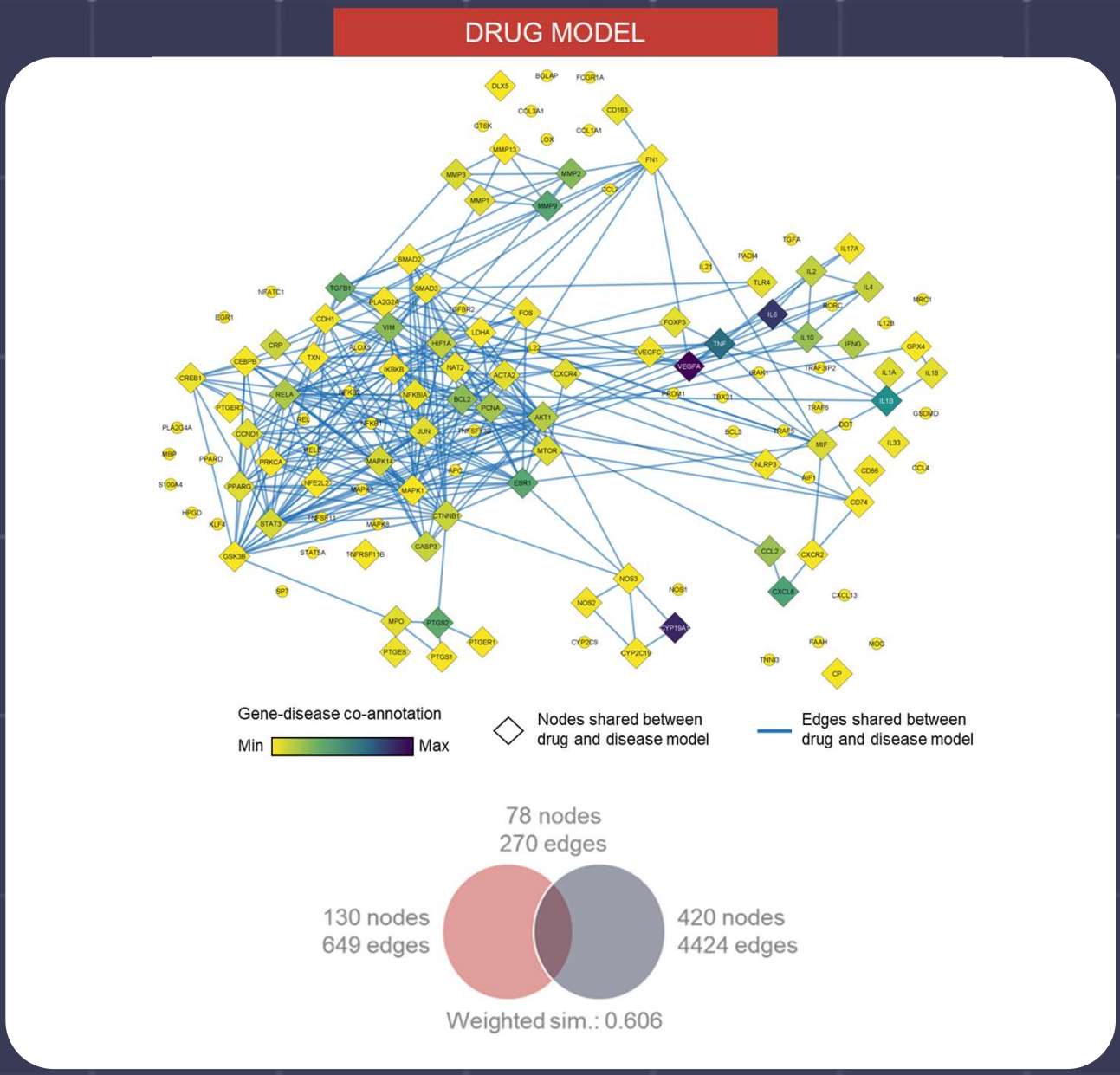


Figure 16: *Interference analysis between the iguratimod MoA model and the endometriosis molecular model shown on the level of the iguratimod MoA model.*

