

# MAOA and NOS3 are promising druggable targets for treating Lung Neoplasms that control activity of RXRA, TCF3 and FOXP1 transcription factor on promoters of genes encoding enzymes metabolizing given metabolites

Demo User

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Genome Enhancer release 3.2 (TRANSFAC®, TRANSPATH® and HumanPSD™ release 2023.1)

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## Abstract

In the present study we applied the software package "Genome Enhancer" to a data set that contains *metabolomics* data. The study is done in the context of *Lung Neoplasms*. The goal of this pipeline is to identify potential drug targets in the molecular network that governs the studied pathological process. In the first step of analysis pipeline discovers transcription factors (TFs) that regulate genes activities in the pathological state. The activities of these TFs are controlled by so-called master regulators, which are identified in the second step of analysis. After a subsequent druggability checkup, the most promising master regulators are chosen as potential drug targets for the analyzed pathology. At the end the pipeline comes up with (a) a list of known drugs and (b) investigational active chemical compounds with the potential to interact with selected drug targets.

From the data set analyzed in this study, we found the following TFs to be potentially involved in the regulation of the genes encoding enzymes metabolizing given metabolites: RXRA, TCF3 and FOXP1. The subsequent network analysis suggested

- eNOS
- arginase-2, mitochondrial
- amine oxidase (flavin-containing) A
- eNOS

as the most promising molecular targets for further research, drug development and drug repurposing initiatives on the basis of identified molecular mechanism of the studied pathology. Having checked the actual druggability potential of the full list of identified targets, both, via

information available in medical literature and via cheminformatics analysis of drug compounds, we have identified the following drugs as the most promising treatment candidates for the studied pathology: tariquidar, Nitroarginine and UBIQUINONE-1.

## 1. Introduction

Recording "-omics" data to measure gene activities, protein expression or metabolic events is becoming a standard approach to characterize the pathological state of an affected organism or tissue. Increasingly, several of these methods are applied in a combined approach leading to large "multiomics" datasets. Still the challenge remains how to reveal the underlying molecular mechanisms that render a given pathological state different from the norm. The disease-causing mechanism can be described by a re-wiring of the cellular regulatory network, for instance as a result of a genetic or epigenetic alterations influencing the activity of relevant genes. Reconstruction of the disease-specific regulatory networks can help identify potential master regulators of the respective pathological process. Knowledge about these master regulators can point to ways how to block a pathological regulatory cascade. Suppression of certain molecular targets as components of these cascades may stop the pathological process and cure the disease.

Conventional approaches of statistical "-omics" data analysis provide only very limited information about the causes of the observed phenomena and therefore contribute little to the understanding of the pathological molecular mechanism. In contrast, the "upstream analysis" method [1-4] applied here has been devised to provide a casual interpretation of the data obtained for a pathology state. This approach comprises two major steps: (1) analysing promoters and enhancers of genes encoding enzymes metabolizing given metabolites for the transcription factors (TFs) involved in their regulation and, thus, important for the process under study; (2) reconstructing the signaling pathways that activate these TFs and identifying master regulators at the top of such pathways. For the first step, the database TRANSFAC® [6] is employed together with the TF binding site identification algorithms Match [7] and CMA [8]. The second step involves the signal transduction database TRANSPATH® [9] and special graph search algorithms [10] implemented in the software "Genome Enhancer".

The "upstream analysis" approach has now been extended by a third step that reveals known drugs suitable to inhibit (or activate) the identified molecular targets in the context of the disease under study. This step is performed by using information from HumanPSD™ database [5]. In addition, some known drugs and investigational active chemical compounds are subsequently predicted as potential ligands for the revealed molecular targets. They are predicted using a pre-computed database of spectra of biological activities of chemical compounds of a library of 2245 known drugs and investigational chemical compounds from HumanPSD™ database. The spectra of biological activities for these compounds are computed using the program PASS on the basis of a (Q)SAR approach [11-13]. These predictions can be used for the research purposes - for further drug development and drug repurposing initiatives.

## 2. Data

For this study the following experimental data was used:

*Table 1. Experimental datasets used in the study*

File name	Data type
TGF_72h vs. NO_TGF_72h	Metabolomics



Figure 1. Annotation diagram of experimental data used in this study. With the colored boxes we show those sub-categories of the data that are compared in our analysis.

### 3. Results

We have analyzed the following condition: TNF vs NO\_TNF 72h.

#### **3.1. Identification of target genes**

In the first step of the analysis **target genes** were identified from the uploaded experimental data. The metabolites were mapped to Recon2 database. Then, genes encoding enzymes, which are involved in synthesis, degradation or modification of these metabolites were identified in Recon2 database. These genes (**target genes**) were then used for further upstream analysis.

Table 2. Top ten genes encoding enzymes, metabolising target metabolites in TNF vs NO\_TNF 72h.

[See full table](#) →

ID	Gene description	Gene symbol	Recon2 ID	Title	logFC
<a href="#">ENSG00000109107</a>	aldolase, fructose-bisphosphate C	ALDOC	M_pmtcrn	L-palmitoylcarnitine	12.21
<a href="#">ENSG00000110090</a>	carnitine palmitoyltransferase 1A	CPT1A	M_dgsn,M_pmtcrn	Deoxyguanosine,L-palmitoylcarnitine	12.21
<a href="#">ENSG00000136872</a>	aldolase, fructose-bisphosphate B	ALDOB	M_pmtcrn	L-palmitoylcarnitine	12.21
<a href="#">ENSG00000149925</a>	aldolase, fructose-bisphosphate A	ALDOA	M_pmtcrn	L-palmitoylcarnitine	12.21
<a href="#">ENSG00000157184</a>	carnitine palmitoyltransferase 2	CPT2	M_dgsn,M_pmtcrn	Deoxyguanosine,L-palmitoylcarnitine	12.21
<a href="#">ENSG00000169169</a>	carnitine palmitoyltransferase 1C	CPT1C	M_dgsn,M_pmtcrn	Deoxyguanosine,L-palmitoylcarnitine	12.21
<a href="#">ENSG00000178537</a>	solute carrier family 25 member 20	SLC25A20	M_pmtcrn	L-palmitoylcarnitine	12.21
<a href="#">ENSG00000205560</a>	carnitine palmitoyltransferase 1B	CPT1B	M_dgsn,M_pmtcrn	Deoxyguanosine,L-palmitoylcarnitine	12.21
<a href="#">ENSG00000129673</a>	aralkylamine N-acetyltransferase	AANAT	M_Nacsertn	N-acetylserotonin	11.77
<a href="#">ENSG00000196433</a>	acetylserotonin O-methyltransferase	ASMT	M_Nacsertn,M_ahcys	N-acetylserotonin,S-Adenosyl-L-homocysteine	11.77

### **3.2. Functional classification of genes**

A functional analysis of genes encoding enzymes metabolizing given metabolites was done by mapping the genes to several known ontologies, such as Gene Ontology (GO), disease ontology (based on HumanPSD™ database) and the ontology of signal transduction and metabolic pathways from the [TRANSPATH®](#) database. Statistical significance was computed using a binomial test.

Figures 2-4 show the most significant categories.

### **Genes encoding enzymes, metabolising target metabolites in TNF vs NO\_TNF 72h:**

237 genes encoding enzymes, metabolising target metabolites in TNF vs NO\_TNF 72h genes were taken for the mapping.

#### **GO (biological process)**

The treemap displays the KEGG metabolic pathway database, organized hierarchically. The largest categories are at the top, and more specific pathways branch out. The colors represent different metabolic categories, and the size of the rectangles indicates the number of pathways. The treemap shows a dense network of metabolic processes, with many pathways branching from a few central nodes. The overall structure is complex and detailed, reflecting the vastness of the KEGG database.

