Repositioning Drugs for Rare Immune Diseases: Hopes and Challenges for a Precision Medicine

Erica Valencic[1,#], Alenka Šmid[2,#], Žiga Jakopin[2], Alberto Tommasini[3] and Irena Mlinarič-Raščan[2,*]

[1]*Laboratory of Immunopathology, Institute for Maternal and Child Health, IRCCS Burlo Garofolo, Trieste, Italy;* [2]*University of Ljubljana, Faculty of Pharmacy, Aškerčeva 7, Ljubljana, Slovenia;* [3]*Department of Pediatrics, Institute for Maternal and Child Health, IRCCS Burlo Garofolo, Trieste, Italy*

Abstract: Human primary immunodeficiency diseases (PIDs) are a large group of rare diseases and are characterized by a great genetic and phenotypic heterogeneity. A large subset of PIDs is genetically defined, which has a crucial impact for the understanding of the molecular basis of disease and the development of precision medicine.

Discovery and development of new therapies for rare diseases has long been de-privileged due to the length and cost of the processes involved. Interest has increased due to stimulatory regulatory and supportive reimbursement environments enabling viable business models.

Accepted: July 18, 2017

Advancements in biomedical and computational sciences enable the development of rational, designed approaches for identification of novel indications of already approved drugs allowing faster delivery of new medicines. Drug repositioning is based either on clinical analogies of diseases or on understanding of the molecular mode of drug action and mechanisms of the disease. All of these are the basis for the development of precision medicine.

Keywords: Drug repositioning, repositioning, primary immunodeficiency diseases; pediatrics, new therapies, rare diseases.

1. REPURPOSING OF DRUGS FOR FASTER ACQUISITION OF THERAPIES FOR RARE DISEASES

Advances in the post-genomic era enabled delineation of disease etiology and identification of their molecular and genetic basis, allowing for the development of precision medicine, a treatment approach that takes advantage of the genetic components of specific disease as well as inter-individual variability to tailor treatment to patients most likely to respond [1]. The term "precision medicine" implies a deeper molecular knowledge and usually indicates therapies that target the underlying cause of a disease. The knowledge of genetic background of the disease and applying the precision medicine approach is particularly important in the case of rare diseases, which are hard to diagnose and often lack appropriate treatments. More than 7000 rare diseases are known, most of which (80% or more) are believed to be of genetic background [2] and only 5% of these diseases have approved treatments. The development of novel therapeutics for this unmet medical need is a major challenge for the pharmaceutical sciences. Drug discovery lags behind current abilities to delineate disease etiology. This is so due to lengthy and costly drug development process. It takes in average 15 years and 1,5 billion € per registered medicine [3]. Academic and industrial researchers are facing societal and economic pressures to shorten the path from the lab to the patient, and to provide cures faster and at a reduced cost. Using already developed and approved drugs for new indications is one of the fastest and easiest ways to provide new treatments to patients. This strategy is also referred to as drug repurposing or drug repositioning, and there has been a heavy reliance on it in recent years.

*Address correspondence to this author at the University of Ljubljana, Faculty of Pharmacy, Aškerčeva 7, Ljubljana, Slovenia; Tel: +386 1 47 69 501; Fax: +386 1 42 58 031;
E-mail: Irena.Mlinaric-Rascan@ffa.uni-lj.si

[#]*These authors contributed equally to the manuscript*

To understand the rationale behind the repurposing concept, one must appreciate that translating high potential idea into a novel medicinal product is a challenging undertaking. The process of drug discovery is unpredictable, and most often requires extensive basic research before all links to the pathogenesis of a human disease are understood. It is estimated that 2 to 3 years are needed for the identification of the target, followed by a year of compound library screening or design of drug with biological efficacy. Lead compound identification is followed by 3 or more years of optimization. Estimation of pharmacokinetic and pharmacodynamic properties in animal models takes approximately 2 years, followed by 5 to 6 years of clinical studies to evaluate the safety and efficacy of the product and another 1 to 2 years for obtaining marketing authorization. The selection process for new chemical entities is very stringent. Starting with 5,000 to 10,000 compounds, only one to five molecules classify as "candidate drugs," and are selected for clinical trials [4].

Alternatively, when looking from another perspective, from pre-clinically successful molecules only 63% enter the first phase of clinical testing, 30% enter the second phase, only 7% enter the third phase and only 3% of medicines receive marketing authorization [5].

Repurposing of drugs is a rational approach to identify novel indications of approved drugs and enables faster development of therapies for rare and newly described diseases. Its major advantage is that drug safety risks, both medical and regulatory, are eliminated *a priori*; the medicine has already undergone clinical testing, thereby reducing the risk of failure in future late-stage clinical trials due to toxicity and thus shortening the time needed to receive marketing authorization [6].

Early cases of repurposed medicines were based on random observations which led to off label prescriptions by doctors and were then followed by approved novel indication labels. Examples include thalidomide and colchicine which were successfully applied in the therapy of PIDs, and antimalarials which were applied in the treatment of systemic lupus erythematosus (SLE).

Thalidomide was adopted for Behçet disease (BD) based on the finding of its efficacy in erythema nodosum leprosum, a condition with several clinical and immunological similarities to BD. Only very recently, it has been discovered that dysregulated signaling of the NFκB has a crucial role in BD in particular in the monogenic form associated with TNFAIP3 mutation [7]. Thalidomide may exert its immunomodulatory action by inhibiting the activation of NFκB and the production of TNF-α [8].

The repositioning of antimalarials to rheumatologic conditions like SLE came from the serendipitous observation of the effects of quinacrine in US soldiers with rheumatologic complaints during the Second World War who experienced symptomatic improvement while taking this agent [9]. Using contemporary computational approaches, it was shown that inhibition of the cGAS enzyme, which is involved in the production of interferons, is a crucial mechanism in the antinflammatory action of antimalarials, setting the ground for their use in monogenic type I interferonopathies [10].

Colchicine is one of the oldest anti-inflammatory drugs used to treat arthritis and gout. For several years it was also used as an unapproved drug to treat Mediterranean fever (FMF) [11]. Most of colchicine activities were attributed to dysfunctioning of the cytoskeleton due to the binding of colchicine to tubulin, thereby inhibiting microtubule formation. This led to metaphase arrest of the dividing cells and hampered neutrophil motility, contributing to decreased inflammation. In-depth molecular studies revealed the ability of colchicine to inhibit the assembly and activation of inflammasome *via* interaction with the NALP3, the Nod-like receptor 3 protein [12]. More recently, colchicine was shown to activate RhoA, a regulatory protein modulating the activation of the pyrin inflammasome in FMF [13]. With these mechanisms, colchicine interferes with Interleukin-1beta production [14]. Consistent with the central role of NALP3 in the inflammation process and thereby in induced inflammatory injuries, colchicine has gained attention as a potential therapeutic agent in several other indications in the field of rheumatology and cardiology [11].

The first part of the presented manuscript aims to address successfully repurposed drugs as well as novel cases currently under investigation in primary immunodeficiency diseases (PIDs). The second part is devoted to the overview of rational design of drug repurposing studies based on numerous available computational approaches.

2. DRUG REPURPOSING IN PRIMARY IMMUNODEFICIENCY DISEASES

Human primary immunodeficiency diseases (PIDs) are genetically and phenotypically heterogeneous group of diseases manifesting symptoms including infections, autoimmunity, autoinflammation, malignancies and allergies. Currently, 260 PIDs are genetically

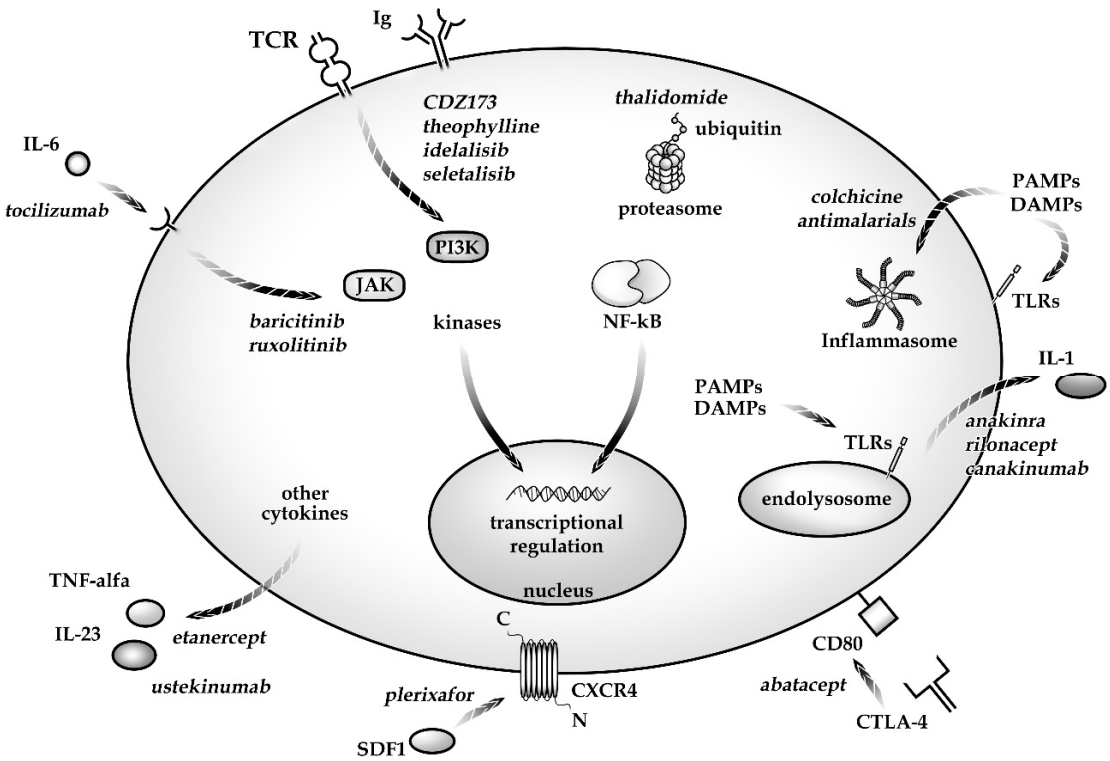


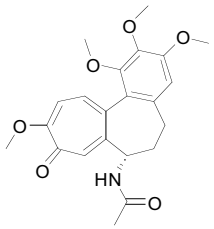
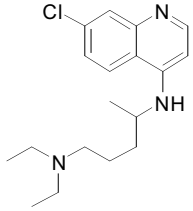
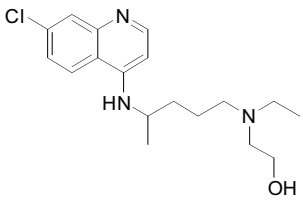
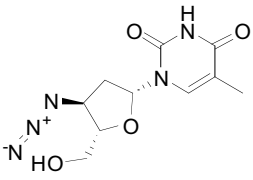
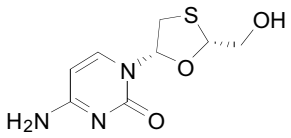
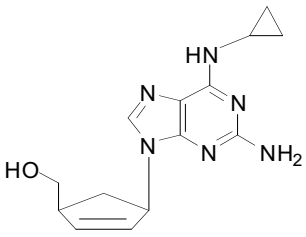
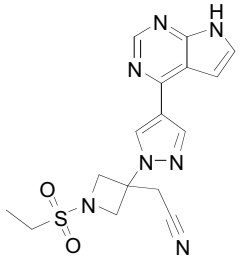
Fig. (1). Schematic representation of drugs and their targets, which are discussed in the article. Abbreviations: Ig, Immunoglobulin; TCR, T cell receptor; IL, interleukin; JAK, Janus kinase; PI3K, PhosphatidylInositol 3-kinase; TNF, Tumor necrosis factor; SDF1, stromal cell-derived factor 1; CXCR4, C-X-C chemokine receptor type 4; CTLA-4, Cytotoxic T-lymphocyte antigen 4; TLR, Toll-like receptor; NF-kB, Nuclear factor kappa beta; PAMP, Pathogen associated molecular patterns; DAMP, Damage-associated molecular pattern.

defined, allowing the delineation of the molecular basis of the disease, which is the background for the development of precision medicine. Of particular pharmacological interest is the use of small molecular inhibitors to modulate hyperactive functions (which are hyperactive as a consequence of gain-of-function -GOF- mutations) leading to over-activated or over-expressed target proteins which appear "druggable". An example is a GOF mutation in the PIK3CD gene, leading to hyperactivation of the p110delta kinase [15], a key component of signal transduction. This molecule represents a potential target for applying precision medicine using tyrosine kinase inhibitors. Conversely, loss-of-function mutations require either replacement therapies or the targeting downstream molecules of the involved pathway(s), such as the use of JAK inhibitors in the treatment of SAVI (stimulator of IFN genes (STING)-associated vasculopathy with onset in infancy) [16]. As elaborated above, the development of new therapeutic approaches include repurposing of drugs already in use for other diseases [17]. The repurposed drugs together with their molecular targets, which are discussed in the following sections, are schematically presented in Fig. (**1**), whereas the chemical structures of the repurposed small-molecule drugs are listed in Table **1**.