Network interference analysis is displayed on the iguratimod MoA model as well as on the endometriosis model. The two presented models show a significant overlap (match) in their network architecture and a high similarity score weighted by gene-disease associations (Fig. 16 and 17). The interference analysis depicted a coverage of 78 nodes and 270 edges between the iguratimod MoA model and the endometriosis model. Nodes enrichment analysis of endometriosis MoA indicated regulation of macrophage activation, prostaglandin metabolic process and secretion, which were also highlighted in the biological processes linked to iguratimod drug targets.

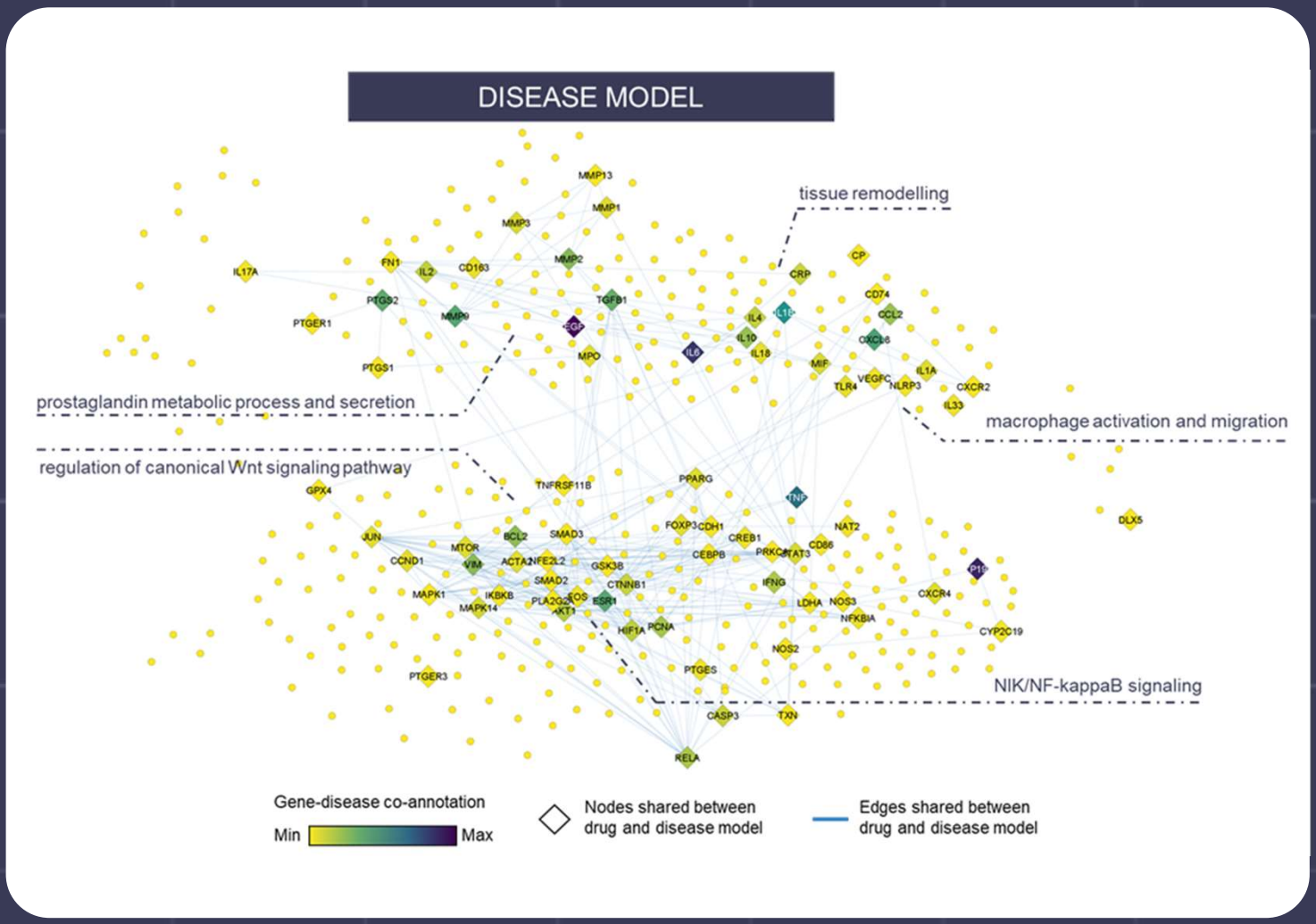


Figure 17: *Interference analysis between the iguratimod MoA model and the endometriosis molecular model shown on the level of the endometriosis molecular model.*

7.1.2 Drug combination analysis with respect to SoC

Currently, there is no cure and no known way how to prevent endometriosis. Surgery can be used to alleviate pain by removing endometrial tissue, dividing adhesions, or removing cysts. Targeting COX1/2 signaling via NSAIDs and GnRH analogues are therapeutic options to improve endometriosis-associated pain[14,33].

The primary effect of iguratimod is clearly distinct from the SoC against endometriosis, namely nonsteroidal anti-inflammatory drugs (NSAIDs: targets COX/PTGS) or neuromodulators (amitriptyline, duloxetine: target SLC6A4, SLC6A2) against pain and GnRH modulators used for ovarian suppression[14]. However, as NF-κB can be inhibited by sex hormones[34] and iguratimod in addition has an inhibitory effect on PTGS2, there is a partial overlap between the predicted effect of iguratimod and the SoC (Fig. 18).

In line with this, it appears a priori plausible that iguratimod could potentially be beneficial as stand-alone therapy but also as an add-on therapy to SoC treatment. Iguratimod shows strong molecular interference with the inflammatory aspects of endometriosis. Furthermore, iguratimod has been shown to ameliorate inflammation-associated pain in other contexts before[35,36]. Of note, due to inhibition of COX-2 and PGE2 signaling, iguratimod might not be ideally suitable for women who want to become pregnant[37,38].