### Full classification →

**TRANSPATH® Pathways (2023.1)**

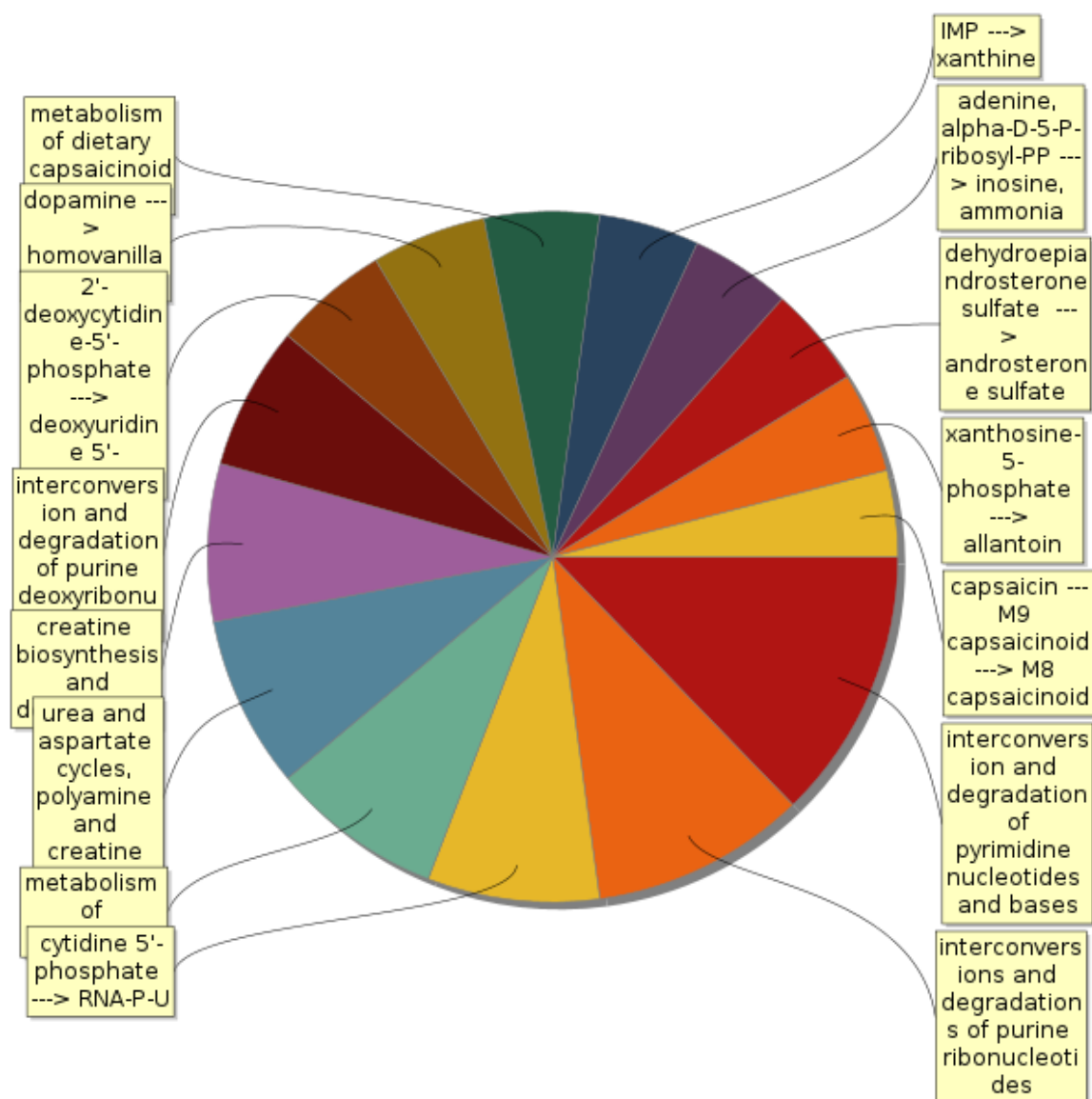


Figure 3. Enriched TRANSPATH® Pathways (2023.1) of genes encoding enzymes, metabolising target metabolites in TNF vs NO\_TNF 72h.

[Full classification →](#)

## HumanPSD(TM) disease (2023.1)

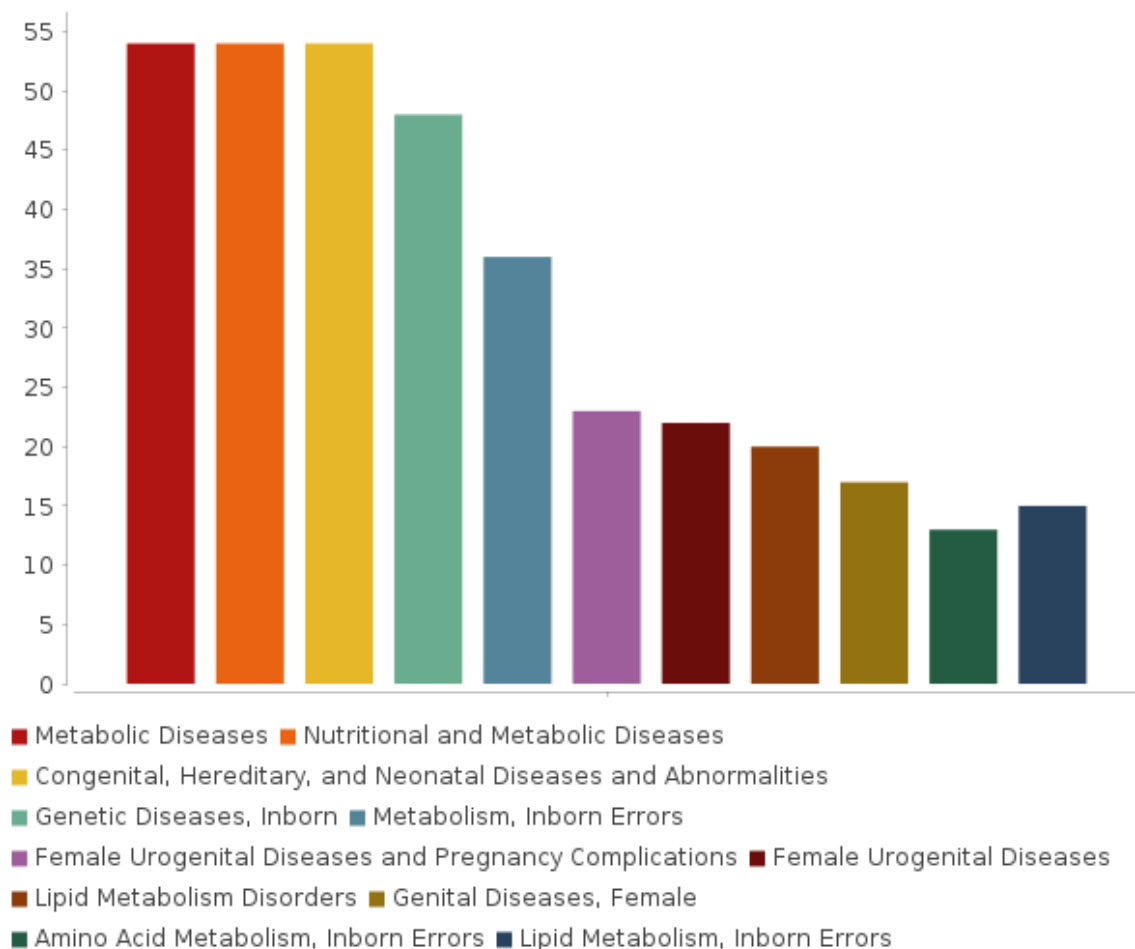
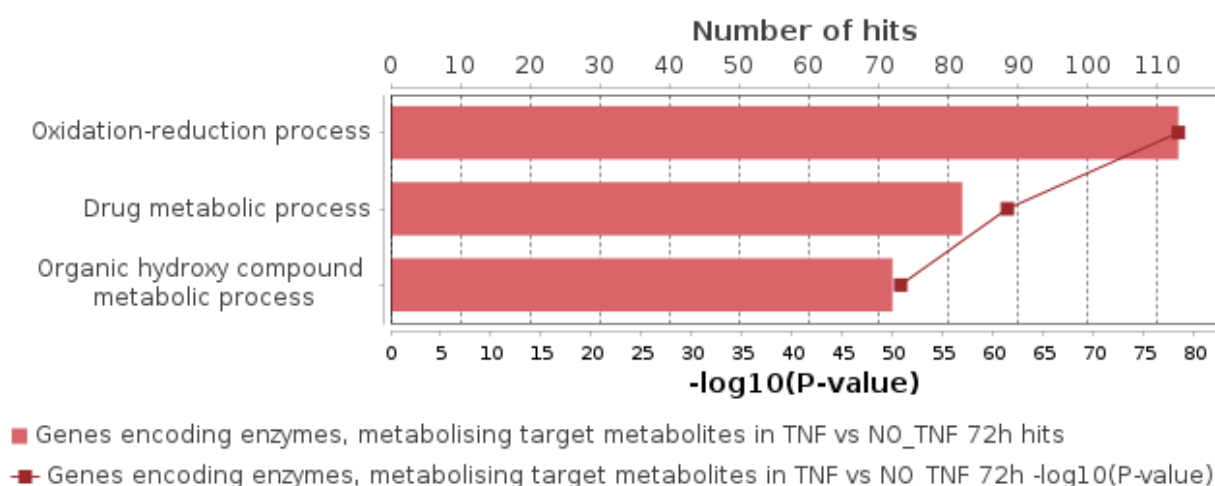


