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Signaling networks in MS: A systems based approach to developing new pharmacological therapies

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Abstract

The pathogenesis of MS involves alterations to multiple pathways and processes, which represent a significant challenge for developing more effective therapies. Systems biology approaches that study pathway dysregulation will offer benefits by integrating in molecular networks and dynamic models with current biological knowledge for understanding disease heterogeneity and response to therapy. In MS, abnormalities have been identified in several cytokine signaling pathways, as well as those of other immune receptors. Among the downstream molecules implicated are Jak/Stat, NF-Kb, ERK1/3, p38 or Jun/Fos. Together, these data suggest that MS is likely to be associated with abnormalities in apoptosis / cell death, microglia activation, blood-brain barrier functioning, immune responses, cytokine production, and/or oxidative stress. While current MS drugs target some of these pathways, others remain untouched. Here, we propose a pragmatic systems analysis approach that involves the large-scale extraction of processes and pathways relevant to MS. This data serves as a scaffold upon which computational modeling can be performed to identify disease subgroups based in the contribution of different processes. Such an analysis, targeting these relevant MS signaling pathways, offers the opportunity to accelerate the development of novel individual or combination therapies.

Key words

Multiple Sclerosis, pathways, signaling, systems biology, drug discovery

Introduction

Multiple Sclerosis (MS) is a chronic disease that is known to involve both inflammatory and neurodegenerative responses. Despite the significant progress made in recent decades, we are still relatively far from achieving a comprehensive understanding of the pathogenesis of this disease. The revolution in molecular biology, immunology and genetics, along with the development of new high-throughput technologies has driven the production of large amounts of data in recent years. However, while numerous genes and proteins have been associated with the disease, significant gaps remain in the quest to understand the pathological mechanisms responsible for MS. Although there is still hope that new studies will reveal specific genes, proteins or cells that will explain an important proportion of the causes of the disease, current perspectives suggest that we already have identified the majority of cells and molecules involved, and that what is urgently needed is to integrate the available and any future data into a comprehensive dynamic picture of MS[1].

Unfortunately, knowing that a gene or cell type is associated with MS is far from providing an explanation about the disease. This is related to the fact that genes or proteins associated with MS to date are not mutated and therefore they do play the physiological role expected for them, complicating the analysis[2]. Moreover, in a complex disease such as MS, genes, proteins and cells dynamically interact with each other in response to the stimuli and challenges the immune and nervous system face[3]. This quantitative and dynamic information is extremely difficult to capture from patients and even from animal models. Second, each individual harbors a different genetic background and also the development of the immune and nervous system is customized for their environment during development, being one of the basis of disease heterogeneity. Therefore, without the integration of molecular information in pathways and considering molecular and cellular heterogeneity, it will be difficult to achieve a good understanding of the pathogenesis of MS. At the individual patient level, it will be critical to collect personalized data to customize the analysis to pave the way towards stratified medicine.

Systems biology approaches may offer important benefits integrating current biological knowledge with clinical information and data on therapeutic responses, thereby allowing models to be generated that might help explain the pathogenesis of the disease[3, 4]. Therefore, in this review we will focus on how a systems biology approach applied to medicine (systems medicine) from the pathway perspective, incorporating molecular information about MS pathogenesis and drug targets, could improve our understanding of the disease and help in the development or identification of new improved therapies.

Pathways regulating MS pathogenesis: a puzzle of the immune system, the CNS and missing pieces