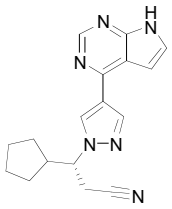
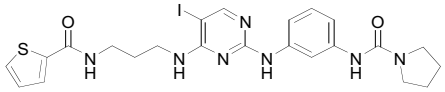
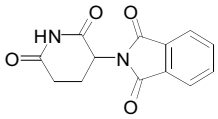
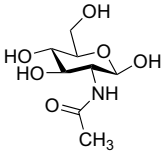
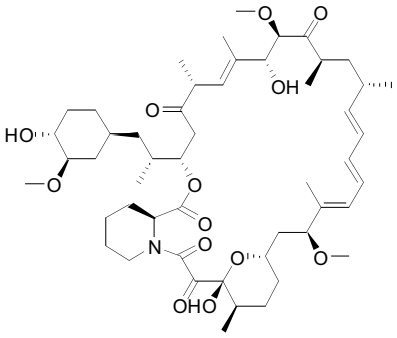
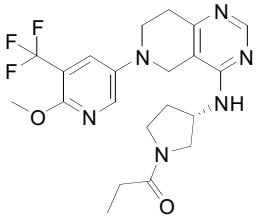
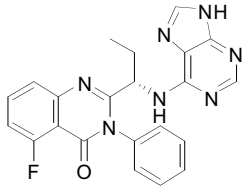
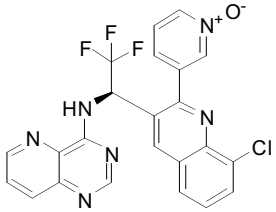
2.1. Repurposing of Drugs for IL1 Inflammasomopathies

Cryopyrin-associated periodic syndromes (CAPS) are a group of diseases caused by mutations in NLRP3 (NLR Family Pyrin Domain Containing 3) gene encoding for cryopyrin. CAPS encompasses a wide range of clinical phenotypes, from Familial Cold Autoinflammatory Syndrome (FCAS) at the milder end, to Muckle-Wells Syndrome (MWS) as an intermediate phenotype and Neonatal-Onset Multisystem Inflammatory Disease (NOMID) (also called Chronic Infantile Neurologic Cutaneous Articular, or CINCA, Syndrome) as the most severe. The common clinical features include rash, fever or chills, and joint pain; while mental impairment, facial malformation, papilledema and arthropathy can occur in the severe cases of diseases [18]. These symptoms are the result of the over-expression of IL1-beta, due to the constitutive activation of the NLRP3 inflammasome [19]. Discovering the role of IL1-beta in CAPS led to a series of

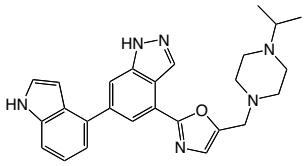
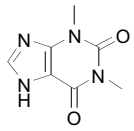
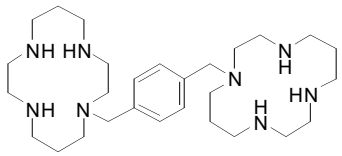
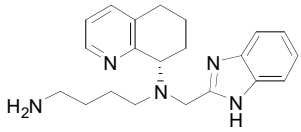
Table 1. Chemical structures of small-molecule drugs which have been or are suggested to be repurposed in primary immunodeficiency diseases (PIDs).

Repurposed Drug	Chemical Structure	Suggested or Actual Use in PID
Colchicine		In clinical use for the treatment of Familial Mediterranean Fever
Chloroquine		Suggested as a candidate drug for Aicardi-Goutières syndrome (AGS) based on its mode of action. No clinical trials have been performed so far.
Hydrochloroquine		Suggested as a candidate drug for AGS based on its mode of action. No clinical trials have been performed so far.
Zidovudine		Currently in a phase 2 clinical trial for the treatment of patients with AGS (NCT02363452).
Lamivudine		Currently in a phase 2 clinical trial for the treatment of patients with AGS (NCT02363452).
Abacavir		Currently in a phase 2 clinical trial for the treatment of patients with AGS (NCT02363452).
Baricitinib		Currently in a compassionate use program for the treatment of SAVI (stimulator of IFN genes (STING)-associated vasculopathy with onset in infancy) and AGS (NCT01724580)

(Table 1) contd….

Repurposed Drug	Chemical Structure	Suggested or Actual Use in PID
Ruxolitinib		Reported compassionate use cases in patients with SAVI.
BX795		Suggested as a candidate drug for the treatment of SAVI based on findings on patient cells (*in vitro*).
Thalidomide		Used for the treatment of Behçet disease.
N-acetylglucosamine		Currently in phase 1 clinical trial in patients with mutations in PGM3 (NCT02511041)
Rapamycin		Experimental use in patients with Activated Phosphoinositide 3-kinase δ Syndrome (APDS) and Autoimmune Lymphoproliferative Syndrome (ALPS).
Leniolisib (CDZ173)		Currently in phase 2/3 clinical trials for the treatment of APDS (NCT02859727, NCT02435173)
Idelalisib		Suggested as a candidate drug for the treatment of patients with APDS, since it is a specific inhibitor of PIK3δ.
Seletalisib		Currently in a phase 1b clinical trial for the treatment of patients with APDS (EudraCT Number: 2015-002900-10)

(Table 1) contd….

Repurposed Drug	Chemical Structure	Suggested or Actual Use in PID
Nemiralisib		Currently in a phase 2 clinical trial for the treatment of APDS (NCT02593539).
Theophylline		Suggested as a candidate drug for the treatment of patients with APDS due to its inhibitory action on the p110δ subunit of PI3K.
Plerixafor		Currently in phase 1 and 3 clinical trials for the treatment of Warts, hypogammaglobulinemia, infections, and myelokathexis (WHIM) (NCT02231879, NCT00967785)
X4P-001		Currently in a phase 2/ 3 clinical trial for the treatment of WHIM (NCT03005327)

successful clinical trials evaluating the efficacy of blocking IL1 signaling in CAPS. First clinical studies demonstrating significant improvement in symptoms of CAPS were performed with anakinra (an IL1 receptor antagonist), which was developed and approved for the treatment of rheumatoid arthritis in 1991, however the new indication for the treatment of CAPS was granted in 2013. In fact, the first FDA-approved therapy for CAPS was treatment with the long-acting IL1 inhibitor rilonacept, which was approved in 2008, followed by another long-acting IL1-beta inhibitor canakinumab, which was approved in 2009 [20].

Familial Mediterranean Fever (FMF) causes short attacks of fever with serositis (peritonitis, pleuritis, arthritis), and is often associated with skin rashes. The main complication of the disease is amyloid A amyloidosis, which affects the kidney and other organs. The disease is caused by gain of function mutations in *MEFV* (familial Mediterranean fever) gene, that encodes for pyrin; this protein interacts with ASC (Apoptosis-associated Speck-like protein containing a CARD), which, in turn, leads to activation of caspase-1 and secretion of the inflammatory cytokine IL1-beta [21]. The main treatment of FMF is with colchicine. Recently, Park *et al.* showed that RhoA, a member of the Ras homolog gene family, acts as an inhibitor of the pyrin inflammasome in physiological conditions [13]. Colchicine is an activator of RhoA and a regulator of the tubulin cytoskeleton and is thought to act by inhibition of pyrin. Patient response to colchicine in subjects with FMF is so striking that it is a major diagnostic criterion for the disease [22]. Subjects who are not responsive or tolerant to colchicine may nevertheless respond to biological inhibitors of IL1 [23, 24]. Evidences that link *MEFV*-mutation and IL1 impairment [25, 26] have led to the hypothesis that IL1 inhibitors, such as anakinra and canakinumab, already used in other autoinflammatory conditions, could be used for the treatment of colchicine-resistant FMF. Very recently, thanks to the substantial results obtained in a phase 3 clinical trial (NCT02059291) canakinumab has been approved by the US Food and Drug Administration (FDA) and by European Medicines Agency (EMA) for the treatment of patients with FMF.

Tumor necrosis factor Receptor-Associated Periodic Syndrome (TRAPS) is a hereditary inflammatory disease characterized by recurrent attacks of fever with high peaks lasting one to two weeks. Fever is often accompanied by gastrointestinal symptoms (abdominal pain, vomiting, and diarrhea), painful skin rashes, muscle pain and periorbital swelling. Heterozygous mutations in the *TNFRSF1A* gene (coding for tumor necrosis factor (TNF)-alpha receptor I [TNFR1]) are the cause of the disease. TNFR1 is a transmembrane protein that binds TNF-alpha either leading to a NF-κB dependent inflammatory response or to caspase-8-dependent apoptosis. Distinct mutation may lead to inflammation with different mechanisms, either by reducing the ratio between shed inhibitory and membrane bound stimulatory TNFR1, or by intracellular retention

of a misfolded TNFR1 activating the proteasome and production of IL1-beta [27, 28]. Even if both TNFalpha and IL1 inhibitors were used, only the latter resulted in lasting disease remission [29, 30].

Mevalonate kinase deficiency (MVK) is characterized by recurrent fever, inflammatory reactions often associated with swollen lymph nodes, abdominal pain, vomiting, diarrhea, joint pain, headache, skin rash, mouth ulcers, and enlarged liver and spleen. In severe cases (mevalonic aciduria) the clinical phenotype includes developmental delay, lack of coordination, visual disturbances, growth retardation and impaired skeletal morphology [31]. Inflammatory manifestations are caused by overproduction of IL1-beta and treatment with biological inhibitors (such as canakinumab) has been used with success to control inflammatory recurrence [32]. Innovative treatments aimed at the correction of the metabolic defect may represent a more specific precision therapy, but complex biochemical regulation makes it a difficult task to accomplish [33].

2.2. Repurposing of Drugs for Interferonopathies

Interferonopathies are a group of monogenic disorders characterized by constitutively upregulated type I interferon (IFN) pathway, causing a wide spectrum of inflammatory phenotypes, often with a partial overlap with systemic lupus erythematosus.

Aicardi-Goutières syndrome (AGS) is progressive encephalopathy, which occurs in the first year of life and is characterized by microcephaly, intracranial calcifications, leukodystrophy, lymphocytosis and increased interferon alpha (IFNα) in the cerebrospinal fluid. AGS is caused by several genetic defects affecting the degradation of cytoplasmic nucleic acids, both exogenous and endogenous, and leading to a constitutive activation of the type I IFN pathway. Repurposing the reverse transcriptase inhibitors has been proposed as a precision therapy to block the accumulation of endogenous retro-element-derived single strand DNA or DNA:RNA hybrids, which play a crucial role in the stimulation of the type I IFN pathway. Administration of a combination of antiretroviral drugs in animal models (*TREX1*-null mice) held promising results [34, 35] and zidovudine, lamivudine and abacavir are currently being studied in a phase 2 clinical trial (NCT02363452) in patients with AGS. Some antimalarial drugs (such as chloroquine, hydro-chloroquine) have been shown to target the type I IFN pathway by inhibiting the enzyme cGAS, which is a crucial sensor of cytoplasmic nucleic acids. Although antimalarials could potentially be used as repurposed drugs, they have not been studied in AGS so far [10]. In both cases, precision medicine could be realized by the repositioning of drugs approved for other indications. Furthermore, JAK inhibitor ruxolitinib has been used in two patients with AGS. Both patients showed an important reduction of interferon (IFN)-stimulated genes expression as well as clinical improvement [36].

SAVI (stimulator of IFN genes (STING)-associated vasculopathy with onset in infancy) is an autoinflammatory disease caused by GOF mutations in *TMEM173* encoding STING, a key protein in the cGAS-IFN pathway. The pathogenic mutations lead to constitutive activation of STING and dysregulated production of type I interferons. The clinical picture is characterized by fever, skin vasculopathy and interstitial lung disease, and a tendency to develop destructive skin lesions, pulmonary fibrosis and respiratory failure [37, 38]. The first attempt for a precision medicine in SAVI used repurposed Janus kinase (JAK) inhibitors already marketed for other indications. For example, baricitinib (approved for rheumatoid arthritis) and ruxolitinib (approved for myelofibrosis and polycythemia vera). These drugs can block the signaling cascade induced by secreted interferons on target cells *in vitro* and are currently being used in compassionate use programmes [37]. However, JAK inhibitors can also interfere with the signaling of other cytokines, with the risk of serious adverse effects. To overcome these limitations, a recent proposal suggests blocking the secretion of IFN itself, by acting on TBK1 kinase, just downstream of activated STING. The compound BX795, which was developed as an inhibitor of 3-phosphoinositide-dependant protein kinase 1 (PDK1) [39], was later shown to also be a specific inhibitor of TBK1, and of the Inhibitor of nuclear factor kappa-B kinase epsilon subunit (IKKE). Preliminary data showed that BX795 was able to inhibit the IFN secretion in primary peripheral blood mononuclear cells from patients with SAVI, without affecting cell survival [40, 41]. Based on these results, BX795 might represent a promising therapeutic opportunity not only for patients with SAVI but also for patients with other interferonopathies with hyperactivation of STING-TBK1 signaling. However, the safety of BX795 has not been studied in clinical trials.