Figure 4. Enriched HumanPSD(TM) disease (2023.1) of genes encoding enzymes, metabolising target metabolites in TNF vs NO\_TNF 72h. The size of the bars correspond to the number of biomarkers of the given disease found among the input set.

[Full classification →](#)

The result of overall Gene Ontology (GO) analysis of the genes encoding enzymes metabolizing given metabolites of the studied pathology can be summarized by the following diagram, revealing the most significant functional categories overrepresented among the observed (genes encoding enzymes metabolizing given metabolites):



### **3.3. Analysis of enriched transcription factor binding sites and composite modules**

In the next step a search for transcription factors binding sites (TFBS) was performed in the regulatory regions of the **target genes** by using the TF binding motif library of the [TRANSFAC®](#)

database. We searched for so called **composite modules** that act as potential condition-specific **enhancers** of the **target genes** in their upstream regulatory regions (-1000 bp upstream of transcription start site (TSS)) and identify transcription factors regulating activity of the genes through such **enhancers**.

Classically, **enhancers** are defined as regions in the genome that increase transcription of one or several genes when inserted in either orientation at various distances upstream or downstream of the gene [8]. Enhancers typically have a length of several hundreds of nucleotides and are bound by multiple transcription factors in a cooperative manner [9].

We applied the Composite Module Analyst (CMA) [8] method to detect such potential enhancers, as targets of multiple TFs bound in a cooperative manner to the regulatory regions of the genes of interest. CMA applies a genetic algorithm to construct a generalized model of the enhancers by specifying combinations of TF motifs (from [TRANSFAC®](#)) whose sites are most frequently clustered together in the regulatory regions of the studied genes. CMA identifies the transcription factors that through their cooperation provide a synergistic effect and thus have a great influence on the gene regulation process.

### **Enhancer model potentially involved in regulation of target genes (genes encoding enzymes, metabolising target metabolites in TNF vs NO\_TNF 72h).**

To build the most specific composite modules we choose genes as the input of CMA algorithm.



The model consists of 2 module(s). Below, for each module the following information is shown:

- PWMs producing matches,
- number of individual matches for each PWM,
- score of the best match.

#### Module 1:

V\$FOXP1\_05  
0.96; N=1

V\$FXR\_01  
0.93; N=2

V\$SP3\_01  
0.96; N=2

V\$RXRA\_16  
0.87; N=3

V\$POU6F1\_01  
0.84; N=2

V\$FRA1\_Q6\_01  
0.92; N=2

Module width: 116

#### Module 2:

V\$FOXP1\_05  
0.96; N=3

V\$E2A\_Q6\_01  
0.97; N=2

V\$TCF7L2\_07  
0.89; N=1

V\$RBPJ\_02  
1.00; N=3

V\$HNF1A\_Q5  
0.92; N=2

V\$SOX10\_07  
0.92; N=1

Module width: 144

**Model score ( $-p \cdot \log_{10}(pval)$ ): 15.56**

**Wilcoxon p-value (pval): 1.65e-33**

**Penalty (p): 0.475**

**Average yes-set score: 4.57**

**Average no-set score: 2.67**

**AUC: 0.80**

**Separation point: 3.91**

**False-positive: 20.42%**

**False-negative: 30.38%**

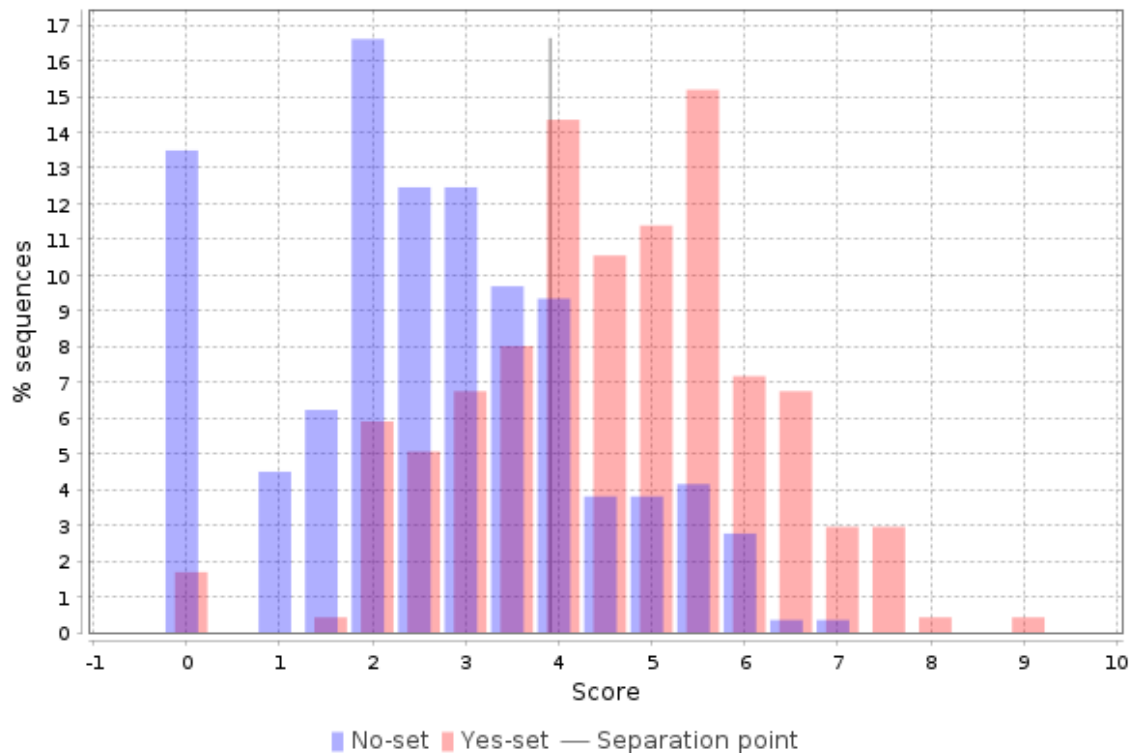


Table 3. List of top ten genes encoding enzymes, metabolising target metabolites in TNF vs NO\_TNF 72h with identified enhancers in their regulatory regions. **CMA score** - the score of the CMA model of the enhancer identified in the regulatory region.