Decades of cellular and molecular research in the field of MS have revealed many genes, proteins and cell subpopulations of the immune system associated with the disease, and such information has expanded massively with the new omics technologies. In order to identify the pathways involved in a given disease, abundant information is available in databases such as Gene-Disease Association Database, the Protein Sequence Database, the Comparative Toxicogenomics Database, the Online Mendelian Inheritance in Man, the Genetic Association Database, or the Literature-derived Human Gene-Disease Network. Moreover, genetic susceptibility for MS has been revealed by genome association studies (GWAS) and the ImmunoChip study in MS, which have identified more than 100 SNPs associated with the disease[5, 6], which have been implicated mainly in immune system pathways (leukocyte activation, apoptosis, and positive regulation of macro-molecule metabolism, JAK-STAT signaling pathway, acute myeloid leukemia, and T cell receptor signaling)[7]. Moreover, several databases containing information about pathways are available such as the Kyoto Encyclopedia of Genes and Genomes (KEGG), Reactome, PathwayCommons or the ConsensusPathDB. Finally, chemoinformatic resources such as DrugBank, ChEMBL and Drug Information Online contain information about drugs, including their targets within human pathways. By combining the information available in these databases, more than 40 pathways associated with MS can be found (Fig. 1A). The overall picture obtained reveals the involvement of a wide range of cellular processes and pathways implicated: apoptosis / cell death, microglia activation, blood-brain barrier functioning, immune response, cytokine production, or oxidative stress. In addition, the search of these cellular processes can be combined with the targets of MS treatments, such as fingolimod, dimethyl-fumarate or interferon-beta. The targets of these therapies can be included by identifying the pathways that link them to the processes above, namely lipid-mediated signaling and its crosstalk with survival and NF-Kb pathways, antioxidant pathways and the Stat-mediated IFNb response, respectively. Interestingly, pathway analysis revealed certain

processes that are not yet targeted by current therapies, e.g. the Notch pathway [8-10]. Another pathway related with MS is vitamin D metabolism, and at present there are several trials testing the efficacy of vitamin D supplementation [11].

New roles in MS of components in known pathways

Once data is retrieved from databases, bioinformatic tools allow identification of interactions between genes, proteins and cells that can be used as hypotheses (Fig1B). For instance, and of pivotal clinical importance, these tools help to study the involvement of CNS pathways in MS pathogenesis. MS is a condition associated with substantial neuronal and axonal damage, and this neurodegeneration probably drives long-term neurological disability[12]. Hence, pathways that are related to neuronal death and axonal damage may be of particular interest for the development of neuroprotective therapies, an approach pursued for decades without success to date. Because our current understanding of the immune system is significantly more advanced than that of the CNS, automatic searches of databases have been likely to reveal significantly more pathways associated with the immune system than the nervous system. Moreover, the study of MS has been focused strongly on its immune aspects, as well as on the development of immunomodulatory medicines that target the inflammatory process and prevent relapses. Fortunately, the picture is now changing, and some of the pathways associated with MS relate to CNS damage, including apoptosis, oxidative stress, or microglia activation. Potential neuroprotective therapies under development are aimed to target such pathways such as green tea catechin epigallocatechin-gallate, trophic factors, Methylthioadenosine or drugs enhancing remyelination [13-16].

Patient-to-patient genetic variability hinders understanding of signalling pathways involved in MS.

It is striking that the recent massive genetic studies (e.g. GWAS, Immunochip) explain so little of individual disease risk[5, 6]. It is also surprising that no single MS therapeutic yet has had well

validated genetic stratification. We believe that this fact, common to many complex diseases, is at least in part due to the lack of a functional, network-based perspective to the pathogenesis of the disease. Signaling networks are very robust to variation in cells and their environment to enable cellular functioning. For instance, it has been shown that even clonal populations strongly vary in the concentration of the same protein [17]. The cellular function regulated by that protein needs to remain unaltered for healthy cellular behavior, therefore a number of network motifs grant robustness to signaling networks such as negative feedback loops [18]. Other variations to which signaling pathways can be robust are genetic polymorphisms. Therefore, including genotyping data when modeling signaling pathways of MS patients is key to understanding MS pathogenesis.

Furthermore, some pathways may not be etiologically relevant because they are associated due to co-segregation of alleles with diseases. One approach for integrating the role of genetic susceptibility in systems biology methods is by considering that risk alleles mildly modify the parameters governing the functioning of the pathways. Therefore, one single allele may not have a significant effect in a given pathway, but the collection of all the risk alleles in a given individual may influence the function of immune pathways to the level of producing autoimmune activation. These considerations may help to improve the prediction of autoimmune response at the individual level. The same reasoning may apply to the fact that no drug stratifies by any single risk allele, although this may change if new therapies target MS associated genes and one of the alleles modulate the biological effects of the drug.