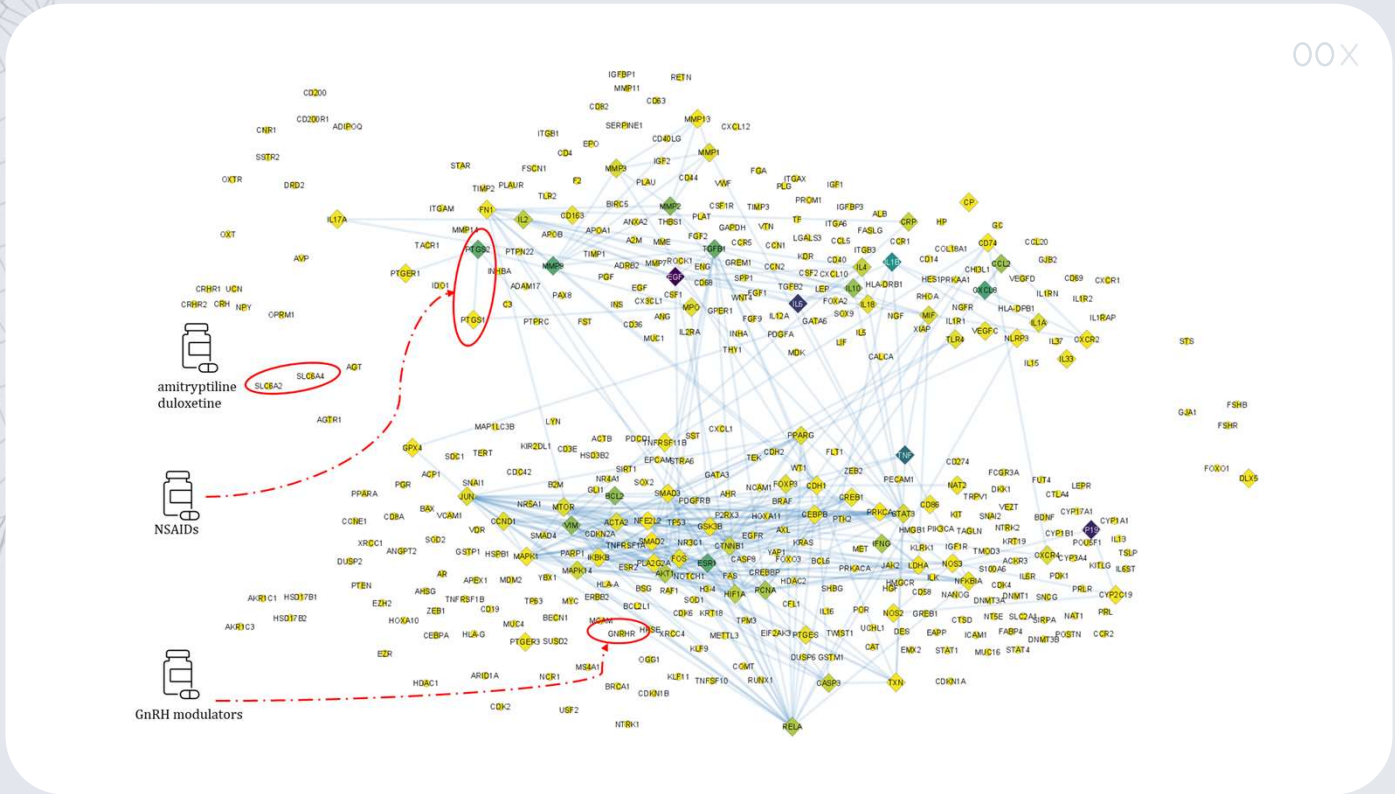


Figure 18: *Impact of SoC on endometriosis shown in the context of the phenotype molecular model. Genes of the molecular model being affected by treatment are outlined by a red circle.*

Among other effects, iguratimod has been described as an inhibitor of the NF-κB signaling pathway. NF-κB is involved in a plethora of cellular processes. Therefore, caution has to be taken with dose-dependent side effects. However, this risk might not be too high as iguratimod has been approved against rheumatoid arthritis for many years and another drug with NF-κB inhibiting effect, omaveloxolone, was recently approved against Friedreich's ataxia[39].

7.1.3 Proposal for biomarker candidates

Information on endometriosis biomarker candidates was extracted from Delta4's Hyper-C platform. The table 4 below holds the most promising biomarker candidates holding annotation as endometriosis biomarker candidates also being at the interference of endometriosis pathophysiology and the iguratimod MoA. These biomarkers therefore are candidates to be measured and evaluated in further preclinical activities of iguratimod in endometriosis.

BIOMARKER	PROGNOSTIC / MECHANISTIC	SAMPLE	ASSAY	EVIDENCE*