2.3. Repurposed Drugs for Other PIDs

Behçet Disease (BD) is a complex disorder with both autoinflammatory and autoimmune pathologic features, and is characterized by recurrent oral and genital ulcers, uveitis and vasculitis. Recent findings showed that rare cases of familial BD may be due to

dominant loss-of-function (LOF) mutations in the gene *TNFAIP3* (TNF Alpha Induced Protein 3), encoding the protein A20 involved in de-ubiquitination of TAF6, NEMO and RIP1 and consequent downregulation of NFkB signaling [42]. The mutated protein, because of inefficient deubiquitination of the target proteins, failed to suppress the TNF-induced activation of NFkB. Although this was shown only in a rare monogenic form of BD, the studies support the idea that modulation of NFkB ubiquitination may have a role in the treatment of BD in general. Indeed, other evidence also suggests that the role of NFkB activation in the pathogenesis of BD is crucial [43]. Of note, thalidomide and its analogs, which are commonly used to treat BD, have been proposed to act by down-regulation of NFkB signaling [8]. Recent reports suggest that thalidomide and its analogs may modulate the ubiquitination and degradation of several targets involved in NFkB activation [44, 45]. Although there is no direct proof of a synergistic action between thalidomide and A20, it is intriguing to speculate that both molecules may regulate inflammation by acting on ubiquitination.

X-linked immunodeficiency with magnesium defect, Epstein-Barr virus infection, and neoplasia (XMEN) is caused by LOF mutations in the *MAGT1* gene, which encodes for a protein able to transport magnesium within cells. The decrease in the magnesium level inside the cells leads to impaired responses to antigen receptor engagement. The clinical manifestation are characterized by CD4 lymphopenia, chronic viral infections (Epstein Barr Virus-EBV), defects of the T-Cell Receptor (TCR) signal and an increased risk of lymphoma [46]. The simple administration of magnesium in the form of oral magnesium gluconate or intravenous magnesium sulphate has been found sufficient to dramatically improve the symptoms of the disease [47].

Immunodeficiency-vasculitis-myoclonus syndrome is an immunodeficiency characterized by recurrent respiratory and skin infections and neurocognitive delay. It is caused by LOF mutations in phosphoglucomutase 3 (*PGM3*) [48]. PGM3 is an enzyme involved in the glycosylation pathway, in particular in the synthesis of uridine diphosphate N-acetylglucosamine (UDP-GlcNAc), a molecule necessary for the correct N-linked glycosylation and O-linked glycosylation of the proteins. Supplementation of GlcNAc, which is available on the market as a food supplement, is able to increase UDP-GlcNAc and bypass the metabolic defect and is currently being in a phase 1 clinical trial (NCT02511041) which aims to study the effect of N-acetylglucosamine on immune function and changes in cellular glycosylation patterns.

Activated phosphoinositide 3-kinase δ syndrome (APDS) is caused by heterozygous GOF mutations in the *PIK3CD* gene encoding for the catalytic subunit p110δ of the phosphatidylinositol 3 kinase (PI3K). The mutations described lead to hyperactivation of the PI3K-AKT-mTOR pathway, resulting in immunodeficiency and increased cell proliferation and survival. The disease is characterized by recurrent lung and ear infections, lymphoproliferation, hepatosplenomegaly, chronic herpesvirus infections, autoimmune phenomena such as cytopenia and an increased risk of lymphoma [49]. LOF mutations in one of the inhibitory and regulatory subunits of the same kinase (p85α) are responsible for another genetic disorder called APDS-like or APDS2, clinically very similar to the APDS [50-52]. Again, the described mutations cause hyperactivation of the same pathway.

Rapamycin (also called sirolimus) is a repurposed drug for these diseases. It was initially discovered as an antifungal metabolite and later found to possess immunosuppressive and antiproliferative properties in mammalian cells. Rapamycin binds a cytosolic receptor and inhibits the "mammalian target of rapamycin" (mTOR) complex, a key regulatory kinase. The use of rapamycin improved symptoms such as the size of the tonsils, lymphadenopathy and hepatosplenomegaly, but it was not sufficient to rescue the defective immune functions [53, 54]. It is expected that specific inhibitors of PIK3δ (leniolisib (CDZ173), idelalisib, seletalisib, nemiralisib) will be better drug candidates. These drugs were developed to treat autoimmune (Sjogren syndrome, psoriasis) or oncohematologic (chronic lymphocytic leukaemia, follicular lymphoma) disorders and are currently in clinical trials in APDS (NCT02435173, NCT02859727, NCT02593539, EudraCT number 2015-002900-10).

Theophylline also has a well-documented inhibitory action on the p110δ subunit of PI3K at pharmacological concentrations [55] and is thus a good candidate for drug repurposing. Theophylline is classified as a phosphodiesterase inhibitor and has been used as a diuretic and a bronchodilator in various indications such as asthma and chronic obstructive pulmonary disease.

Immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX); common variable immunodeficiency-8 (CVID) and autoimmune lymphoproliferative syndrome type V (ALPS5) are immune disorders characterized by the loss of immune tolerance. In all cases, the co-

stimulatory molecule CTLA4 plays a significant role in the pathogenesis of the immune-dysregulation. The treatment with abatacept, a recombinant fusion CTLA4 protein, coupled with the Fc fragment of human immunoglobulin IgG, which was developed for the treatment of rheumatoid arthritis, has been proposed as a promising targeted therapy in these disorders. In a normal immune response, the engagement of CTLA4 serves as a negative feedback of T cell activation, interfering with the co-stimulatory signals between CD28 on T cells and B7-1 (CD80) and B7-2 (CD86) on antigen presenting cells. CTLA-4 (CD152) binds to B7 with a higher affinity than CD28 which contributes to the switching-off the activation. Abatacept is expected to act by replacement of the defective mutated CTLA4 in ALPS5 [56]; in the other two disorders the mechanism of action of the drug is more complex. As LRBA is involved in recycling CTLA4 from the endosome to the cell membrane, the deficiency of LRBA reflects on a reduced expression of CTLA4 on the membrane and impaired immunoregulatory function. Lo *et al.* showed that the treatment with abatacept is able to partially rescue the defective immunoregulation in subjects with LRBA deficiency [57]. The potential of abatacept to be repurposed for treatment of the IPEX syndrome is supported by the efficacy of this drug in mice with Foxp3 mutations [58]. In humans, the drug has only been evaluated in a limited fashion (EudraCT Number: 2008-003368-21).

Autoimmune Lymphoproliferative Syndrome (ALPS) or Canale-Smith syndrome is a disorder of lymphocyte homeostasis due to the defective apoptosis of activated lymphocytes. The most common form is due to dominant negative mutations in the *TNFRSF6* (tumor necrosis factor receptor superfamily, member 6) gene encoding the FAS protein. After T-cell activation, FAS expression on the membrane increases, making the cell more susceptible to FAS-Ligand induced programmed cell death and contributing to the switching-off of the immune response. Trimerization of FAS on the membrane triggers a signaling mediated by the adapter proteins FADD/MORT1, eventually leading to activation of caspase-8 and cell apoptosis. In subjects with defective FAS function, there is a prevalence of proliferative/survival signals transduced by antigen or cytokine receptors, which are in part mediated by the PIK3-AKT-mTOR pathway [59]. Indeed, inhibition of mTOR with rapamycin in mice was highly effective in contrasting the lymphoproliferative and autoimmune features typical of ALPS [60]. Even though no randomized controlled trial has been completed in humans, the experimental use of rapamycin in various clinical cases of ALPS held promising results [61, 62].

Warts, hypogammaglobulinemia, infections, and myelokathexis (WHIM) is a disease characterized by neutropenia, lymphopenia and monocytopenia, with clinical features including recurrent bacterial infections (pharyngitis, sinusitis, ear infections, meningitis and pneumonia) and warts on the hands and feet, caused by human papilloma virus [63]. It is a rare genetic disease and is due to mutations in C-X-C chemokine receptor type 4 (*CXCR4*) gene. CXCR4 is expressed on mature leukocytes and is involved in the signal transduction pathways that control adhesion and cell homing in the bone marrow, the myelopoiesis and lymphopoiesis. GOF mutations cause prolonged activation of the receptor that determines the retention of neutrophils and other white blood cells in the bone marrow [63].

Plerixafor is an antagonist of the alpha chemokine receptor CXCR4 and approved in adults with lymphoma and multiple myeloma in combination with granulocyte colony-stimulating factor (G-CSF), to enhance mobilization of hematopoietic stem cells in peripheral blood during aphaeretic procedures. Currently, two clinical trials are recruiting patients to evaluate the use of plerixafor in the treatment of WHIM (NCT02231879, NCT00967785). In a previous study, subcutaneous administration of plerixafor twice daily for 6 months in 3 patients with WHIM led to an increase in peripheral leukocyte count and to the reduction of infections; however, immunoglobulin levels and specific vaccine responses were not fully restored [64]. Although the response to plerixafor was not complete, it fostered the development of novel oral inhibitors of CXCR4, such as X4P-001, which is being developed for use as a life-long treatment for patients with WHIM and is currently being studied in a Phase 2/3 trial (NCT03005327).

Leukocyte adhesion deficiency 1 is a rare immunodeficiency due to a homozygous LOF mutation in *ITGB2* gene, encoding the beta-2-integrin (CD18), which plays a key role in adhesion of neutrophils to the endothelium and their transmigration in tissues. The disease is characterized by recurrent bacterial infections, which start in infancy and affect the skin, the oral cavity and the respiratory tract [65]. Adult patients often present severe periodontitis, which can result in premature tooth loss. Evaluation of cytokines production in gingival biopsies showed increased levels of IL17 and IL23; as further proof animal models of the disease spontaneously develop periodontitis, which can be controlled by blocking interleukin-23-interleukin-17

axis. Ustekinumab is a human monoclonal antibody that recognizes the p40 subunit of IL12 and IL23 and inhibits the downstream production of IL17. It is approved to treat plaque psoriasis and psoriatic arthritis. Experimental administration of this drug to a patient with LAD1 led to an improvement of inflammatory oral lesions, paralleled by a normalization of IL23-IL17 levels [66].

Chronic Mucocutaneous Candidiasis (CMC) due to STAT1 GOF. is characterized by non-invasive recurrent infections of the skin, nails, and mucosa by *Candida albicans* [67] and includes autoimmune features [68]. CMC is associated with GOF mutations in *STAT1* (Signal transducer and activator of transcription 1) gene which results in increased STAT1 phosphorylation and overexpression of cytokines such as IFNα/β and IL27, which inhibit Th17 differentiation [69]. Since STAT1 phosphorylation is induced by Janus Kinase (JAK) signaling, ruxolitinib, a JAK1/JAK2 inhibitor, developed and approved for myelofibrosis and polycythemia vera, is a promising candidate for drug repurposing [70]. Indeed its efficacy seems to decrease the phosphorylation of STAT1, thus normalizing both Th differentiation and interferon response, seemingly without impairing STAT3 signaling [71].

Immunodeficiency due to mutations in STAT3 gene. Immunodeficiency with a broad clinical spectrum including early-onset autoimmunity, lymphoproliferation, recurrent fungal, bacterial and viral infections and postnatal short stature is caused by GOF mutations in the *STAT3* gene. This condition causes impaired STAT3 dephosphorylation kinetics which result in the hyperactivity of this protein; furthermore STAT1 and STAT5 phosphorylation levels are decreased after stimulation with IFNγ and IL2 respectively. Since signal transduction of IL6 involves activation of STAT3, tocilizumab, an anti-IL6 receptor monoclonal antibody (originally developed and approved for rheumatoid arthritis and systemic juvenile idiopathic arthritis) is a candidate for drug repurposing [72].

3. RATIONAL DRUG REPURPOSING APPROACHES

Traditionally, drug repositioning was based on clinical analogies of diseases and drug phenotypes. The advancement in the understanding of molecular basis of diseases and mechanisms of drug actions, drug molecular structures and properties, 3D target protein structures, effects on changing gene expression upon treatment allow the rational designing of repurposing studies. Numerous computational methods using this new knowledge are being developed and will be addressed in the following section.