In summary, understanding how each individuals' genetic polymorphisms lead to their specific signaling network activity would enable characterization of the different MS phenotypes. This would, however, suggest a further question: how does patient-to-patient variability in signaling activity affect drug efficacy? To answer this question, signaling networks need to be elucidated not only depending on donor genetic variability, but also in a cell-specific manner, thereby

determining how each pathway contributes to the cell phenotype (pro-inflammatory, degeneration, repair), and to identify the missing steps (molecules and interactions) that participate in such MS pathways. For example, IL-10 is one of the main immunosuppressor cytokines but clinical trials with IL-10 failed to show benefits in MS, probably because IL-10 receptor signaling is at least partially deregulated in immune cells[19], and this can influence the individual response. We envision that a fine mapping of specific pathways such as cytokines pathways in specific cell types (e.g. CD4, CD8, B cells) in parallel with large high throughput studies will allow us to improve pathway annotation. Coupled computational modeling and experimental validation will enable characterization of signaling networks in a cell-type, donor and genetic variant specific manner, as reviewed in detail below.

Other challenges in pathway analysis

The fact that database searches identify many pathways associated with MS in immune cells might suggest that there is significant cross-talk between the major pathways within the same cell, with important proteins participating in several signaling cascades (Jak/Stat, NF-Kb, ERK1/2, p38, Jun/Fos). Crosstalk within pathways in the same cell is complex and thus difficult to study only based on existing experiments. Second, there is a substantial gap in our understanding of how such crosstalk interactions are translated into a cell-type specific response at the system level (e.g., interferon-beta produces different effects on macrophages and T cells, which are related with different clinical effects). Third, it is particularly difficult to make sense out of the existing MS data, since it is a disease that affects the arguably two most complex tissues/organ systems in our body, i.e. the immune system and the CNS, as described above. Fourth, annotation of gene function is still incomplete, and the role of the same genes in the CNS is often even less well understood or as yet unknown. For example, TNFα may have detrimental effects in the immune system in MS but they might also be beneficial in the CNS during remyelination [20]. Indeed, TNFα promotes oligodendrocyte progenitor proliferation, as well as

remyelination, which probably explain why the application of the TNF-antagonist lenercept produced an unexpected deterioration of MS [21]. Finally, a principal limitation in pathway analysis using existing data for functional annotation is that these approaches do not provide a mechanistic model that can be simulated, hence hindering the prediction of novel signaling mechanisms. To solve the challenges described here, combined analysis of newly acquired experimental data and mathematical models can be used[22]. Next, we review in detail such predictive models.

Predictive and mechanistic models to understand MS pathogenesis and therapies

The past decade has seen an explosion in the information regarding the cellular networks that transmit and process signals from the cell's environment. To gain novel understanding of the basic mechanisms that the cell uses to integrate these signals, as well as how such mechanisms are impaired by disease, mechanistic –mathematical- models are a powerful tool [23]. The first step to mathematical modeling is a literature search to gather the current understanding regarding the molecular process of interest, in this case MS. To that end, we can query public resources, a process that yields the pathways known to be involved in MS, which in turn are combined to form a signaling network. Signaling networks can be used as initial scaffolds upon which we can formulate mechanistic hypothesis and evaluate similarity with experimental data and disease-driven changes (Fig. 2, upper row). Therefore, experimental data needs to be acquired that measures as many readouts as possible relevant to the disease of study, i.e. present in the signaling network. To that end, phosphoproteomic measurements are key in the analysis of signaling pathways because measuring abundance of phosphorylated proteins closely indicates propagation of a signal through a pathway and can be used in functional models[24]. Previous work in the field has provided clear examples that phosphoproteomic analysis is able to provide accurate models of some pathways in cells such as hepatocytes [25]. Bead-based ELISA assays of xMAP technology (Luminex, Austin, TX) are well suited for this task [24], enabling measurement of the abundance of a large number of phosphorylated proteins in the MS pathways above-mentioned, in immune cells of individual patients of differents cohorts. Combining phosphoproteomics with genotyping in mathematical models, both the activity of MS pathways and the genetic variability that may explain the patient-to-patient difference in terms of response to treatment can be studied (Fig. 2, upper row). Once a signaling network has been assembled via literature search, and the data to compare it has to been measured, mathematical approaches enable formalization of the network as a mechanistic model. Intuitively, the formulation as mathematical model of such a signaling network

addresses two limitations: they are neither specific to individual patients (or even often to specific cell types), nor are they computable, i.e., can be used to predict the outcome of perturbations with drugs and ligands.