[See full table →](#)

Ensembl IDs	Gene symbol	Gene description	CMA score	Factor names
ENSG00000167600	CYP2S1	cytochrome P450 family 2 subfamily S member 1	8.77	POU6F1(h), E2A(h), Fra-1(h), HNF-1alpha(h), FXR(h), RXRalpha(h), SOX-10(h)...
ENSG00000158125	XDH	xanthine dehydrogenase	8.14	SOX-10(h), Fra-1(h), RXRalpha(h), FXR(h), TCF-7L2(h), HNF-1alpha(h)
ENSG00000101846	STS	steroid sulfatase	7.7	Fra-1(h), RXRalpha(h), FOXP1(h), FXR(h), POU6F1(h), TCF-7L2(h), E2A(h)
ENSG00000156222	SLC28A1	solute carrier family 28 member 1	7.7	FOXP1(h), E2A(h), Fra-1(h), RXRalpha(h), FXR(h)
ENSG00000160688	FLAD1	flavin adenine dinucleotide synthetase 1	7.69	SOX-10(h), FOXP1(h), RXRalpha(h), FXR(h), POU6F1(h), Fra-1(h)
ENSG00000178537	SLC25A20	solute carrier family 25 member 20	7.52	E2A(h), Sp3(h), RXRalpha(h), RBP-Jkappa(h), FOXP1(h), FXR(h), SOX-10(h)
ENSG00000184254	ALDH1A3	aldehyde dehydrogenase 1 family member A3	7.45	SOX-10(h), TCF-7L2(h), RXRalpha(h), FXR(h), E2A(h), FOXP1(h)
ENSG00000186115	CYP4F2	cytochrome P450 family 4 subfamily F member 2	7.39	TCF-7L2(h), E2A(h), Fra-1(h), RXRalpha(h), FXR(h), POU6F1(h), Sp3(h)
ENSG00000160870	CYP3A7	cytochrome P450 family 3 subfamily A member 7	7.38	FOXP1(h), E2A(h), HNF-1alpha(h), SOX-10(h), RXRalpha(h), FXR(h)
ENSG00000107902	LHPP	phospholysine phosphohistidine inorganic pyrophosphate phosphatase	7.07	E2A(h), TCF-7L2(h), FXR(h), RXRalpha(h), RBP-Jkappa(h)

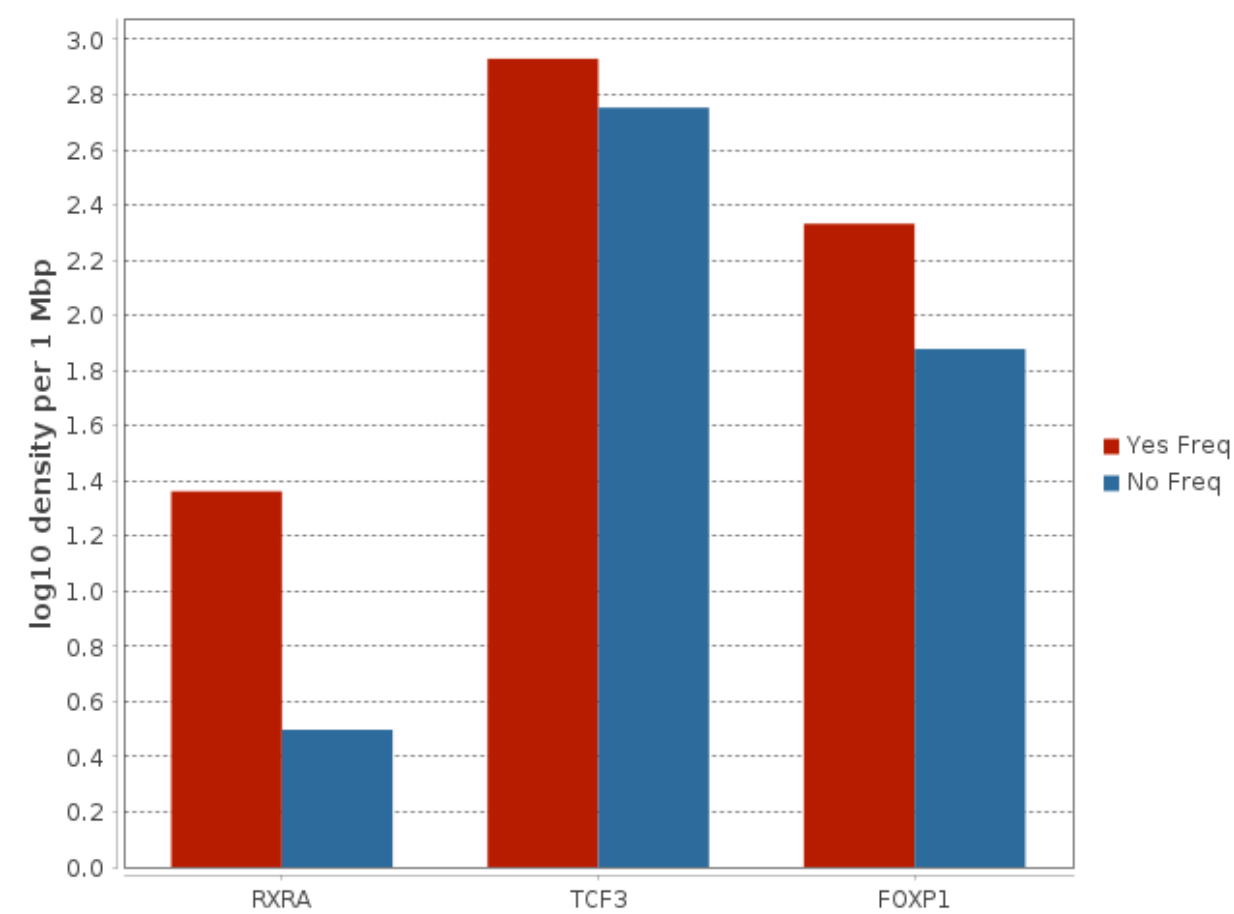
On the basis of the enhancer models we identified transcription factors potentially regulating the **target genes** of our interest. We found 11 transcription factors controlling expression of genes encoding enzymes, metabolising target metabolites in TNF vs NO\_TNF 72h (see Table 4).

Table 4. Transcription factors of the predicted enhancer model potentially regulating the genes encoding enzymes metabolizing given metabolites (genes encoding enzymes, metabolising target metabolites in TNF vs NO\_TNF 72h). **Yes-No ratio** is the ratio between frequencies of the sites in Yes sequences versus No sequences. It describes the level of the enrichment of binding sites for the indicated TF in the regulatory target regions. **Regulatory score** is the measure of involvement of the given TF in the controlling of expression of genes that encode master regulators presented below (through positive feedback loops).