3.1. Computational Methods in Drug Repurposing

The field of chemoinformatics is already well-established in classical drug-design methodologies, and plays an important role from the earliest phase of compound design on to preclinical studies, during which computational methods are used to identify and characterize potential and actual toxicology-related adverse effects. The promiscuity of a compound is usually inversely correlated to its safety. However, recently computational pharmacology has lately found another valuable application by facilitating the process of drug repurposing. By taking advantage of high-throughput data generated from diverse areas, such as proteomics, genomics, chemoproteomics and phenomics [4], it offers several advantages over other employed methodologies, including low-cost, rapidly obtained results and screening against an array of targets, therefore it is widely used in both industry and academia. In most cases are considered a cost-effective and synergistic complement to experimental techniques thereby leading to optimal results [73]. In particular, *in silico* methods exploit the existing data to focus/prioritize the *in vitro* testing of large chemical datasets and ensure that the compounds with predicted high probabilities of activity are assayed first.

Conventional *in silico* methods can be classified into two major categories, the structure-based and ligand-based approaches. The structure-based approaches, such as molecular docking and receptor-based pharmacophore searching, explore target site structural features to find potential binding molecules. These approaches are only useful in cases where the 3D structures of targets are available and are of sufficient quality. They enable virtual screening of large datasets/databases of ligands and targets using a reversed methodology. Specifically, a small molecule ligand is screened for structural complementarity against multiple known drug targets thus constituting a rather unconventional scenario of »one ligand-many targets«. The calculated binding complementarities, expressed as numerical values according to different scoring functions, allow for the ranking and identification of potentially relevant macromolecular targets.

Ligand-based methods, which are simpler, use searches based on molecular similarity. A similarity measure is usually comprised of three parts: (*i*) the molecular representation used for comparison; (*ii*) the weighting scheme, which assigns degrees of

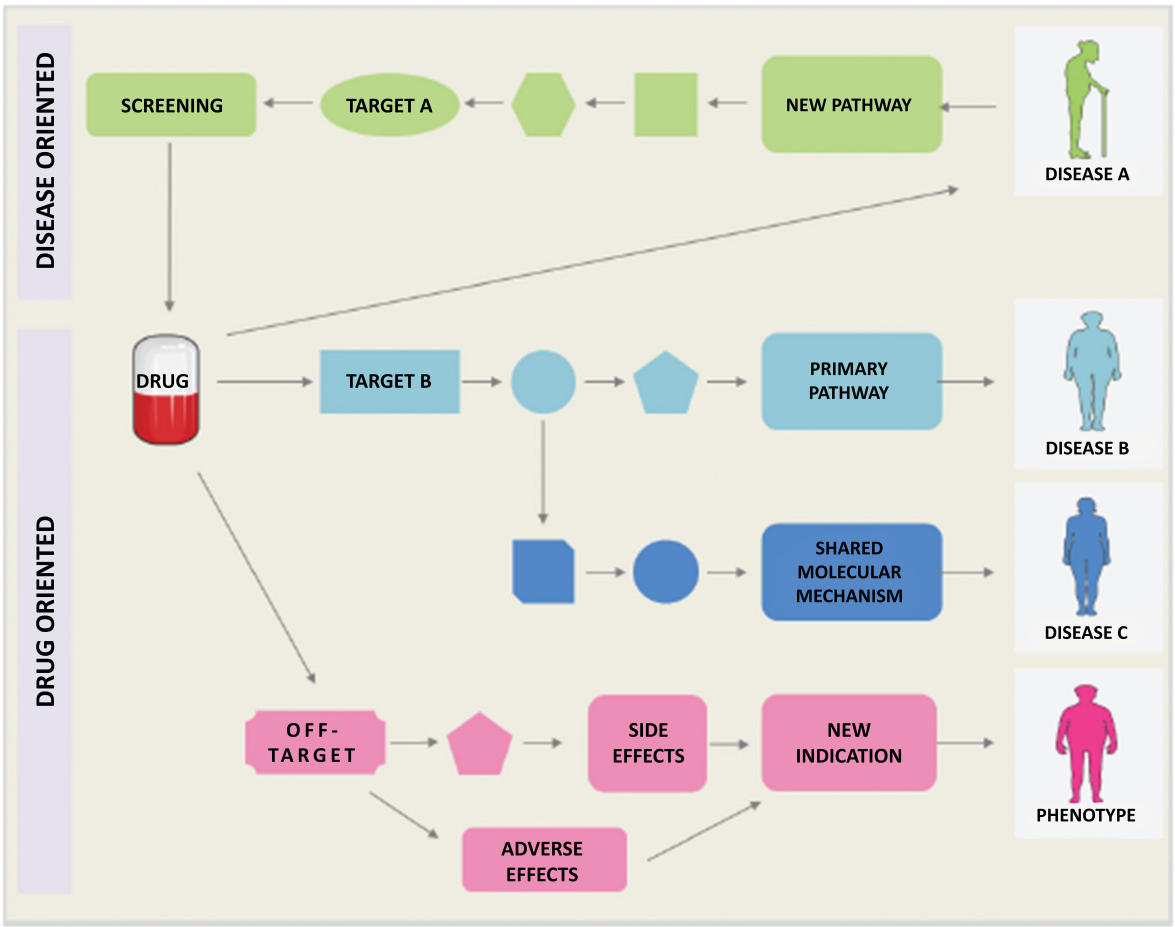


Fig. (2). Rational drug repurposing approaches. The approaches to drug repurposing studies can be classified as either drug-oriented or disease-oriented. The disease-oriented approach is based on clinical manifestations and novel insights into molecular pathways of a disease (disease **A**), leading to new target identification (target **A**), followed by screening for target modulators, one of which may be an existent drug and can be repositioned. Drug-oriented approaches use the knowledge of a drug's molecular mode of action on the target (target **B**) or on other, off-target systems. If target (**B**), originally indicated for disease (**B**) modulates different underlying pathways, which are involved in the pathogenesis of other diseases (disease **C**), the drug can be repositioned. Understanding of drug's action on off-targets responsible for side effects or adverse drug reactions, leads to identification of a novel indication.

importance to individual elements and (*iii*) the similarity coefficient, which provides a quantitative measure for comparison of structural relatedness [74]. Different methods for the molecular representation are available. For instance, molecules can be described with 1D descriptors (depicting a whole molecule), 2D descriptors (calculated from a two-dimensional representation) and 3D descriptors (calculated from a three-dimensional representation) encoding different types of information [74]. The ligand-based approach encompasses diverse techniques, such as (quantitative) structure-activity relationships ((Q)SAR), 2D molecule fingerprints, 3D similarity search and pharmacophores and machine-learning methods. Machine learning methods are particularly useful for targets whose SAR information and 3D structural information are scarce or not available.

3.1.1. Structure-based Approaches

3.1.1.1. Reverse Docking

The predominantly used structure-based method in the process of drug repurposing is reverse molecular docking. The most basic requirements to conduct a reverse docking protocol are a collection of ligands and a library of macromolecular targets [75]. The ligands are usually obtained from one of several valuable databases of approved and experimental/investigational drugs, including DrugBank, NIH Chemical Genomics Center Pharmaceutical Collection, World Drug Index and ChEMBL [75]. A substantial number of known proteins have already been crystallized and their 3D structures solved to date. In addition, in the post-genomic era novel targets are being identified on a reg-

ular basis as a direct result of advancement of the fields of functional genomics, systems biology, and network pharmacology. A rich repertoire of more than one thousand known 3D structures of diverse macromolecular targets encompassing various classes (mostly enzymes but also receptors, ion channels and nucleic acids) have been compiled in Potential Drug Target Database (PDTD) in PDB format [76]. A recently developed web-server Target Fishing Dock (TarFisDock) uses PTDT to facilitate identification of potential macromolecular binding targets [77]. Therapeutic targets database represents yet another comprehensive database containing known as well as explored/investigated targets, while several focused, disease-oriented protein structure databases have also emerged recently, *e.g.* Tropical Disease Research Database [78]. As opposed to a number of software available for conventional ligand-protein docking, only a few programs, such as TarFisDock, MDock, INVDOCK and Autodock Vina are currently available for reverse docking; for these types of programs automation of protein structure preparation and computational efficiency are required [75]. When interpreting and comparing results one must take into consideration that these programs use different docking protocols/algorithms as well as different scoring functions. A successful example of the use of the reverse docking approach, combined with *in vitro* experiments, is the identification of antipsychotics acetophenazine, fluphenazine and periciazine as androgen receptor antagonists [79].

3.1.1.2. Reverse Pharmacophore Mapping

Alternative structure-based strategies allowing for prediction of additional targets of known drugs rely on the comparative analysis of the binding site structural characteristics or make use of the established receptor-based pharmacophore models [80]. The latter is also called reverse pharmacophore mapping and constitutes a technique in which pharmacophore models of clinically relevant proteins are constructed and then constitute a starting point for subsequent comparison of each of these models against small molecule ligands. Several freely available web applications have recently been made available, which facilitate drug repurposing in a high-throughput manner and represent a valuable resource to the scientific community. For instance, PharmMapper screens entered compounds against a database composed of several thousand receptor-based pharmacophore models covering the chemical space of over 1500 drug targets, and produces the best mapping pose of the query [81]. Better outcomes are usually achieved when both structure- and ligand-based approaches are used. The reverse pharmacophore mapping approach has already been employed in order to determine off-target effects of known drugs, which is the idea underlying the drug-oriented method of drug repositioning. A subset of approved drugs has been screened against pharmacophores of various clinically relevant transporters, such as hPEPT1 [82], P-gp [83], hOCTN2 [84, 85] and the ASBT [86]. Those queries led to the discovery of novel transporter inhibitors possessing overlapping pharmacophore features despite the fact that they belonged to diverse therapeutic classes.

3.1.2. Ligand-based Approaches

3.1.2.1. SAR/QSAR Approach

Different molecular representations, including numerical descriptors, chemical graphs, fingerprints and 3D models can be used to describe a structure. The evaluation of structure-activity relationships (SAR), which is important in medicinal chemistry, usually involves similarity and potency/activity comparisons. Consequently, SAR analysis also holds great potential in drug repurposing. Furthermore, computational QSAR (quantitative structure activity relationships) approaches are often used to assist in SAR analysis, thus allowing for different structures to be compared for similarity from both qualitative as well as quantitative perspectives [87]. A quantitative computational assessment of similarity entails the choice of regularly applied molecular descriptors and a measure of similarity or distance. This approach, however, has serious limitations, considering SAR/QSAR analysis are usually valid for series of structurally similar compounds and that they are also highly dependent on the choice of descriptors and measures. SAR/QSAR approaches often makes use of molecular descriptors, such as 2D fingerprints. It has been observed that SAR phenotypes changed considerably if alternative descriptors/ fingerprints (which include structural fragment, atom environment, and pharmacophore fingerprints) were employed, in turn, resulting in fundamental SAR differences [87].

3.1.2.2. 2D Fingerprints

2D molecular descriptors comprise (*i*) topological indices, denoting the size, degree of branching and shape of a molecule, as well as (*ii*) substructural descriptors, characterizing the substructural features of a given molecule either by its chemical graph or its fingerprint. Similarity search using 2D fingerprints is one of the simplest *in silico* tools successfully applied in medicinal chemistry, hence its use could be a valuable

asset in drug repurposing. Briefly, the fingerprints are binary in nature and determine the presence or absence of 2D substructural fragments in a given molecule. Therefore, the similarity between a pair of molecules depends solely on the number of common substructural fragments. It is described by a similarity coefficient (also called an association coefficient), for example the most frequently used Tanimoto coefficient, which then reveals a degree of relatedness between two structural representations [74]. Computational 2D similarity search using different topological (complementary shape) as well as electric (electrostatic interactions) descriptors has been effectively used to predict cross-reactivity in immunoassays by screening the SCUT database of FDA-approved drugs and to identify novel inhibitors of toxicology immunoassays [88-90]. Similarly, Keiser *et al.* showed that 2D structural similarity can be successfully applied in the prediction of new targets for known drugs [91, 92]. These cases clearly demonstrate that 2D similarity search alone can be used for finding small molecules possessing similar pharmacophore features to those of known drugs.

3.1.2.3. 3D Similarity Search

3D molecular structure comparison represents an alternative or complementary method to 2D similarity search, but is inherently more complex. 3D descriptors, including atomic distance, molecular shape, molecular field and pharmacophore, also take into account the conformational flexibility of a molecule [93]. It possesses the capacity to capture different structural patterns related to biological activity and provides a valuable alternative insight [94]. This approach involves the construction of a 3D pharmacophore model common to a set of known actives, which is then used for 3D structural similarity search using the available databases (*e.g.* DrugBank). Several alternative methodologies can be used to generate 3D drug similarity data drug conformational analysis, molecular alignments or 3D similarity functions [94]. The pharmocophore model is subsequently integrated with a source of targets available in different databases, *e.g.* ChEMBL, thus developing a predictor for drug-target interactions. Comparison of 2D and 3D structure methods has demonstrated that each captures a diverse chemical space, resulting in different sets of candidates [94]. One successful use of pharmacophore model was the identification of antidiabetic glybenclamide as an antithrombotic agent on the basis of its pharmacophore similarity with a known TP receptor antagonist [95].