Several mathematical modeling approaches have become well established in the field of systems biology and can be applied to signaling pathways, ranging from logic to physicochemical models [26]. The lack of quantitative information for building the models can be bridged by using logic (Boolean) modeling, which includes only causal information and that due to this simplicity, has many fewer parameters (quantitative properties) to evaluate. This advantage can be used to represent large signaling networks that can be generated with limited data [27]. To implement logic models, tools such as CellNOpt [28] enable formalization of the signaling network as logic model and subsequent simulation. Next, these tools enable calibration of the model, which is performed by changing the network topology, i.e. the shape of the network in terms of the interactions between the present signaling intermediates. These changes consist in introducing or removing interactions, and systematic comparison of the simulations upon different topologies against the experimental data predicts the topology that best fits the data. The simplicity in logic modeling that enables simulation of large networks at the same time hinders highly detailed modeling of small networks. In more detailed analyses, appropriate tools are physicochemical models that describe the underlying biochemical reactions explicitly [23, 29, 30]. Here the model parameters are quantitative characteristics such as kinetic rates of the reactions that they represent, which are revealed by model calibration against the experimental data. In both modeling approaches, the main challenge lies in calibrating the model in order to make the model specific for MS, while at the same time determining the factors contributing to patient-to-patient variability. To address patient-to-patient variability, instead of starting from a single signaling network one solution is to calibrate an ensemble of networks featuring a high number of different starting topologies in order to test many different hypotheses that are compared separately to the xMAP and genotype of single patients[31] ,

including in these signaling topologies the mechanisms that grant robustness to signaling pathways, such as negative feedback loops. Thereby, we could determine which of the ensemble of topologies best fits each individual patient (Fig 2, middle row). Overall, the modeling of signaling pathways in MS, using either logic networks or mathematical models, offers the opportunity to predict new signaling mechanisms that help better understand disease pathogenesis. For example, a recent mathematical model of the type 1 interferon pathway revealed the translocation of Stat-1 to the nucleus as the most critical step in the signaling of IFN-b, a finding that could not be predicted solely based in molecular analysis but required dynamic simulations[32].

Drug development and combination therapy in predictive models of MS

One obvious question is if recent technological developments have provided a large amount of data about MS, why is drug discovery still so complex and provides so limited results? Although the limitations of the drug discovery process have been reviewed in detail[33], several specific issues regarding how biological information is translated into models of the disease and pathways are of importance. In the process of developing useful pathway models for drug development it is critical to take in consideration many aspects that at present are not well covered, such as (i) the availability of quantitative and kinetic data from human/patients; (ii) integration of individual heterogeneity and genetic background for defining the response to therapy, or (iii) the need to develop approaches for integrating and simulating complex networks of not just cells but also tissues. As described in Fig. 2, here we propose that coupling several omics and genotyping to mathematical models of signaling networks can address these issues. Further, existent drugs can be repurposed to target MS-related components by including their targets in a signaling network that can then be formalized as a mathematical model and simulated. This would enable to test millions of different options in terms of topology of signaling networks, therapeutic regimens, and drug/target combinations. This should allow prediction of the signaling mechanisms by which these existing drugs could be repurposed to

MS, discard therapeutic approaches that may not work and point at the ones that deserve careful experimental and clinical testing.