ESR1	+/-	peripheral blood	qRT-PCR	human[40]
CTNNB1	+/-	peripheral whole venous blood	qRT-PCR	human[41]
AKT1	+/-	endometrial stromal fibroblasts; ectopic endometriotic lesions	qRT-PCR; western blot (phosphorylation)	mouse[42], human[43]
IL6	+/+	peritoneal fluid; blood	ELISA; qRT-PCR	human[44,45]
VEGFA	+/+	eutopic and ectopic endometrial tissue	qRT-PCR; western blot	human[46,47]
TNF	+/+	Serum	Bioplex Protein Array	human[48]
CXCL8	+/+	peritoneal fluid	ELISA	human[49]

Table 4: *List of potential biomarkers predicted to be regulated by iguratimod in endometriosis.*

(reported marker evidence either in the human setting or in animal models)*

Of note, there are currently no protein biomarkers approved for clinical use in endometriosis as pointed out by a large-scale meta-analysis[50]. In this study the authors systematically reviewed over 100 potential blood biomarkers in the context of endometriosis across 141 studies with more than 15,000 participants in total. None of these biomarkers appeared to have sufficient accuracy to be used clinically outside a research setting. Nevertheless, the biomarkers listed above might still add value in monitoring the impact of iguratimod on relevant processes driving endometriosis.