3.1.3. Signature-based Approaches

Signature-based methods make use of gene signatures derived from whole genome expression data of the disease with and without applied treatment. One of the first widely used tools developed for this purpose is connectivity mapping, the basic concept of which is to use a reference database (CMap) containing drug-specific gene expression profiles and compare it with a disease specific gene expression signature, which corresponds to a list of differentially expressed genes (up- and down-regulated) and uniquely represents the studied phenotype (*i.e.* disease state) [96]. The resultant 'connectivity score' reflects the closeness or connection between the expression profiles and can be used to identify known drugs which would potentially be able to reverse the transcriptomic profile representative of a disease of interest. The CMap database was first introduced in 2006 and currently contains more than 7,000 expression profiles representing 1,309 compounds over 5 different cell lines (build 2 dataset version). In 2014 the Broad Institute released another large database as a part of the Library of Integrated Network-based Cellular Signatures (LINCS) project which contains numerous gene expression profiles of broad range of human cell lines before and after chemical and genetic perturbations such as different compound treatments, gene knockdown or gene overexpression [97]. The database contains approximately 1.3M experimental reference profiles and as such represents a very valuable resource for drug repurposing studies. CMap has been employed in many drug repurposing studies, which led to identification of numerous promising candidate drugs for different diseases. For example, celastrol, a plant-derived triterpene with antioxidant and anti-inflammatory action, was identified by connectivity mapping as a potential radiosensitizing agent for use in non–small cell lung carcinoma [98] and as a potential anti-AML stem cell agent [99]. Using the CMap thioridazine, an antipsychotic drug used to treat schizophrenia and psychosis, was identified as potential anti-glioblastoma agent [100] and as an inhibitor of phosphatidylinositol-3'-kinase (PI3K)/AKT pathway in ovarian cancer cells [101].

Since the first introduction of the CMap principle and methodology, there have been numerous applications of this approach by many research groups and some new methods of pattern matching and data normalization were developed. The sscMap (statistically significant connectivity map) is a popular tool which added statistical stringency to guard the results against false positives in the analysis and is a useful implemen-

tation of the connectivity mapping method [102]. Another approach, the 'CMapBatch', is a computational meta-analysis pipeline where instead of applying CMap to one individual gene signature, it is applied to multiple gene signatures for the same disease and then the resulting outcomes are combined [103]. The QUADrATiC (QUB Accelerated Drug And Transcriptomic Connectivity) software package has been developed for the exploration of gene expression connectivity on the subset of the LINCS data set corresponding to FDA-approved small molecule compounds [104]. Recently, the DrugSig database been established which provides a user-friendly web interface for drug repurposing studies. The database currently contains more than 6000 expression signatures (mostly from CMap database) of more than 1300 drugs as well as 800 targets which have been constructed according to descriptions from the literature and publically available databases such as DrugBank, KEGG, CTD and TTD [105]. DeSigN (Differentially Expressed Gene Signatures - Inhibitors) is another CMap-inspired bioinformatics pipeline which allows the comparison of experimental gene expression signatures to those associated with drug response phenotype based on IC_{50} data, which is contained in the Genomics of Drug Sensitivity in Cancer (GDSC) database [106].

Methods for connectivity mapping use simplistic models of pattern matching techniques. Since they do not consider any mechanistic aspects, they cannot provide detailed information about signaling pathways which lead to the observed signature [107]. A more in-depth insight into molecular targets and mode of drug action could be achieved by integrating other available data sources and developing new pathway or network-based models.

3.1.4. Network-based Approaches

Network based computational methods aim at describing a complex system as a connected graph in which nodes represent the individual molecular entity of interest (such as gene, protein or drug) while edges represent an interaction between the nodes [108]. Networks can be constructed by using numerous publicly accessible databases containing various types of biological and molecular data. Based on the type of data is included, different types of interaction networks can be distinguished. Currently, the major types are protein–protein interaction (PPI), gene regulatory and metabolic networks.

Gene regulatory networks are based on transcriptomic data and assume that drugs with similar gene signatures target the same proteins. Examples of such transcriptome-based methods are Mode of action by network analysis (MANTRA) and NFFinder. MANTRA is a drug-drug similarity network based on the CMap data which makes use of a post-processed version of the cMap dataset, where compounds are catalogued into a drug similarity network. The user can integrate a drug under investigation into the network and deduct its mode of action by analysing the surrounding subnetwork [109]. The NFFinder has been initially developed in the context of orphan diseases like Neurofibromatosis (NF) but was later generalized to any other disorder. NFFinder uses transcriptomic data from GEO and CMap to find relationships between drugs, diseases and a phenotype of interest, as well as identifying experts having published on that domain [110].

Protein–protein interaction networks (PPINs) represent interactions between known drug targets and other proteins, or between proteins that have indirect interaction with targets. Most of them assume that proteins which are targeted by similar drugs are also functionally related and therefore close in the PPIN [111]. An example of computational method which integrates drug therapy information, chemical structure information, and PPIN to predict drug-target interactions is drugCIPHER [112] which has been successfully applied to predict targets of traditional Chinese medicine (TCM) [107].

In metabolic networks each node represent a metabolite and the edges represent the reactions and enzymes that catalyze them [113]. An important class of methods based on metabolic networks is flux balance analysis (FBA) which is mostly used for identification of drug targets. For example, Li *et al.* developed a computational method to predict the metabolic reactions of 60 human cancer cell lines (the NCI-60 set) influenced by approved anti-cancer drugs as well as to predict new targets for the latter [91]. The analysis of cancer specific metabolic networks in a study by Folger *et al.* resulted in identification of 52 selective drug targets, of which 40% were targeted by known anti-cancer drugs, and the rest were new target-candidates [114].

CONCLUSION

The fastest way to meet the unmet medical needs related to human primary immunodeficiency diseases (PIDs) is to reposition/repurpose existent, already approved drugs whenever possible. Several case studies presented in this paper demonstrate that this approach is successful. It is worth highlighting that the molecular

or functional diagnosis of the disease in each patient is key to the application of targeted precision medicines. Thus, it could be expected that the list of druggable diseases will expand even more in the following years, in particular for cases associated with hyperfunctioning of kinases.

Post-genomic biomedical advances, especially advances in relevant computation methods and tools, and the growth of bio-structural and genomic databases, enable a rapid identification and comparison of disease state phenotypes, small molecule ligands, and biomolecular targets. These tools enable the identification of existent drugs that are precision medicines, with methods of action that are understood at the mechanistic level.

A review of current state-of-the-art computational tools and methodologies suggest that repurposing and repositioning will become an even more viable strategy as tools and databases continue to develop and as ever more intricate comparisons of disease state phenotypes and drug targets reveal previously unknown relationships between diseases.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Toward Precision Medicine. Building a Knowledge Network for Biomedical Research and a New Taxonomy of Disease. National Research Council (US) Committee on A Framework for Developing a New Taxonomy of Disease. Washington (DC): National Academies Press (US), **2011**; ISBN-13: 978-0-309-22222-8 ISBN-10: 0-309-22222-2.

[2] Rare diseases and orphan products. accelerating research and development. Institute of Medicine (US) Committee on accelerating rare diseases research and orphan product development; Eds.; Marilyn J Field and Thomas F Boat. Washington (DC): National Academies Press (US), **2010**; ISBN-13: 978-0-309-15806-0ISBN-10: 0-309-15806-0.

[3] Paul, S.M.; Mytelka, D.S.; Dunwiddie, C.T.; Persinger, C.C.; Munos, B.H.; Lindborg, S.R.; Schacht, A.L. How to improve R&D productivity: the pharmaceutical industry's grand challenge. *Nat. Rev. Drug Discov.,* **2010**, *9*(3), 203-214.

[4] Jin, G.; Wong, S.T.C. Toward better drug repositioning: prioritizing and integrating existing methods into efficient pipelines. *Drug Discov. Today,* **2014**, *19*(5), 637-644.

[5] AstraZeneca SL. Available at: https://www. astrazeneca.si/od-laboratorija-do-bolnika (Accessed June 20, 2017).

[6] Li, Y.Y.; Jones, S.J. Drug repositioning for personalized medicine. *Genome Med.,* **2012**, *4*(3), 27.

[7] Hatemi, G.; Seyahi, E.; Fresko, I.; Talarico, R.; Hamuryudan, V. One year in review 2016: Behçet's syndrome. *Clin. Exp. Rheumatol.,* **2016**, *34*(6)(Suppl. 102), 10-22.

[8] Lv, P.; Li, H-Y.; Ji, S-S.; Li, W.; Fan, L-J. Thalidomide alleviates acute pancreatitis-associated lung injury *via* down-regulation of NFκB induced TNF-α. *Pathol. Res. Pract.,* **2014**, *210*(9), 558-564.

[9] Lee, S-J.; Silverman, E.; Bargman, J.M. The role of antimalarial agents in the treatment of SLE and lupus nephritis. *Nat. Rev. Nephrol.,* **2011**, *7*(12), 718-729.

[10] An, J.; Woodward, J. J.; Sasaki, T.; Minie, M.; Elkon, K. B. Cutting Edge: Antimalarial drugs inhibit IFN-β production through blockade of cyclic GMP-AMP synthase-DNA interaction. *J. Immunol.,* **2015**, *194*(9), 4089-4093.

[11] Abbate, A.; Mauro, A.G.; Thurber, C. Colchicine in acute myocardial infarction: Teaching new tricks to an old dog. *Transl. Med. (Sunnyvale),* **2015**, *5*(4), e133.

[12] Taskiran, E.Z.; Cetinkaya, A.; Balci-Peynircioglu, B.; Akkaya, Y.Z.; Yilmaz, E. The effect of colchicine on pyrin and pyrin interacting proteins. *J. Cell. Biochem.,* **2012**, *113*(11), 3536-3546.

[13] Park, Y.H.; Wood, G.; Kastner, D.L.; Chae, J.J. Pyrin inflammasome activation and RhoA signaling in the autoinflammatory diseases FMF and HIDS. *Nat. Immunol.,* **2016**, *17*(8), 914-921.

[14] Dalbeth, N.; Lauterio, T.J.; Wolfe, H.R. Mechanism of action of colchicine in the treatment of gout. *Clin. Ther.,* **2014**, *36*(10), 1465-1479.

[15] Hartman, H.N.; Niemela, J.; Hintermeyer, M.K.; Garofalo, M.; Stoddard, J.; Verbsky, J.W.; Rosenzweig, S.D.; Routes, J.M. Gain of function mutations of PIK3CD as a cause of primary sclerosing cholangitis. *J. Clin. Immunol.,* **2015**, *35*(1), 11-14.

[16] Volpi, S.; Picco, P.; Caorsi, R.; Candotti, F.; Gattorno, M.; Type, I. Type I interferonopathies in pediatric rheumatology. *Pediatr. Rheumatol. Online J.,* **2016**, *14*(1), 35.

[17] Notarangelo, L.D.; Fleisher, T.A. Targeted strategies directed at the molecular defect: Toward precision medicine for select primary immunodeficiency disorders. *J. Allergy Clin. Immunol.,* **2017**, *139*(3), 715-723.

[18] Giat, E.; Lidar, M. Cryopyrin-associated periodic syndrome. *Isr. Med. Assoc. J.,* **2014**, *16*(10), 659-661.

[19] Martinon, F.; Tschopp, J. Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. *Cell,* **2004**, *117*(5), 561-574.

[20] Goldbach-Mansky, R.; Kastner, D.L. Autoinflammation: the prominent role of IL-1 in monogenic autoinflammatory diseases and implications for common illnesses. *J. Allergy Clin. Immunol.,* **2009**, *124*(6), 1141-1149.

[21] de Torre-Minguela, C.; Mesa Del Castillo, P.; Pelegrín, P. The NLRP3 and pyrin inflammasomes: Implications in the pathophysiology of autoinflammatory diseases. *Front. Immunol.,* **2017**, *8*, 43.

[22] Demirkaya, E.; Erer, B.; Ozen, S.; Ben-Chetrit, E. Efficacy and safety of treatments in familial Mediterranean fever: A systematic review. *Rheumatol. Int.,* **2016**, *36*(3), 325-331.

[23] Calligaris, L.; Marchetti, F.; Tommasini, A.; Ventura, A. The efficacy of anakinra in an adolescent with colchicine-resistant familial Mediterranean fever. *Eur. J. Pediatr.,* **2008**, *167*(6), 695-696.