Given the complexity and heterogeneity of MS, combination therapies that modulate various pathogenic pathways simultaneously are an attractive treatment strategy [34]. A synergistic effect of two drugs with different mechanisms of action may potentially improve efficacy, safety and tolerability. By contrast, defining the optimal combination of drugs requires a more comprehensive understanding of the networks of pathways in different cells initiating and driving the progression of MS, an effort that can be addressed using systems biology techniques [35]. The integration of clinical, biological and pharmaceutical data in computational models that reproduce the complexity of such diseases can be used to identify synergistic effects by evaluating the downstream effects of drugs [36].

Finally, another significant challenge in improving drug development is predicting side-effects of therapies. Predictive toxicity was something highly theoretical until recently, but in the last years new significant insights have been provided by developing new algorithms combining drug databases and safety databases. Prior knowledge extracted from such databases can be introduced in mathematical models (Fig. 2) that are starting to provide useful predictions regarding potential side-effects that can be tested in preclinical or early clinical phases of drug development [37, 38] . However, this complex issue is still far from being solved.

Conclusions

The pathogenesis of MS is complex, involving hundreds of genes and proteins that act in numerous pathways and evolve along time and disease progression, each of which can contribute to the phenotype. These genes and proteins may respond distinctly to different therapies, and even behave differently in different patients. In order to integrate current knowledge and generate a

comprehensive model of MS pathogenesis, pathway analysis represents a promising strategy. Combining experimental and medical data with distinct systems biology approaches should provide new insights on disease pathogenesis, allow us to screen *in silico* new drugs for repurposing, as well as test combinations of drugs, before exposing patients to therapy.

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Figure legends

Figure 1. Signaling pathways associated with MS. A) An example of the pathways implicated in MS that can be indentified from databases. Right column shows the heatmap of the pathways associated with MS and the left columns the heatmap of the pathways associated with each of the drugs. On the right, each cluster is followed by the list of the pathways it includes (in bold the pathways specifically target by a drug). Blue squares: known mechanisms of action for a given drug. Green box: pathways associated with MS but not targeted by any drug. B) Integration of signaling pathways implicated in MS in network models: the genes/proteins associated with MS are displayed in orange, drugs in green; and the main MS pathways targeted by therapies in yellow.

Figure 2. Pipeline for the identification of new therapies based on the modeling of signaling pathways associated with MS and MS drugs. Flow from first to second row panels: experimental set-ups, such as proteomics and genotyping, can be tailored to interrogate MS specific signatures in terms of phosphoproteomics (rows, phosphorylation profile of specific proteins e.g. xMAP assays; Columns, MS-related treatments) and the risk variants. A literature search enables MS- and immune-specific pathways to be compiled and drug-protein networks can be assembled (the hot scale shows the density of proteins in the signaling pathways, and the upper layer shows green and blue clusters of proteins targeted by MS-related drugs). **Flow from second to third row panels:** logic and dynamic models can be constructed based on MS- and immune-specific signaling pathways. In order to study how signaling is deregulated in MS, one model can be calibrated against a patient-specific dataset, thereby yielding an ensemble of patient-specific models that enables common signaling mechanisms and those that explain patient-to-patient variability to be discriminated. In parallel, the signaling pathways and drug-protein networks can be used as an input for machine learning approaches in order to reposition existing drugs that can be used to infer new drug indications or to predict toxicity. **From new drug indication to model:** literature search of existing drugs and their targets, combined with search of those targets in MS-specific networks yields MS-specific drug-protein networks, which suggests new drug indications by identifying the

interactions from drug targets to MS networks. These newly-indicated drugs can then be introduced in the predictive models to understand their mechanisms of action in order to select those drugs with the best potential efficacy.