[See full table →](#)

ID	Gene symbol	Gene description	Regulatory score	Yes-No ratio
<a href="#">MO000019619</a>	RXRA	retinoid X receptor alpha	1.77	7.32
<a href="#">MO000032492</a>	TCF3	transcription factor 3	1.77	1.5
<a href="#">MO000028068</a>	FOXP1	forkhead box P1	1.48	2.85
<a href="#">MO000088742</a>	NR1H4	nuclear receptor subfamily 1 group H member 4	1.48	2.19
<a href="#">MO000025684</a>	FOSL1	FOS like 1, AP-1 transcription factor subunit	1.42	4.88
<a href="#">MO000082618</a>	HNF1A	HNF1 homeobox A	1.4	2.31
<a href="#">MO000030964</a>	RBPJ	recombination signal binding protein for immunoglobulin kappa J region	1.37	1.43
<a href="#">MO000028320</a>			1.21	10.97
<a href="#">MO000046079</a>	SP3	Sp3 transcription factor	1.2	1.55
<a href="#">MO000026882</a>	TCF7L2	transcription factor 7 like 2	1.11	1.93

The following diagram represents the key transcription factors, which were predicted to be potentially regulating genes encoding enzymes metabolizing given metabolites in the analyzed pathology: RXRA, TCF3 and FOXP1.



**3.4. Finding master regulators in networks**

In the second step of the upstream analysis common regulators of the revealed TFs were identified. These master regulators appear to be the key candidates for therapeutic targets as they have a master effect on regulation of intracellular pathways that activate the pathological process of our study. The identified master regulators are shown in Table 5.



The identified master regulators that may govern pathology associated genes were checked for druggability potential using HumanPSD™ [5] database of gene-disease-drug assignments and PASS [11-13] software for prediction of biological activities of chemical compounds on the basis of a (Q)SAR approach. Respectively, for each master regulator protein we have computed two Druggability scores: HumanPSD Druggability score and PASS Druggability score. Where Druggability score represents the number of drugs that are potentially suitable for inhibition (or activation) of the corresponding target either according to the information extracted from medical literature (from HumanPSD™ database) or according to cheminformatics predictions of compounds activity against the examined target (from PASS software).

The cheminformatics druggability check is done using a pre-computed database of spectra of biological activities of chemical compounds from a library of all small molecular drugs from HumanPSD™ database, 2507 pharmaceutically active known chemical compounds in total. The spectra of biological activities has been computed using the program PASS [11-13] on the basis of a (Q)SAR approach.

If both Druggability scores were below defined thresholds (see Methods section for the details) such master regulator proteins were not used in further analysis of drug prediction.

As a result we created the following two tables of prospective drug targets (top targets are shown here):



Table 6. Prospective drug targets selected from full list of identified master regulators filtered by Druggability score from HumanPSD™ database. **Druggability score** contains the number of drugs that are potentially suitable for inhibition (or activation) of the target. The drug targets are sorted according to the **Total rank** which is the sum of three ranks computed on the basis of the three scores: keynode score, CMA score and expression change score (logFC, if present). See Methods section for details.

[See full table](#) →

Gene symbol	Gene Description	Druggability score	Total rank
MAOA	monoamine oxidase A	26	52
ARG2	arginase 2	2	63
NOS3	nitric oxide synthase 3	16	63
MPO	myeloperoxidase	22	72
NOS1	nitric oxide synthase 1	38	72
CYP1A2	cytochrome P450 family 1 subfamily A member 2	33	72



Table 7. Prospective drug targets selected from full list of identified master regulators filtered by Druggability score predicted by PASS software. Here, the **Druggability score** for master regulator proteins is computed as a sum of PASS calculated probabilities to be active as a target for various small molecular compounds. The drug targets are sorted according to the **Total rank** which is the sum of three ranks computed on the basis of the three scores: keynode score, CMA score and expression change score (logFC, if present). See Methods section for details.

[See full table](#) →

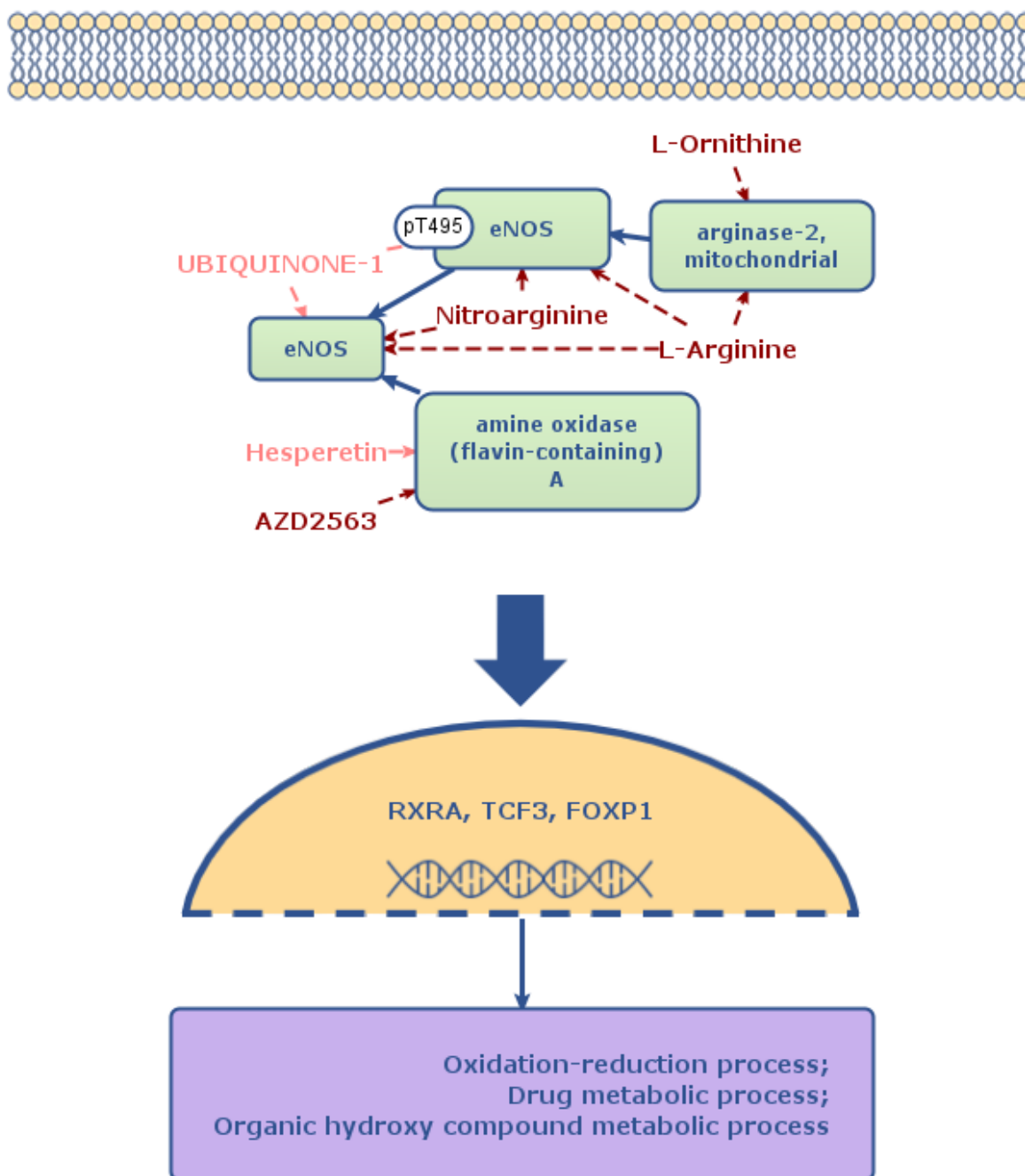
Gene symbol	Gene Description	Druggability score	Total rank
MAOA	monoamine oxidase A	4.95	52
NOS3	nitric oxide synthase 3	32.7	63
MPO	myeloperoxidase	96.88	72
NOS1	nitric oxide synthase 1	1.56	72
CYP1A2	cytochrome P450 family 1 subfamily A member 2	12.28	72
TPO	thyroid peroxidase	103.91	74