[24] Laskari, K.; Boura, P.; Dalekos, G.N.; Garyfallos, A.; Karokis, D.; Pikazis, D.; Settas, L.; Skarantavos, G.; Tsitsami, E.; Sfikakis, P.P. Longterm beneficial effect of canakinumab in colchicine-resistant familial Mediterranean fever. *J. Rheumatol.,* **2017**, *44*(1), 102-109.

[25] Chae, J.J.; Wood, G.; Masters, S.L.; Richard, K.; Park, G.; Smith, B.J.; Kastner, D.L. The B30.2 domain of pyrin, the familial Mediterranean fever protein, interacts directly with caspase-1 to modulate IL-1beta production. *Proc. Natl. Acad. Sci. USA,* **2006**, *103*(26), 9982-9987.

[26] Omenetti, A.; Carta, S.; Delfino, L.; Martini, A.; Gattorno, M.; Rubartelli, A. Increased NLRP3-dependent interleukin 1β secretion in patients with familial Mediterranean fever: correlation with MEFV genotype. *Ann. Rheum. Dis.,* **2014**, *73*(2), 462-469.

[27] Bachetti, T.; Ceccherini, I. Tumor necrosis factor receptor-associated periodic syndrome as a model linking autophagy and inflammation in protein aggregation diseases. *J. Mol. Med. (Berl.),* **2014**, *92*(6), 583-594.

[28] Kimberley, F.C.; Lobito, A.A.; Siegel, R.M.; Screaton, G.R. Falling into TRAPS--receptor misfolding in the TNF receptor 1-associated periodic fever syndrome. *Arthritis Res. Ther.,* **2007**, *9*(4), 217.

[29] Cantarini, L.; Rigante, D.; Lucherini, O.M.; Cimaz, R.; Laghi Pasini, F.; Baldari, C.T.; Benucci, M.; Simonini, G.; Di Sabatino, V.; Brizi, M.G.; Galeazzi, M. Role of etanercept in the treatment of tumor necrosis factor receptor-associated periodic syndrome: personal experience and review of the literature. *Int. J. Immunopathol. Pharmacol.,* **2010**, *23*(3), 701-707.

[30] Gattorno, M.; Pelagatti, M.A.; Meini, A.; Obici, L.; Barcellona, R.; Federici, S.; Buoncompagni, A.; Plebani, A.; Merlini, G.; Martini, A. Persistent efficacy of anakinra in patients with tumor necrosis factor receptor-associated periodic syndrome. *Arthritis Rheum.,* **2008**, *58*(5), 1516-1520.

[31] De Pieri, C.; Taddio, A.; Insalaco, A.; Barbi, E.; Lepore, L.; Ventura, A.; Tommasini, A. Different presentations of mevalonate kinase deficiency: a case series. *Clin. Exp. Rheumatol.,* **2015**, *33*(3), 437-442.

[32] Bodar, E.J.; van der Hilst, J.C.H.; Drenth, J.P.H.; van der Meer, J.W.M.; Simon, A. Effect of etanercept and anakinra on inflammatory attacks in the hyper-IgD syndrome: introducing a vaccination provocation model. *Neth. J. Med.,* **2005**, *63*(7), 260-264.

[33] Marcuzzi, A.; Piscianz, E.; Valencic, E.; Monasta, L.; Vecchi Brumatti, L.; Tommasini, A. To extinguish the fire from outside the cell or to shutdown the gas valve inside? novel trends in anti-inflammatory therapies. *Int. J. Mol. Sci.,* **2015**, *16*(9), 21277-21293.

[34] Beck-Engeser, G.B.; Eilat, D.; Wabl, M. An autoimmune disease prevented by anti-retroviral drugs. *Retrovirology,* **2011**, *8*, 91.

[35] Crow, Y.J.; Manel, N. Aicardi-Goutières syndrome and the type I interferonopathies. *Nat. Rev. Immunol.,* **2015**, *15*(7), 429-440.

[36] Tüngler, V.; König, N.; Günther, C.; Engel, K.; Fiehn, C.; Smitka, M.; von der Hagen, M.; Berner, R.; Lee-Kirsch, M.A. Response to: 'JAK inhibition in STING-associated interferonopathy' by Crow *et al. Ann. Rheum. Dis.,* **2016**, *75*(12), e76.

[37] Liu, Y.; Jesus, A.A.; Marrero, B.; Yang, D.; Ramsey, S.E.; Sanchez, G.A.M.; Tenbrock, K.; Wittkowski, H.; Jones, O.Y.; Kuehn, H.S.; Lee, C.R.; DiMattia, M.A.; Cowen, E.W.; Gonzalez, B.; Palmer, I.; DiGiovanna, J.J.; Biancotto, A.; Kim, H.; Tsai, W.L.; Trier, A.M.; Huang, Y.; Stone, D.L.; Hill, S.; Kim, H.J.; Hilaire, C.S.; Gurprasad, S.; Plass, N.; Chapelle, D.; Horkayne-Szakaly, I.; Foell, D.; Barysenka, A.; Candotti, F.; Holland, S.M.; Hughes, J.D.; Mehmet, H.; Issekutz, A.C.; Raffeld, M.; McElwee, J.; Fontana, J.R.; Minniti, C.P.; Moir, S.; Kastner, D.L.; Gadina, M.; Steven, A.C.; Wingfield, P.T.; Brooks, S.R.; Rosenzweig, S.D.; Fleisher, T.A.; Deng, Z.; Boehm, M.; Paller, A.S.; Goldbach-Mansky, R. Activated STING in a vascular and pulmonary syndrome. *N. Engl. J. Med.,* **2014**, *371*(6), 507-518.

[38] Jeremiah, N.; Neven, B.; Gentili, M.; Callebaut, I.; Maschalidi, S.; Stolzenberg, M-C.; Goudin, N.; Frémond, M-L.; Nitschke, P.; Molina, T.J.; Blanche, S.; Picard, C.; Rice, G.I.; Crow, Y.J.; Manel, N.; Fischer, A.; Bader-Meunier, B.; Rieux-Laucat, F. Inherited STING-activating mutation underlies a familial inflammatory syndrome with lupus-like manifestations. *J. Clin. Invest.,* **2014**, *124*(12), 5516-5520.

[39] Feldman, R.I.; Wu, J.M.; Polokoff, M.A.; Kochanny, M.J.; Dinter, H.; Zhu, D.; Biroc, S.L.; Alicke, B.; Bryant, J.; Yuan, S.; Buckman, B.O.; Lentz, D.; Ferrer, M.; Whitlow, M.; Adler, M.; Finster, S.; Chang, Z.; Arnaiz, D.O. Novel small molecule inhibitors of 3-phosphoinositide-dependent kinase-1. *J. Biol. Chem.,* **2005**, *280*(20), 19867-19874.

[40] Frémond, M-L.; Uggenti, C.; Van Eyck, L.; Melki, I.; Bondet, V.; Kitabayashi, N.; Hertel, C.; Hayday, A.; Neven, B.; Rose, Y.; Duffy, D.; Crow, Y.J.; Rodero, M.P. Blockade of TANK-binding kinase 1/IKKε Inhibits Mutant Stimulator of Interferon Genes (STING)-mediated inflammatory responses in human peripheral blood mononuclear cells. *Arthritis Rheumatol.,* **2017**, *69*(7), 1495-1501.

[41] Clark, K.; Plater, L.; Peggie, M.; Cohen, P. Use of the pharmacological inhibitor BX795 to study the regulation and physiological roles of TBK1 and IkappaB kinase epsilon: A distinct upstream kinase mediates Ser-172 phosphorylation and activation. *J. Biol. Chem.,* **2009**, *284*(21), 14136-14146.

[42] Zhou, Q.; Wang, H.; Schwartz, D.M.; Stoffels, M.; Park, Y.H.; Zhang, Y.; Yang, D.; Demirkaya, E.; Takeuchi, M.; Tsai, W.L.; Lyons, J.J.; Yu, X.; Ouyang, C.; Chen, C.; Chin, D.T.; Zaal, K.; Chandrasekharappa, S.C.; P Hanson, E.; Yu, Z.; Mullikin, J.C.; Hasni, S.A.; Wertz, I.E.; Ombrello, A.K.; Stone, D.L.; Hoffmann, P.; Jones, A.; Barham, B.K.; Leavis, H.L.; van Royen-Kerkof, A.; Sibley, C.; Batu, E.D.; Gül, A.; Siegel, R.M.; Boehm, M.; Milner, J.D.; Ozen, S.; Gadina, M.; Chae, J.; Laxer, R.M.; Kastner, D.L.; Aksentijevich, I. Loss-of-function mutations in TNFAIP3 leading to A20 haploinsufficiency cause an early-onset autoinflammatory disease. *Nat. Genet.,* **2016**, *48*(1), 67-73.

[43] Todaro, M.; Zerilli, M.; Triolo, G.; Iovino, F.; Patti, M.; Accardo-Palumbo, A.; di Gaudio, F.; Turco, M.C.; Petrella, A.; de Maria, R.; Stassi, G. NF-kappaB protects Behçet's disease T cells against CD95-induced apoptosis upregulating antiapoptotic proteins. *Arthritis Rheum.,* **2005**, *52*(7), 2179-2191.

[44] Liu, Y.; Huang, X.; He, X.; Zhou, Y.; Jiang, X.; Chen-Kiang, S.; Jaffrey, S.R.; Xu, G. A novel effect of thalidomide and its analogs: suppression of cereblon ubiquitination enhances ubiquitin ligase function. *FASEB J.,* **2015**, *29*(12), 4829-4839.

[45] Krönke, J.; Fink, E.C.; Hollenbach, P.W.; MacBeth, K.J.; Hurst, S.N.; Udeshi, N.D.; Chamberlain, P.P.; Mani, D.R.; Man, H.W.; Gandhi, A.K.; Svinkina, T.; Schneider, R.K.; McConkey, M.; Järås, M.; Griffiths, E.; Wetzler, M.; Bullinger, L.; Cathers, B.E.; Carr, S.A.; Chopra, R.; Ebert, B.L. Lenalidomide induces ubiquitination and degradation of CK1α in del(5q) MDS. *Nature,* **2015**, *523*(7559), 183-188.

[46] Li, F-Y.; Chaigne-Delalande, B.; Kanellopoulou, C.; Davis, J.C.; Matthews, H.F.; Douek, D.C.; Cohen, J.I.; Uzel, G.; Su, H.C.; Lenardo, M.J. Second messenger role for Mg2+ revealed by human T-cell immunodeficiency. *Nature,* **2011**, *475*(7357), 471-476.

[47] Chaigne-Delalande, B.; Li, F-Y.; O'Connor, G.M.; Lukacs, M.J.; Jiang, P.; Zheng, L.; Shatzer, A.; Biancalana, M.; Pittaluga, S.; Matthews, H.F.; Jancel, T.J.; Bleesing, J.J.; Marsh, R.A.; Kuijpers, T.W.; Nichols, K.E.; Lucas, C.L.; Nagpal, S.; Mehmet, H.; Su, H.C.; Cohen, J.I.; Uzel, G.;

Lenardo, M.J. Mg2+ regulates cytotoxic functions of NK and CD8 T cells in chronic EBV infection through NKG2D. *Science,* **2013**, *341*(6142), 186-191.

[48] Hay, B.N.; Martin, J.E.; Karp, B.; Davis, J.; Darnell, D.; Solomon, B.; Turner, M.; Holland, S.M.; Puck, J.M. Familial immunodeficiency with cutaneous vasculitis, myoclonus, and cognitive impairment. *Am. J. Med. Genet. A.,* **2004**, *125A*(2), 145-151.

[49] Angulo, I.; Vadas, O.; Garçon, F.; Banham-Hall, E.; Plagnol, V.; Leahy, T.R.; Baxendale, H.; Coulter, T.; Curtis, J.; Wu, C.; Blake-Palmer, K.; Perisic, O.; Smyth, D.; Maes, M.; Fiddler, C.; Juss, J.; Cilliers, D.; Markelj, G.; Chandra, A.; Farmer, G.; Kielkowska, A.; Clark, J.; Kracker, S.; Debré, M.; Picard, C.; Pellier, I.; Jabado, N.; Morris, J.A.; Barcenas-Morales, G.; Fischer, A.; Stephens, L.; Hawkins, P.; Barrett, J.C.; Abinun, M.; Clatworthy, M.; Durandy, A.; Doffinger, R.; Chilvers, E.R.; Cant, A.J.; Kumararatne, D.; Okkenhaug, K.; Williams, R.L.; Condliffe, A.; Nejentsev, S. Phosphoinositide 3-kinase δ gene mutation predisposes to respiratory infection and airway damage. *Science,* **2013**, *342*(6160), 866-871.