Figure 1

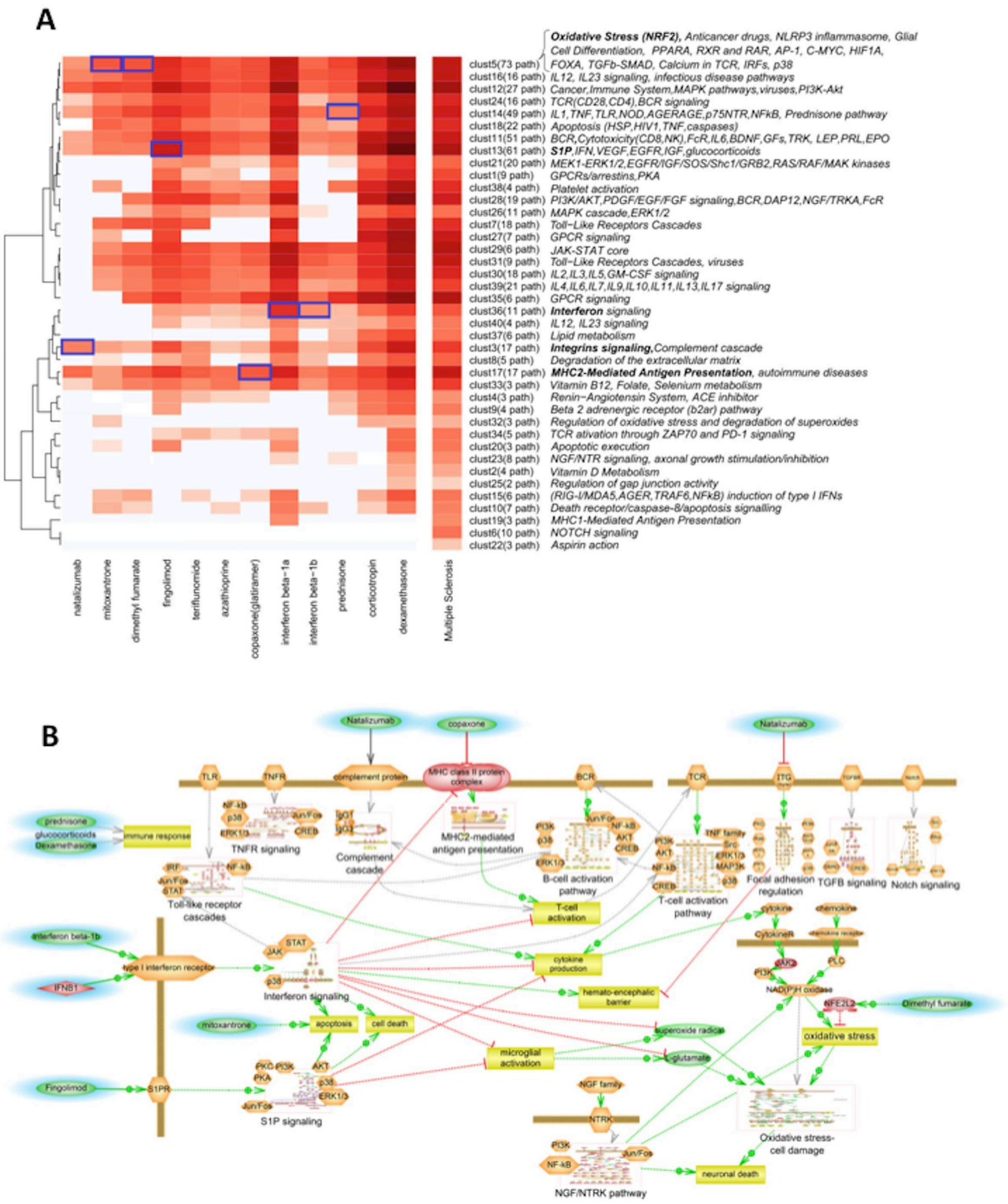