Below we represent schematically the main mechanism of the studied pathology. In the schema we considered the top two drug targets of each of the two categories computed above. In addition we have added two top identified master regulators for which no drugs may be identified yet, but that are playing the crucial role in the molecular mechanism of the studied pathology. Thus the

molecular mechanism of the studied pathology was predicted to be mainly based on the following key master regulators:

- eNOS
- arginase-2, mitochondrial
- amine oxidase (flavin-containing) A
- eNOS

This result allows us to suggest the following schema of affecting the molecular mechanism of the studied pathology:



Drugs which are shown on this schema: L-Arginine, Hesperetin, AZD2563, UBIQUINONE-1, Nitroarginine and L-Ornithine, should be considered as a prospective research initiative for further drug repurposing and drug development. These drugs were selected as top matching treatments to the most prospective drug targets of the studied pathology, however, these results should be considered with special caution and are to be used for research purposes only, as there is not enough clinical information for adapting these results towards immediate treatment of patients.

The drugs given in dark red color on the schema are FDA approved drugs or drugs which have gone through various phases of clinical trials as active treatments against the selected targets.

The drugs given in pink color on the schema are drugs, which were cheminformatically predicted to be active against the selected targets.

## 5. Identification of potential drugs

In the last step of the analysis we strived to identify known activities as well as drugs with cheminformatically predicted activities that are potentially suitable for inhibition (or activation) of the identified molecular targets in the context of specified human diseases(s).

Proposed drugs are top ranked drug candidates, that were found to be active on the identified targets and were selected from 4 categories:

1. FDA approved drugs or used in clinical trials drugs for the studied pathology;
2. Repurposing drugs used in clinical trials for other pathologies;
3. Drugs, predicted by PASS to be active against identified drug targets and against the studied pathology;
4. Drugs, predicted by PASS to be active against identified drug targets but for other pathologies.

Proposed drugs were selected on the basis of Drug rank which was computed from the ranks sum based on the individual ranks of the following scores:

- Target activity score (depends on ranks of all targets that were found for the selected drug);
- Disease activity score (weighted sum of number of clinical trials on disease(s) under study where the selected drug is known to be applied or PASS Disease activity score - cheminformatically predicted property of the compound to be active against the studied disease(s));
- Clinical validity score (applicable only for drugs predicted on the basis of literature curation in HumanPSD™ database (Tables 8 and 9), reflects the number of the highest clinical trials phase on which the drug was tested for any pathology).

You can refer to the Methods section for more details on drug ranking procedure.

Based on the Drug rank, a numerical value of Drug score was calculated, which reflects the potential activity of the respective drug on the overall molecular mechanism of the studied pathology. Drug score values belong to the range from 1 to 100 and are calculated as a quotient of maximum drug rank and the drug rank of the given drug multiplied by 100.

Top drugs of each category are given in the tables below:



## Drugs approved in clinical trials



Table 8. FDA approved drugs or drugs used in clinical trials for the studied pathology (most promising treatment candidates selected for the identified drug targets on the basis of literature curation in [HumanPSD™](#) database)

[See full table](#) →

Name	Target names	Drug score	Disease activity score	Disease trial phase
tariquidar	CYP2B6, CYP3A4, CYP1A2, CYP2E1, CYP2C9, CYP2C8, CYP2D6	95	6	Phase 3: Lung Neoplasms, Carcinoma, Non-Small-Cell Lung, Neoplasms
lidocaine patch	CYP1A2	88	3	Phase 2: Lung Neoplasms, Anxiety, Anxiety Disorders, Arthritis, Atrophy, Behavior, Behavior, Addictive, Blood Loss, Surgical, Breast Neoplasms, Carcinoma, Carpal Tunnel Syndrome, Cervical Intraepithelial Neoplasia, Cocaine-Related Disorders, Colic, Cystitis, Cystitis, Interstitial, Cysts, Diabetic Neuropathies, Enthesopathy, Epilepsy, Feeding and Eating Disorders, Fibromyalgia, Ganglion Cysts, Geographic Atrophy, Head and Neck Neoplasms, Headache, Heart Diseases, Heart Valve Diseases, Hemorrhage, Humeral Fractures, Infections, Infertility, Irritable Bowel Syndrome, Joint Diseases, Lacerations, Leiomyoma, Liver Diseases, Macular Degeneration, Migraine Disorders, Migraine with Aura, Migraine without Aura, Mucositis, Muscle Cramp, Myofascial Pain Syndromes, Myoma, Neoplasms, Nervous System Diseases, Neuralgia, Obesity, Osteoarthritis, Osteoarthritis, Knee, Pain, Peripheral Nervous System Diseases, Poroma, Post-Dural Puncture Headache, Premature Birth, Premature Ejaculation, Prostatic Neoplasms, Pulpitis, Rheumatic Diseases, Rotator Cuff Injuries, Scleroderma, Localized, Scleroderma, Systemic, Shoulder Impingement Syndrome, Somatoform Disorders, Spasm, Stomatitis, Stroke, Syndrome, Synovial Cyst, Temporomandibular Joint Disorders, Temporomandibular Joint Dysfunction Syndrome, Tendinopathy, Tennis Elbow, Trigeminal Nerve Diseases, Trigeminal Neuralgia, Urinary Incontinence, Urinary Incontinence, Stress, Urinary Tract Infections, Uterine Cervical Dysplasia, Wounds and Injuries
L-Arginine	NOS3, NOS2, ARG2	85	2	Phase 2: Lung Neoplasms, Alzheimer Disease, Amino Acid Metabolism, Inborn Errors, Anemia, Anemia, Sickle Cell, Aneurysm, Aortic Aneurysm, Aortic Aneurysm, Abdominal, Argininosuccinic Aciduria, Arthritis, Arthritis, Rheumatoid, Asthma, Carcinoma, Small Cell, Coronary Artery Disease, Coronary Disease, Cystic Fibrosis, Cysts, Death, Sudden, Cardiac, Dental Caries, Diabetes Mellitus, Diabetes Mellitus, Type 2, Diabetic Foot, Erectile Dysfunction, Essential Hypertension, Fibrosis, Foot Ulcer, Galactosemias, Head and Neck Neoplasms, Heart Arrest, Heart Defects, Congenital, Heart Diseases, Hepatitis, Hepatitis A, Hepatitis, Alcoholic, Hernias, Diaphragmatic, Congenital, Hypercholesterolemia, Hypertension, Inflammation, Ischemia, Lymphoma, Lymphoproliferative Disorders, MELAS Syndrome, Malaria, Malaria, Falciparum, Medulloblastoma, Meningioma, Metabolism, Inborn Errors, Mood Disorders, Myocardial Ischemia, Neoplasms, Nervous System Diseases, Neuroblastoma, Neuroendocrine Tumors, Pain, Peripheral Arterial Disease, Peripheral Nervous System Diseases, Peripheral Vascular Diseases, Prostatic Neoplasms, Prostatic