[50] Lucas, C.L.; Zhang, Y.; Venida, A.; Wang, Y.; Hughes, J.; McElwee, J.; Butrick, M.; Matthews, H.; Price, S.; Biancalana, M.; Wang, X.; Richards, M.; Pozos, T.; Barlan, I.; Ozen, A.; Rao, V.K.; Su, H.C.; Lenardo, M.J. Heterozygous splice mutation in PIK3R1 causes human immunodeficiency with lymphoproliferation due to dominant activation of PI3K. *J. Exp. Med.,* **2014**, *211*(13), 2537-2547.

[51] Deau, M-C.; Heurtier, L.; Frange, P.; Suarez, F.; Bole-Feysot, C.; Nitschke, P.; Cavazzana, M.; Picard, C.; Durandy, A.; Fischer, A.; Kracker, S. A human immunodeficiency caused by mutations in the PIK3R1 gene. *J. Clin. Invest.,* **2014**, *124*(9), 3923-3928.

[52] Lougaris, V.; Faletra, F.; Lanzi, G.; Vozzi, D.; Marcuzzi, A.; Valencic, E.; Piscianz, E.; Bianco, A.; Girardelli, M.; Baronio, M.; Loganes, C.; Fasth, A.; Salvini, F.; Trizzino, A.; Moratto, D.; Facchetti, F.; Giliani, S.; Plebani, A.; Tommasini, A. Altered germinal center reaction and abnormal B cell peripheral maturation in PI3KR1-mutated patients presenting with HIGM-like phenotype. *Clin. Immunol.,* **2015**, *159*(1), 33-36.

[53] Lucas, C.L.; Kuehn, H.S.; Zhao, F.; Niemela, J.E.; Deenick, E.K.; Palendira, U.; Avery, D.T.; Moens, L.; Cannons, J.L.; Biancalana, M.; Stoddard, J.; Ouyang, W.; Frucht, D.M.; Rao, V.K.; Atkinson, T.P.; Agharahimi, A.; Hussey, A.A.; Folio, L.R.; Olivier, K.N.; Fleisher, T.A.; Pittaluga, S.; Holland, S.M.; Cohen, J.I.; Oliveira, J.B.; Tangye, S.G.; Schwartzberg, P.L.; Lenardo, M.J.; Uzel, G. Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110δ result in T cell senescence and human immunodeficiency. *Nat. Immunol.,* **2014**, *15*(1), 88-97.

[54] Ballou, L.M.; Lin, R.Z. Rapamycin and mTOR kinase inhibitors. *J. Chem. Biol.,* **2008**, *1*(1-4), 27-36.

[55] Foukas, L.C.; Daniele, N.; Ktori, C.; Anderson, K.E.; Jensen, J.; Shepherd, P.R. Direct effects of caffeine and theophylline on p110 delta and other phosphoinositide 3-kinases. Differential effects on lipid kinase and protein kinase activities. *J. Biol. Chem.,* **2002**, *277*(40), 37124-37130.

[56] Lee, S.; Moon, J.S.; Lee, C-R.; Kim, H-E.; Baek, S-M.; Hwang, S.; Kang, G.H.; Seo, J.K.; Shin, C.H.; Kang, H.J.; Ko, J.S.; Park, S.G.; Choi, M. Abatacept alleviates severe autoimmune symptoms in a patient carrying a *de novo* variant in CTLA-4. *J. Allergy Clin. Immunol.,* **2016**, *137*(1), 327-330.

[57] Lo, B.; Zhang, K.; Lu, W.; Zheng, L.; Zhang, Q.; Kanellopoulou, C.; Zhang, Y.; Liu, Z.; Fritz, J.M.; Marsh, R.; Husami, A.; Kissell, D.; Nortman, S.; Chaturvedi, V.; Haines, H.; Young, L.R.; Mo, J.; Filipovich, A.H.; Bleesing, J.J.; Mustillo, P.; Stephens, M.; Rueda, C.M.; Chougnet, C.A.; Hoebe, K.; McElwee, J.; Hughes, J.D.; Karakoc-Aydiner, E.; Matthews, H.F.; Price, S.; Su, H.C.; Rao, V.K.; Lenardo, M.J.; Jordan, M.B. AUTOIMMUNE DISEASE. Patients with LRBA deficiency show CTLA4 loss and immune dysregulation responsive to abatacept therapy. *Science,* **2015**, *349*(6246), 436-440.

[58] Singh, N.; Chandler, P.R.; Seki, Y.; Baban, B.; Takezaki, M.; Kahler, D.J.; Munn, D.H.; Larsen, C.P.; Mellor, A.L.; Iwashima, M. Role of CD28 in fatal autoimmune disorder in scurfy mice. *Blood,* **2007**, *110*(4), 1199-1206.

[59] Xie, C.; Patel, R.; Wu, T.; Zhu, J.; Henry, T.; Bhaskarabhatla, M.; Samudrala, R.; Tus, K.; Gong, Y.; Zhou, H.; Wakeland, E.K.; Zhou, X.J.; Mohan, C. PI3K/AKT/mTOR hypersignaling in autoimmune lymphoproliferative disease engendered by the epistatic interplay of Sle1b and FASlpr. *Int. Immunol.,* **2007**, *19*(4), 509-522.

[60] Teachey, D.T.; Obzut, D.A.; Axsom, K.; Choi, J.K.; Goldsmith, K.C.; Hall, J.; Hulitt, J.; Manno, C.S.; Maris, J.M.; Rhodin, N.; Sullivan, K.E.; Brown, V.I.; Grupp, S.A. Rapamycin improves lymphoproliferative disease in murine autoimmune lymphoproliferative syndrome (ALPS). *Blood,* **2006**, *108*(6), 1965-1971.

[61] Teachey, D.T.; Greiner, R.; Seif, A.; Attiyeh, E.; Bleesing, J.; Choi, J.; Manno, C.; Rappaport, E.; Schwabe, D.; Sheen, C.; Sullivan, K.E.; Zhuang, H.; Wechsler, D.S.; Grupp, S.A. Treatment with sirolimus results in complete responses in patients with autoimmune lymphoproliferative syndrome. *Br. J. Haematol.,* **2009**, *145*(1), 101-106.

[62] Klemann, C.; Esquivel, M.; Magerus-Chatinet, A.; Lorenz, M.R.; Fuchs, I.; Neveux, N.; Castelle, M.; Rohr, J.; da Cunha, C.B.; Ebinger, M.; Kobbe, R.; Kremens, B.; Kollert, F.; Gambineri, E.; Lehmberg, K.; Seidel, M.G.; Siepermann, K.; Voelker, T.; Schuster, V.; Goldacker, S.; Schwarz, K.; Speckmann, C.; Picard, C.; Fischer, A.; Rieux-Laucat, F.; Ehl, S.; Rensing-Ehl, A.; Neven, B. Evolution of disease activity and biomarkers on and off rapamycin in 28 patients with autoimmune lymphoproliferative syndrome. *Haematologica,* **2017**, *102*(2), e52-e56.

[63] Hernandez, P.A.; Gorlin, R.J.; Lukens, J.N.; Taniuchi, S.; Bohinjec, J.; Francois, F.; Klotman, M.E.; Diaz, G.A. Mutations in the chemokine receptor gene CXCR4 are associated with WHIM syndrome, a combined immunodeficiency disease. *Nat. Genet.,* **2003**, *34*(1), 70-74.

[64] McDermott, D.H.; Liu, Q.; Velez, D.; Lopez, L.; Anaya-O'Brien, S.; Ulrick, J.; Kwatemaa, N.; Starling, J.; Fleisher, T.A.; Priel, D.A.L.; Merideth, M.A.; Giuntoli, R.L.; Evbuomwan, M.O.; Littel, P.; Marquesen, M.M.; Hilligoss, D.; DeCastro, R.; Grimes, G.J.; Hwang, S.T.; Pittaluga, S.; Calvo, K.R.; Stratton, P.; Cowen, E.W.; Kuhns, D.B.; Malech, H.L.; Murphy, P.M. A phase 1 clinical trial of long-term, low-dose treatment of WHIM syndrome with the CXCR4 antagonist plerixafor. *Blood,* **2014**, *123*(15), 2308-2316.

[65] Back, A.L.; Kwok, W.W.; Hickstein, D.D. Identification of two molecular defects in a child with leukocyte adherence deficiency. *J. Biol. Chem.,* **1992**, *267*(8), 5482-5487.

[66] Moutsopoulos, N.M.; Zerbe, C.S.; Wild, T.; Dutzan, N.; Brenchley, L.; DiPasquale, G.; Uzel, G.; Axelrod, K.C.; Lisco, A.; Notarangelo, L.D.; Hajishengallis, G.; Notarangelo, L.D.; Holland, S.M. Interleukin-12 and interleukin-23 blockade in leukocyte adhesion deficiency type 1. *N. Engl. J. Med.,* **2017**, *376*(12), 1141-1146.

[67] van de Veerdonk, F.L.; Plantinga, T.S.; Hoischen, A.; Smeekens, S.P.; Joosten, L.A.B.; Gilissen, C.; Arts, P.; Rosentul, D.C.; Carmichael, A.J.; Smits-van der Graaf, C.A.A.; Kullberg, B.J.; van der Meer, J.W.M.; Lilic, D.;

Veltman, J.A.; Netea, M.G. STAT1 mutations in autosomal dominant chronic mucocutaneous candidiasis. *N. Engl. J. Med.,* **2011**, *365*(1), 54-61.

[68] Toubiana, J.; Okada, S.; Hiller, J.; Oleastro, M.; Lagos Gomez, M.; Aldave Becerra, J.C.; Ouachée-Chardin, M.; Fouyssac, F.; Girisha, K.M.; Etzioni, A.; Van Montfrans, J.; Camcioglu, Y.; Kerns, L.A.; Belohradsky, B.; Blanche, S.; Bousfiha, A.; Rodriguez-Gallego, C.; Meyts, I.; Kisand, K.; Reichenbach, J.; Renner, E.D.; Rosenzweig, S.; Grimbacher, B.; van de Veerdonk, F.L.; Traidl-Hoffmann, C.; Picard, C.; Marodi, L.; Morio, T.; Kobayashi, M.; Lilic, D.; Milner, J.D.; Holland, S.; Casanova, J-L.; Puel, A. Heterozygous STAT1 gain-of-function mutations underlie an unexpectedly broad clinical phenotype. *Blood,* **2016**, *127*(25), 3154-3164.

[69] Boisson-Dupuis, S.; Kong, X-F.; Okada, S.; Cypowyj, S.; Puel, A.; Abel, L.; Casanova, J-L. Inborn errors of human STAT1: allelic heterogeneity governs the diversity of immunological and infectious phenotypes. *Curr. Opin. Immunol.,* **2012**, *24*(4), 364-378.

[70] Higgins, E.; Al Shehri, T.; McAleer, M.A.; Conlon, N.; Feighery, C.; Lilic, D.; Irvine, A.D. Use of ruxolitinib to successfully treat chronic mucocutaneous candidiasis caused by gain-of-function signal transducer and activator of transcription 1 (STAT1) mutation. *J. Allergy Clin. Immunol.,* **2015**, *135*(2), 551-553.

[71] Weinacht, K.G.; Charbonnier, L-M.; Alroqi, F.; Plant, A.; Qiao, Q.; Wu, H.; Ma, C.; Torgerson, T.R.; Rosenzweig, S.D.; Fleisher, T.A.; Notarangelo, L.D.; Hanson, I.C.; Forbes, L.R.; Chatila, T.A. Ruxolitinib reverses dysregulated T helper cell responses and controls autoimmunity caused by a novel signal transducer and activator of transcription 1 (STAT1) gain-of-function mutation. *J. Allergy Clin. Immunol.,* **2017**, *139*(5), 1629-1640.e2.

[72] Milner, J.D.; Vogel, T.P.; Forbes, L.; Ma, C.A.; Stray-Pedersen, A.; Niemela, J.E.; Lyons, J.J.; Engelhardt, K.R.; Zhang, Y.; Topcagic, N.; Roberson, E.D.O.; Matthews, H.; Verbsky, J.W.; Dasu, T.; Vargas-Hernandez, A.; Varghese, N.; McClain, K.L.; Karam, L.B.; Nahmod, K.; Makedonas, G.; Mace, E.M.; Sorte, H.S.; Perminow, G.; Rao, V.K.; O'Connell, M.P.; Price, S.; Su, H.C.; Butrick, M.; McElwee, J.; Hughes, J.D.; Willet, J.; Swan, D.; Xu, Y.; Santibanez-Koref, M.; Slowik, V.; Dinwiddie, D.L.; Ciaccio, C.E.; Saunders, C.J.; Septer, S.; Kingsmore, S.F.; White, A.J.; Cant, A.J.; Hambleton, S.; Cooper, M.A. Early-onset lymphoproliferation and autoimmunity caused by germline STAT3 gain-of-function mutations. *Blood,* **2015**, *125*(4), 591-599.

[73] Ekins, S.; Williams, A.J.; Krasowski, M.D.; Freundlich, J.S. In silico repositioning of approved drugs for rare and neglected diseases. *Drug Discov. Today,* **2011**, *16*(7-8), 298-310.