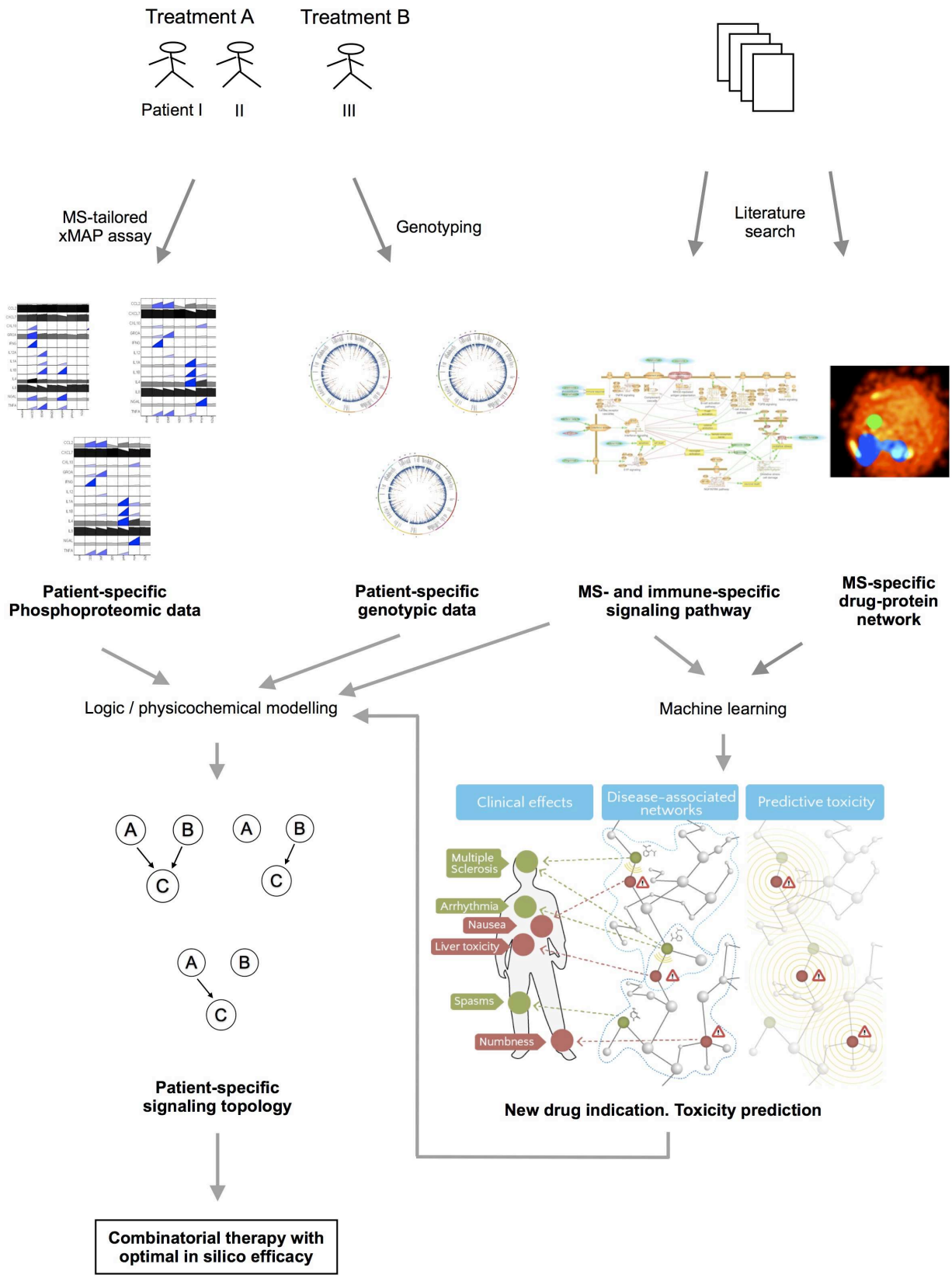