				Neoplasms, Castration-Resistant, Psychotic Disorders, Schizophrenia, Sepsis, Skin Ulcer, Small Cell Lung Carcinoma, Syndrome, Thalassemia, Tic Disorders, Ulcer, Univentricular Heart, Urea Cycle Disorders, Inborn, Urinary Bladder Neoplasms, Vascular Diseases, alpha-Thalassemia, beta-Thalassemia
Fulvestrant	NOS1, CYP1A2	82	3	Phase 2: Lung Neoplasms, Adenocarcinoma, Adenosarcoma, Aggression, Biliary Tract Neoplasms, Brain Neoplasms, Breast Neoplasms, Carcinoma, Carcinoma in Situ, Carcinoma, Ductal, Carcinoma, Ductal, Breast, Carcinoma, Intraductal, Noninfiltrating, Carcinoma, Lobular, Carcinoma, Non-Small-Cell Lung, Carcinoma, Ovarian Epithelial, Carcinoma, Small Cell, Carcinoma, Squamous Cell, Carcinoma, Transitional Cell, Carcinosarcoma, Colorectal Neoplasms, Cystadenocarcinoma, Cystadenocarcinoma, Serous, Cysts, Endometrial Neoplasms, Erythema, Familial Primary Pulmonary Hypertension, Fibrous Dysplasia, Polyostotic, Genetic Diseases, Inborn, Genital Neoplasms, Female, Head and Neck Neoplasms, Hypertension, Hypertension, Pulmonary, Leiomyosarcoma, Lupus Erythematosus, Systemic, Mixed Tumor, Mullerian, Myosarcoma, Neoplasm Metastasis, Neoplasms, Neutropenia, Ovarian Neoplasms, Prostatic Neoplasms, Prostatic Neoplasms, Castration-Resistant, Puberty, Precocious, Pulmonary Arterial Hypertension, Rectal Neoplasms, Recurrence, ST Elevation Myocardial Infarction, Sarcoma, Sarcoma, Endometrial Stromal, Sex Cord-Gonadal Stromal Tumors, Small Cell Lung Carcinoma, Squamous Cell Carcinoma of Head and Neck, Stomach Neoplasms, Syndrome, Triple Negative Breast Neoplasms, Urinary Bladder Neoplasms, Urologic Neoplasms, Uterine Cervical Neoplasms, Uterine Neoplasms
tilarginine	NOS1, NOS3, NOS2	82	1	Phase 1: Lung Neoplasms, Anemia, Anemia, Sickle Cell, Breast Neoplasms, Carcinoma, Carcinoma, Non-Small-Cell Lung, Carcinoma, Squamous Cell, Carcinoma, Transitional Cell, Cerebral Arterial Diseases, Cysts, DNA Repair-Deficiency Disorders, Endotoxemia, Essential Hypertension, Fibrosis, Glomerulonephritis, HIV Infections, Heart Failure, Hodgkin Disease, Hypercapnia, Hypertension, Hypoxia, Insulin Resistance, Kidney Diseases, Kidney Diseases, Cystic, Liver Cirrhosis, Lymphoma, Melanoma, Neoplasms, Nephritis, Obesity, Polycystic Kidney Diseases, Polycystic Kidney, Autosomal Dominant, Postural Orthostatic Tachycardia Syndrome, Sarcopenia, Squamous Cell Carcinoma of Head and Neck, Syncope, Syncope, Vasovagal, Syndrome, Tachycardia, Toxemia, Triple Negative Breast Neoplasms, Urinary Bladder Neoplasms

The ***Disease trial phase*** column reflects the maximum clinical trials phase in which the drug was studied for the analyzed pathology.

## Repurposing drugs



Table 9. Repurposed drugs used in clinical trials for other pathologies (prospective drugs against the identified drug targets on the basis of literature curation in [HumanPSD™](#) database)

[See full table](#) →

Name	Target names	Drug score	Maximum trial phase
Nitroarginine	NOS1, NOS3, NOS2	81	Phase 1: Cardiovascular Diseases, Neoplasms, Vascular Diseases
pimagedine HCl	NOS1, NOS3, NOS2	80	Not Applicable: Asthma, Lung Diseases, Lung Diseases, Obstructive, Pulmonary Disease, Chronic Obstructive
l-name	NOS1, NOS3, NOS2	80	Phase 3: Asthma, Paralysis, Quadriplegia
Raltegravir	CYP3A4, CYP1A2, CYP2C9, CYP2D6	80	Phase 4: Acquired Immunodeficiency Syndrome, Bone Diseases, Bone Diseases, Metabolic, Cardiovascular Diseases, Coinfection, Communicable Diseases, Drug-Related Side Effects and Adverse Reactions, Fatty Liver, Fatty Liver, Alcoholic, HIV Infections, HIV Seropositivity, Hepatitis, Hepatitis A, Hepatitis C, Hepatitis C, Chronic, Immunologic Deficiency Syndromes, Infections, Inflammation, Liver Diseases, Metabolic Diseases, Neurocognitive Disorders, Non-alcoholic Fatty Liver Disease, Osteoporosis, Syndrome, Tuberculosis, Vascular Diseases
Chlorzoxazone	CYP3A4, NOS1, NOS3, NOS2	80	Phase 4: Alcoholism, Arthritis, Diabetes Mellitus, Diabetes Mellitus, Type 2, Osteoarthritis, Osteoarthritis, Hip, Osteoarthritis, Knee, Pain

The **Maximum trial phase** column reflects the maximum clinical trials phase in which the drug was studied for any pathology.



No prospective drugs were found, which would be predicted by PASS software to be active against the identified drug targets and would be predicted to have biological activity against the studied disease(s).



Table 10. Prospective drugs, predicted by [PASS](#) software to be active against the identified drug targets, though without cheminformatically predicted activity against the studied disease(s) (drug candidates predicted with the cheminformatics tool PASS)

[See full table](#) →

Name	Target names	Drug score	Target activity score
UBIQUINONE-1	CYP2B6, CYP3A4, ALDH1A2, CYP1B1, CYP1A2, CYP2J2, NOS3, CYP2E1, CYP2C9, CYP2C8, CYP2D6, CYP1A1	95	0.44
Bisoxatin	CYP2B6, CYP3A4, CYP1B1, CYP1A2, CYP2J2, CYP2E1, CYP2C9, CYP2C8, CYP2D6, CYP1A1	86	0.51
Pravastatin	HMGCR, NOS3	86	0.12
Fluorouracil	CYP2B6, CYP3A4, CYP1B1, CYP1A2, CYP2J2, CYP2E1, CYP2C9, CYP2C8, CYP2D6, CYP1A1	86	0.16
Hesperetin	CYP3A4, TPO, CYP1A2, CYP2J2, XDH, CYP2C9, CYP2C8, CYP2B6, MAOA, MPO, CYP1B1, NOS3, CYP2E1, CYP2D6, CYP1A1	85	0.65

As the result of drug search we propose the following drugs as most promising candidates for treating the pathology under study: tariquidar, Nitroarginine and UBIQUINONE-1. These drugs were selected for acting on the following targets: CYP1A2 and NOS3, which were predicted to be active in the molecular mechanism of the studied pathology.

The selected drugs are top ranked drug candidates from each of the four categories of drugs: (1) FDA approved drugs or used in clinical trials drugs for the studied pathology; (2) repurposing

drugs used in clinical trials for other pathologies; (3) drugs, predicted by PASS software to be active against the studied pathology; (4) drugs, predicted by PASS software to be repurposed from other pathologies.

## **Supplementary drug info**

In addition to the approved and repurposed drugs proposed by Genome Enhancer, below the **Supplementary drug info** table is given, which contains an extended list of drugs used for treatment of neoplasms. Those drugs which were predicted by Genome Enhancer as prospective treatment candidates for the studied case (both approved and repurposed) have a respective **Predicted Drug Score** assigned to them. This value on a scale from 1 to 100 reflects the potential activity of the respective drug on the overall molecular mechanism of the studied pathology. The **Predicted Drug Score** column contains the "-" (Not Identified) value in case the drug targets of the respective treatment were not found in the molecular mechanism of the studied pathology.