[74] Willett, P. Similarity searching using 2D structural fingerprints. *Methods Mol. Biol.,* **2011**, *672*, 133-158.

[75] Kharkar, P.S.; Warrier, S.; Gaud, R.S. Reverse docking: a powerful tool for drug repositioning and drug rescue. *Future Med. Chem.,* **2014**, *6*(3), 333-342.

[76] Gao, Z.; Li, H.; Zhang, H.; Liu, X.; Kang, L.; Luo, X.; Zhu, W.; Chen, K.; Wang, X.; Jiang, H. PDTD: A web-accessible protein database for drug target identification. *BMC Bioinformatics,* **2008**, *9*, 104.

[77] Li, H.; Gao, Z.; Kang, L.; Zhang, H.; Yang, K.; Yu, K.; Luo, X.; Zhu, W.; Chen, K.; Shen, J.; Wang, X.; Jiang, H. TarFisDock: A web server for identifying drug targets with docking approach. *Nucleic Acids Res*., **2006**, *34*(Suppl 2), W219-224.

[78] Zhu, F.; Shi, Z.; Qin, C.; Tao, L.; Liu, X.; Xu, F.; Zhang, L.; Song, Y.; Liu, X.; Zhang, J.; Han, B.; Zhang, P.; Chen, Y. Therapeutic target database update 2012: a resource for facilitating target-oriented drug discovery. *Nucleic Acids Res.,* **2012**, *40*(Database issue), D1128-D1136.

[79] Bisson, W.H.; Cheltsov, A.V.; Bruey-Sedano, N.; Lin, B.; Chen, J.; Goldberger, N.; May, L.T.; Christopoulos, A.; Dalton, J.T.; Sexton, P.M.; Zhang, X-K.; Abagyan, R. Discovery of antiandrogen activity of nonsteroidal scaffolds of marketed drugs. *Proc. Natl. Acad. Sci. USA,* **2007**, *104*(29), 11927-11932.

[80] Liu, X.; Zhu, F.; Ma, X.H.; Shi, Z.; Yang, S.Y.; Wei, Y.Q.; Chen, Y.Z. Predicting targeted polypharmacology for drug repositioning and multi- target drug discovery. *Curr. Med. Chem.,* **2013**, *20*(13), 1646-1661.

[81] Liu, X.; Ouyang, S.; Yu, B.; Liu, Y.; Huang, K.; Gong, J.; Zheng, S.; Li, Z.; Li, H.; Jiang, H. PharmMapper server: A web server for potential drug target identification using pharmacophore mapping approach. *Nucleic Acids Res.,* **2010**, *38*, W609-614.

[82] Ekins, S.; Johnston, J.S.; Bahadduri, P.; D'Souza, V.M.; Ray, A.; Chang, C.; Swaan, P.W. *In vitro* and pharmacophore-based discovery of novel hPEPT1 inhibitors. *Pharm. Res.,* **2005**, *22*(4), 512-517.

[83] Chang, C.; Bahadduri, P.M.; Polli, J.E.; Swaan, P.W.; Ekins, S. Rapid identification of P-glycoprotein substrates and inhibitors. *Drug Metab. Dispos.,* **2006**, *34*(12), 1976-1984.

[84] Diao, L.; Ekins, S.; Polli, J.E. Quantitative structure activity relationship for inhibition of human organic cation/carnitine transporter. *Mol. Pharm.,* **2010**, *7*(6), 2120-2131.

[85] Diao, L.; Ekins, S.; Polli, J.E. Novel inhibitors of human organic cation/carnitine transporter (hOCTN2) *via* computational modeling and *in vitro* testing. *Pharm. Res.,* **2009**, *26*(8), 1890-1900.

[86] Zheng, X.; Ekins, S.; Raufman, J-P.; Polli, J.E. Computational models for drug inhibition of the human apical sodium-dependent bile acid transporter. *Mol. Pharm.,* **2009**, *6*(5), 1591-1603.

[87] Dimova, D.; Stumpfe, D.; Bajorath, J. Quantifying the fingerprint descriptor dependence of structure-activity relationship information on a large scale. *J. Chem. Inf. Model.,* **2013**, *53*(9), 2275-2281.

[88] Krasowski, M.D.; Siam, M.G.; Iyer, M.; Ekins, S. Molecular similarity methods for predicting cross-reactivity with therapeutic drug monitoring immunoassays. *Ther. Drug Monit.,* **2009**, *31*(3), 337-344.

[89] Krasowski, M.D.; Siam, M.G.; Iyer, M.; Pizon, A.F.; Giannoutsos, S.; Ekins, S. Chemoinformatic methods for predicting interference in drug of abuse/toxicology immunoassays. *Clin. Chem.,* **2009**, *55*(6), 1203-1213.

[90] Krasowski, M.D.; Pizon, A.F.; Siam, M.G.; Giannoutsos, S.; Iyer, M.; Ekins, S. Using molecular similarity to highlight the challenges of routine immunoassay-based drug of abuse/toxicology screening in emergency medicine. *BMC Emerg. Med.,* **2009**, *9*, 5.

[91] Keiser, M.J.; Setola, V.; Irwin, J.J.; Laggner, C.; Abbas, A.I.; Hufeisen, S.J.; Jensen, N.H.; Kuijer, M.B.; Matos, R.C.; Tran, T.B.; Whaley, R.; Glennon, R.A.; Hert, J.; Thomas, K.L.H.; Edwards, D.D.; Shoichet, B.K.; Roth, B.L. Predicting new molecular targets for known drugs. *Nature,* **2009**, *462*(7270), 175-181.

[92] Keiser, M.J.; Roth, B.L.; Armbruster, B.N.; Ernsberger, P.; Irwin, J.J.; Shoichet, B.K. Relating protein pharmacology by ligand chemistry. *Nat. Biotechnol.,* **2007**, *25*(2), 197-206.

[93] Shin, W-H.; Zhu, X.; Bures, M.G.; Kihara, D. Three-dimensional compound comparison methods and their application in drug discovery. *Molecules,* **2015**, *20*(7), 12841-12862.

[94] Vilar, S.; Hripcsak, G. Leveraging 3D chemical similarity, target and phenotypic data in the identification of drug-protein and drug-adverse effect associations. *J. Cheminform.,* **2016**, *8*, 35.

[95] Ting, H.J.; Khasawneh, F.T. Glybenclamide: an antidiabetic with *in vivo* antithrombotic activity. *Eur. J. Pharmacol.,* **2010**, *649*(1-3), 249-254.

[96] Lamb, J.; Crawford, E.D.; Peck, D.; Modell, J.W.; Blat, I.C.; Wrobel, M.J.; Lerner, J.; Brunet, J-P.; Subramanian, A.; Ross, K.N.; Reich, M.; Hieronymus, H.; Wei, G.; Armstrong, S.A.; Haggarty, S.J.; Clemons, P.A.; Wei, R.; Carr, S.A.; Lander, E.S.; Golub, T.R. The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. *Science,* **2006**, *313*(5795), 1929-1935.

[97] Duan, Q.; Flynn, C.; Niepel, M.; Hafner, M.; Muhlich, J. L.; Fernandez, N. F.; Rouillard, A. D.; Tan, C. M.; Chen, E. Y.; Golub, T. R.; Sorger, P. K.; Subramanian, A.; Ma'ayan, A. LINCS canvas browser: Interactive web app to query, browse and interrogate LINCS L1000 gene expression signatures. *Nucleic Acids Res.,* **2014**, *42*, W449-460.

[98] Jun, H.Y.; Kim, T-H.; Choi, J.W.; Lee, Y.H.; Lee, K.K.; Yoon, K-H. Evaluation of connectivity map-discovered celastrol as a radiosensitizing agent in a murine lung carcinoma model: Feasibility study of diffusion-weighted magnetic resonance imaging. *PLoS One,* **2017**, *12*(5), e0178204.

[99] Hassane, D.C.; Guzman, M.L.; Corbett, C.; Li, X.; Abboud, R.; Young, F.; Liesveld, J.L.; Carroll, M.; Jordan, C.T. Discovery of agents that eradicate leukemia stem cells using an in silico screen of public gene expression data. *Blood,* **2008**, *111*(12), 5654-5662.

[100] Cheng, H-W.; Liang, Y-H.; Kuo, Y-L.; Chuu, C-P.; Lin, C-Y.; Lee, M-H.; Wu, A.T.H.; Yeh, C-T.; Chen, E.I-T.; Whang-Peng, J.; Su, C-L.; Huang, C-Y. Identification of thioridazine, an antipsychotic drug, as an antiglioblastoma and anticancer stem cell agent using public gene expression data. *Cell Death Dis.,* **2015**, *6*(5), e1753.

[101] Rho, S.B.; Kim, B-R.; Kang, S. A gene signature-based approach identifies thioridazine as an inhibitor of phosphatidylinositol-3′-kinase (PI3K)/AKT pathway in ovarian cancer cells. *Gynecol. Oncol.,* **2011**, *120*(1), 121-127.

[102] Wen, Q.; Kim, C-S.; Hamilton, P.W.; Zhang, S-D. A gene-signature progression approach to identifying candidate small-molecule cancer therapeutics with connectivity mapping. *BMC Bioinformatics,* **2016**, *17*(1), 211.

[103] Fortney, K.; Griesman, J.; Kotlyar, M.; Pastrello, C.; Angeli, M.; Sound-Tsao, M.; Jurisica, I. Prioritizing therapeutics for lung cancer: an integrative meta-analysis of cancer gene signatures and chemogenomic data. *PLOS Comput. Biol.,* **2015**, *11*(3), e1004068.

[104] O'Reilly, P.G.; Wen, Q.; Bankhead, P.; Dunne, P.D.; McArt, D.G.; McPherson, S.; Hamilton, P.W.; Mills, K.I.; Zhang, S-D. QUADrATiC: scalable gene expression connectivity mapping for repurposing FDA-approved therapeutics. *BMC Bioinformatics,* **2016**, *17*(1), 198.

[105] Wu, H.; Huang, J.; Zhong, Y.; Huang, Q. DrugSig: A resource for computational drug repositioning utilizing gene expression signatures. *PLoS One,* **2017**, *12*(5), e0177743.

[106] Lee, B.K.B.; Tiong, K.H.; Chang, J.K.; Liew, C.S.; Abdul Rahman, Z.A.; Tan, A.C.; Khang, T.F.; Cheong, S.C. DeSigN: connecting gene expression with therapeutics for drug repurposing and development. *BMC Genomics,* **2017**, *18*(1)(Suppl. 1), 934.

[107] Iorio, F.; Rittman, T.; Ge, H.; Menden, M.; Saez-Rodriguez, J. Transcriptional data: A new gateway to drug repositioning? *Drug Discov. Today,* **2013**, *18*(7-8), 350-357.

[108] Vitali, F.; Cohen, L.D.; Demartini, A.; Amato, A.; Eterno, V.; Zambelli, A.; Bellazzi, R. A network-based data integration approach to support drug repurposing and multi-target therapies in triple negative breast cancer. *PLoS One,* **2016**, *11*(9), e0162407.

[109] Iorio, F.; Bosotti, R.; Scacheri, E.; Belcastro, V.; Mithbaokar, P.; Ferriero, R.; Murino, L.; Tagliaferri, R.; Brunetti-Pierri, N.; Isacchi, A.; di Bernardo, D. Discovery of drug mode of action and drug repositioning from transcriptional responses. *Proc. Natl. Acad. Sci. USA,* **2010**, *107*(33), 14621-14626.

[110] Setoain, J.; Franch, M.; Martínez, M.; Tabas-Madrid, D.; Sorzano, C.O.S.; Bakker, A.; Gonzalez-Couto, E.; Elvira, J.; Pascual-Montano, A. NFFinder: an online bioinformatics tool for searching similar transcriptomics experiments in the context of drug repositioning. *Nucleic Acids Res.,* **2015**, *43*(W1), W193-W199.

[111] Dai, Y.-F.; Zhao, X.-M. A survey on the computational approaches to identify drug targets in the postgenomic era. *BioMed Res. Int.,* **2015**, *2015*. Doi:10.1155/2015/239654

[112] Zhao, S.; Li, S. Network-based relating pharmacological and genomic spaces for drug target identification. *PLoS One,* **2010**, *5*(7), e11764.

[113] Li, Z.; Wang, R-S.; Zhang, X-S. Two-stage flux balance analysis of metabolic networks for drug target identification. *BMC Syst. Biol.,* **2011**, *5*(Suppl. 1), S11.

[114] Folger, O.; Jerby, L.; Frezza, C.; Gottlieb, E.; Ruppin, E.; Shlomi, T. Predicting selective drug targets in cancer through metabolic networks. *Mol. Syst. Biol.,* **2011**, *7*, 501.