7.1.4 Feasibility aspects regarding further development

Next to the mechanistic fit of drug MoA and disease pathophysiology as discussed in the previous sections, the feasibility aspect is an important factor when considering whether to further develop a compound for a selected disease. We therefore evaluated the availability of preclinical in vivo models for endometriosis and analyzed key performance indicators of clinical trials on endometriosis.

Availability of preclinical in-vivo models

Rodent models seem to be the best option to model endometriosis. While rodents also have menstruation, the complexity of the syndrome might complicate straightforward conclusions[51,52]. In general, animal models of endometriosis mostly investigate the reduction of cell proliferation and lesion size, measure parameters related to apoptosis, or protein and gene expression data, and most of clinical trial treatments aim to only alleviate pain. Hence, from the point of view of therapy for human endometriosis, these models have provided insufficient results. Several studies refer to the procedure of ovarian endometriosis induction as given below (Tab. 5).

SPECIES	PROCEDURE	REFERENCE
Mouse	ovariectomy with estrogen supplement, solely endometrium fragments, intraperitoneal injection	53
Rat	surgical insertion of ovarian endometrioma, induced diffuse intraperitoneal endometriosis, extraperitoneal endometriosis	54
Rat	surgery to initiate endometriosis on the abdominal wall	55
Mouse/Rat	Homologous Models Heterologous Models	52

Table 5: *List of endometriosis animal models.*

Clinical trial duration and patient enrollment

We investigated all listed ongoing clinical phase II and phase III trials on endometriosis and assessed the average number of trial duration as well as the number of enrolled patients. In a typical trial setup, the amelioration of pain due to a tested drug will be evaluated in a 12-week-course. The primary readout parameters in conducted trials are related to the measurement of pelvic pain, severity in dysmenorrhea and in urinary symptoms, fertilization rate and live birth rate.

Clinical phase II ongoing endometriosis trials (n=16) on average run for 2.0 years ranging from 2.5 months to 4.8 years at maximum, whereas average duration of completed trials was 4.9 years. The average number of enrolled patients for clinical phase II trials is 78, ranging from 10 to 400.

Clinical phase III ongoing endometriosis trials (n=14) on average run for 3.6 years ranging from 1.4 to 6.5 years at maximum, whereas average duration of completed trials was 10.4 years. The average number of enrolled patients for clinical phase II trials is 294, ranging from 10 to 1020.

7.1.5 Business aspects regarding further development

Market size

The prevalence of endometriosis is estimated to affect 6-10% of reproductive women globally. The global endometriosis market was valued at $1.2 billion in 2021 and is projected to reach $3.9 billion by 2031, growing at a CAGR of 12.6% from 2022 to 2031. The main driver of dynamic endometriosis market development is patients' awareness of this disease's treatment options. Additionally, government agencies are investing in healthcare infrastructure to provide better treatment facilities to the women suffering from endometriosis.

Competitors

The figure 19 depicts the competitor landscape in the field of endometriosis considering granted patents and ongoing clinical trials in Phase II and/or Phase III sponsored by companies. Bayer has the highest number of granted patents in the field of endometriosis and additionally is currently running Phase IV with application of dienogest, which is an orally-active semisynthetic progestogen acting as an agonist at the progesterone receptor (PR) (NCT04808843). Merck, the second company with numerous patents on the list, is currently not developing any treatments in clinical trials. Among other top listed companies with a high number of patents is AbbVie, who is the sponsor of two clinical trials in Phase III aiming to ameliorate moderate to severe pain associated with endometriosis. On the top of the clinical Phase II and/or Phase III trials sponsors list in terms of its number are Eunice Kennedy Shriver National Institute of Child Health and Human Development and Myovant Sciences GmbH (Tab. 6).