A
clust5(73 path) Oxidative Stress (NRF2), Anticancer drugs, NLRP3 inflammasome, Glial Cell Differentiation, PPARA, RXR and RAR, AP-1, C-MYC, HIF1A, FOXA, TGFb-SMAD, Calcium in TCR, IRFs, p38
clust16(16 path) IL12, IL23 signaling, infectious disease pathways
clust12(27 path) Cancer,Immune System,MAPK pathways,viruses,PI3K-Akt
clust24(16 path) TCR(CD28,CD4),BCR signaling
clust14(49 path) IL1,TNF,TLR,NOD,AGERAGE,p75NTR,NFkB, Prednisone pathway
clust18(22 path) Apoptosis (HSP,HIV1,TNF,caspases)
clust11(51 path) BCR,Cytotoxicity(CD8,NK),FcR,IL6,BDNF,GFs,TRK, LEP,PRL,EPO
clust13(61 path) S1P,IFN,VEGF,EGFR,IGF,glucocorticoids
clust21(20 path) MEK1-ERK1/2,EGFR/IGF/SOS/Shc1/GRB2,RAS/RAF/MAK kinases
clust1(9 path) GPCRs/arrestins,PKA
clust38(4 path) Platelet activation
clust28(19 path) PI3K/AKT,PDGF/EGF/FGF signaling,BCR,DAP12,NGF/TRKA,FcR
clust26(11 path) MAPK cascade,ERK1/2
clust7(18 path) Toll−Like Receptors Cascades
clust27(7 path) GPCR signaling
clust29(6 path) JAK-STAT core
clust31(9 path) Toll−Like Receptors Cascades, viruses
clust30(18 path) IL2,IL3,IL5,GM-CSF signaling
clust39(21 path) IL4,IL6,IL7,IL9,IL10,IL11,IL13,IL17 signaling
clust35(6 path) GPCR signaling
clust36(11 path) Interferon signaling
clust40(4 path) IL12, IL23 signaling
clust37(6 path) Lipid metabolism
clust3(17 path) Integrins signaling,Complement cascade
clust8(5 path) Degradation of the extracellular matrix
clust17(17 path) MHC2-Mediated Antigen Presentation, autoimmune diseases
clust33(3 path) Vitamin B12, Folate, Selenium metabolism
clust4(3 path) Renin−Angiotensin System, ACE inhibitor
clust9(4 path) Beta 2 adrenergic receptor (b2ar) pathway
clust32(3 path) Regulation of oxidative stress and degradation of superoxides
clust34(5 path) TCR ativation through ZAP70 and PD-1 signaling
clust20(3 path) Apoptotic execution
clust23(8 path) NGF/NTR signaling, axonal growth stimulation/inhibition
clust2(4 path) Vitamin D Metabolism
clust25(2 path) Regulation of gap junction activity
clust15(6 path) (RIG-I/MDA5,AGER,TRAF6,NFkB) induction of type I IFNs
clust10(7 path) Death receptor/caspase-8/apoptosis signalling
clust19(3 path) MHC1-Mediated Antigen Presentation
clust6(10 path) NOTCH signaling
clust22(3 path) Aspirin action
natalizumab
mitoxantrone
dimethyl fumarate
fingolimod
teriflunomide
azathioprine
copaxone(glatiramer)
interferon beta-1a
interferon beta-1b
prednisone
corticotropin
dexamethasone
Multiple Sclerosis
B
Natalizumab
copaxone
TLR
TNFR
complement protein
MHC class II protein complex
BCR
TCR
ITG
prednisone
glucocorticoids
Dexamethasone
immune response
TNFR signaling
Complement cascade
MHC2-mediated antigen presentation
B-cell activation pathway
T-cell activation pathway
Focal adhesion regulation
TGFB signaling
Notch signaling
Toll-like receptor cascades
T-cell activation
interferon beta-1b
IFNB1
type I interferon receptor
JAK
STAT
cytokine production
Interferon signaling
hemato-encephalic barrier
cytokine
chemokine
CytokineR
PLC
NAD(P)H oxidase
NFE2L2
Dimethyl fumarate
mitoxantrone
apoptosis
cell death
superoxide radicals
oxidative stress
microglial activation
glutamate
Fingolimod
S1PR
PKC
PKA
AKT
S1P signaling
NGF family
NTRK
Oxidative stress-cell damage
neuronal death
NGF/NTRK pathway

Figure 2


Treatment A
Treatment B
Patient I
II
III
MS-tailored xMAP assay
Genotyping
Literature search
Patient-specific Phosphoproteomic data
Patient-specific genotypic data
MS- and immune-specific signaling pathway
MS-specific drug-protein network
Logic / physicochemical modelling
Machine learning
A
B
C
Clinical effects
Disease-associated networks
Predictive toxicity
Multiple Sclerosis
Arrhythmia
Nausea
Liver toxicity
Spasms
Numbness
Patient-specific signaling topology
New drug indication. Toxicity prediction
Combinatorial therapy with optimal in silico efficacy