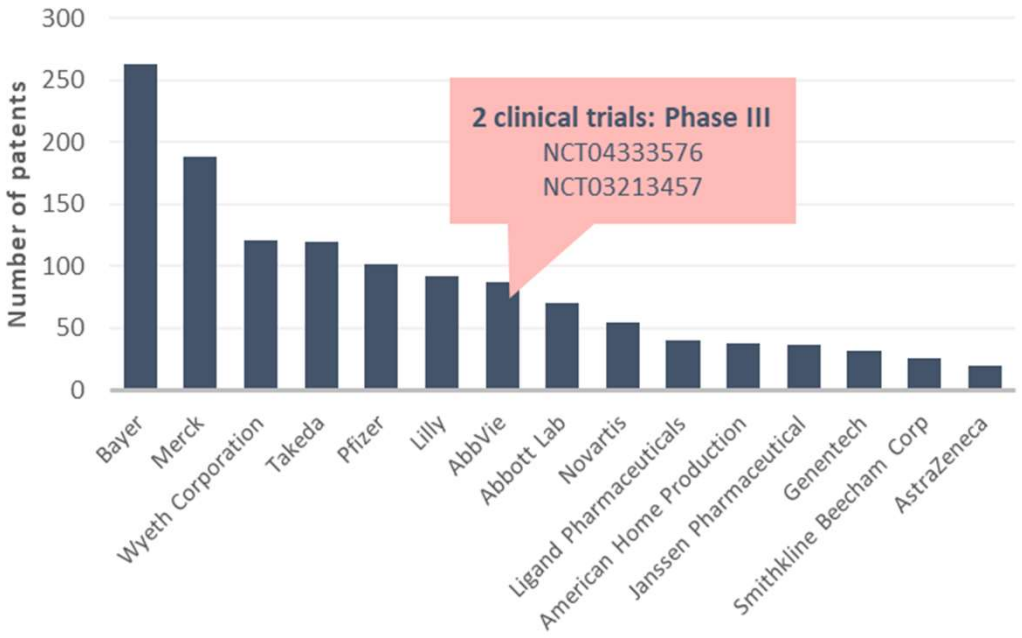


Figure 19: *Companies with endometriosis patents and/or running trials against endometriosis in clinical Phase II and/or III.*

SPONSOR	Number of clinical trials	
	Phase II	Phase III
Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)	2	1
Antaros Medical	1	-
Biós Farmacêutica	1	-
Enteris BioPharma Inc.	1	-
Hera Biotech, Inc.	1	-
Hope Medicine Inc.	1	-
IQVIA	1	-
ONE Fertility	1	-
Organon & Co	1	-
Parexel	1	-
Science Valley Research Institute	1	-
Spago Nanomedical AB	1	-
Syneos Health	1	-
TiumBio Co., Ltd.	1	-
AbbVie	-	2
Myovant Sciences GmbH	-	2
Kissei Pharmaceutical Co., Ltd.	-	1
Qilu Pharmaceutical (Hainan) Co., Ltd.	-	1
Royan Institute	-	1

Table 6: *Company-sponsored clinical Phase II and/or Phase III trials against endometriosis.*

Appendix A: References

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Appendix B: Abbreviations

COX2	cyclooxygenase 2
DMARD	disease-modifying anti-rheumatic drug
GnRH	gonadotropin-releasing hormone
GO	gene ontology
GO bp	gene ontology biological process
IGU	iguratimod
ITP	idiopathic thrombocytopenic purpura
MeSH	Medical Subject Headings
MIF	migration inhibitory factor
MoA	mechanism of action
NFKB1	nuclear factor kappa B 1
NSAID	non-steroidal anti-inflammatory drug
PPI	protein-protein interaction
PTGS2	prostaglandin-endoperoxide synthase 2
RA	rheumatoid arthritis
SoC	Standard of Care
TRAF3IP2	TRAF3 interacting-protein 2

Appendix C: List of figures

Appendix D: List of tables

Appendix E: Supplementary data files

Supplementary datafile 1: Iguratimod MoA model file

Supplementary datafile 2: Phenotype screening result matrix

Supplementary datafile 3: Model interference file for endometriosis and Iguratimod MoA