*Table 11. Supplementary drug info: extended list of drugs used for treatment of neoplasms with respective drug scores predicted for the studied pathology.*

<b>Drug</b>	<b>Disease</b>	<b>Predicted Drug Score</b>
<a href="#">Abarelix</a>	Prostatic Neoplasms	-
<a href="#">Abemaciclib</a>	Breast Neoplasms	-
<a href="#">Abiraterone</a>	Prostatic Neoplasms, Castration-Resistant	55
<a href="#">Abiraterone acetate</a>	Prostatic Neoplasms, Castration-Resistant	-
<a href="#">Acalabrutinib</a>	Lymphoma, Mantle-Cell	-
<a href="#">Acitretin</a>	Psoriasis	-
<a href="#">Ado-trastuzumab emtansine</a>	Breast Neoplasms Neoplasms	-
<a href="#">Afatinib</a>	Carcinoma, Non-Small-Cell Lung	-
<a href="#">Aflibercept</a>	Colorectal Neoplasms Diabetic Retinopathy Edema Vascular Diseases Wet Macular Degeneration	72
<a href="#">Alectinib</a>	Carcinoma, Non-Small-Cell Lung	31
<a href="#">Alemtuzumab</a>	Brain Abscess Leukemia, Lymphocytic, Chronic, B-Cell Multiple Sclerosis Multiple Sclerosis, Relapsing-Remitting Sclerosis	-
<a href="#">Alitretinoin</a>	Sarcoma, Kaposi	-
<a href="#">Alpelisib</a>	Breast Neoplasms	-
<a href="#">Altretamine</a>	Ovarian Neoplasms	-
<a href="#">Aminolevulinic acid</a>	Keratosis Keratosis, Actinic	-
<a href="#">Anagrelide</a>	Thrombocythemia, Essential Thrombocytosis	-
<a href="#">Anastrozole</a>	Breast Neoplasms Hypersensitivity Obesity Obesity, Morbid Recurrence Weight Loss	51
<a href="#">Apalutamide</a>	Prostatic Neoplasms, Castration-Resistant	-
<a href="#">Aprepitant</a>	Nausea Neoplasms Postoperative Nausea and Vomiting	-
<a href="#">Arsenic trioxide</a>	Leukemia, Promyelocytic, Acute	-
<a href="#">Atezolizumab</a>	Carcinoma, Non-Small-Cell Lung Carcinoma, Transitional Cell Triple Negative Breast Neoplasms	-
<a href="#">Avelumab</a>	Carcinoma, Merkel Cell Carcinoma, Renal Cell Carcinoma, Transitional Cell	-
<a href="#">Axitinib</a>	Carcinoma, Renal Cell	49
<a href="#">Azacitidine</a>	Anemia, Refractory Anemia, Refractory, with Excess of Blasts Leukemia, Myelomonocytic, Chronic Myelodysplastic Syndromes Preleukemia Syndrome	34
<a href="#">Belinostat</a>	Lymphoma, T-Cell, Peripheral	-
<a href="#">Bendamustine</a>	Leukemia, Lymphocytic, Chronic, B-Cell Leukemia, Lymphoid	-
<a href="#">Bevacizumab</a>	Breast Neoplasms Colonic Neoplasms Colorectal Neoplasms Corneal Neovascularization Diabetic	-

	Retinopathy Dilatation, Pathologic Edema Epistaxis Glaucoma Hemorrhage Macular Degeneration Macular Edema Neoplasm Metastasis Neoplasms Neovascularization, Pathologic Optic Nerve Diseases Pterygium Rectal Neoplasms Retinal Detachment Retinal Diseases Retinal Vein Occlusion Telangiectasia, Hereditary Hemorrhagic Telangiectasis Vitreous Hemorrhage	
Bexarotene	Lymphoma, T-Cell Lymphoma, T-Cell, Cutaneous	-
Bicalutamide	Prostatic Neoplasms	5
Binimetinib	Melanoma	-
Blinatumomab	Precursor B-Cell Lymphoblastic Leukemia-Lymphoma	-
Bortezomib	Brain Abscess Glomerulonephritis Glomerulonephritis, IGA Kidney Diseases Multiple Myeloma Neoplasms, Plasma Cell Nephritis Renal Insufficiency	-
Bosutinib	Leukemia, Myelogenous, Chronic, BCR-ABL Positive	-
Brentuximab vedotin	Hodgkin Disease Lymphoma Lymphoma, Large-Cell, Anaplastic Lymphoma, T-Cell, Peripheral	-
Brigatinib	Carcinoma, Non-Small-Cell Lung	-
Buserelin	Prostatic Neoplasms	-
Cabazitaxel	Prostatic Neoplasms, Castration-Resistant	-
Cabergoline	Drug-Related Side Effects and Adverse Reactions Pituitary Neoplasms	-
Cabozantinib	Thyroid Neoplasms	-
Capecitabine	Breast Neoplasms Colonic Neoplasms Colorectal Neoplasms	-
Carboplatin	Carcinoma, Non-Small-Cell Lung Lung Neoplasms Neoplasms Neuroendocrine Tumors Ovarian Neoplasms Retinoblastoma	-
Carfilzomib	Multiple Myeloma	-
Carmustine	Astrocytoma Glioblastoma Hodgkin Disease Medulloblastoma Multiple Myeloma Neoplasms	-
Ceritinib	Carcinoma, Non-Small-Cell Lung	-
Cetuximab	Colorectal Neoplasms	-
Cinacalcet	Anemia Calcinosis Cardiovascular Diseases Hyperparathyroidism Hyperparathyroidism, Secondary Kidney Diseases Kidney Failure, Chronic Neoplasm Metastasis Neoplasms Parathyroid Neoplasms Renal Insufficiency Vascular Calcification Vascular Diseases Vision Disorders	-
Cisplatin	Carcinoma, Squamous Cell Neoplasms Uterine Cervical Neoplasms Carcinoma, Non-Small-Cell Lung Esophageal Neoplasms Carcinoma	31
Cladribine	Leukemia, Hairy Cell	-
Clofarabine	Precursor Cell Lymphoblastic Leukemia-Lymphoma	30
Cobimetinib	Melanoma	-
Copanlisib	Lymphoma, Follicular	-
Crizotinib	Carcinoma, Non-Small-Cell Lung	-
Cyproterone acetate	Prostatic Neoplasms	-
Dabrafenib	Melanoma	-
Dacomitinib	Carcinoma, Non-Small-Cell Lung	-
Daratumumab	Multiple Myeloma	-
Dasatinib	Leukemia, Myelogenous, Chronic, BCR-ABL Positive Leukemia, Myeloid, Chronic-Phase Precursor Cell Lymphoblastic Leukemia-Lymphoma	-
Decitabine	Anemia, Refractory Anemia, Refractory, with Excess of Blasts Leukemia, Myelomonocytic, Chronic Myelodysplastic Syndromes	32
Degarelix	Cardiovascular Diseases Prostatic Neoplasms Vascular Diseases	-
Denosumab	Arthritis, Rheumatoid Bone Diseases Bone Diseases, Metabolic Breast Neoplasms Hyperparathyroidism Hyperparathyroidism, Primary Metabolic Diseases Neoplasm	-

	Metastasis Neoplasms Osteoporosis Osteoporosis, Postmenopausal Prostatic Neoplasms	
Dexrazoxane	Breast Neoplasms Cardiomyopathies	-
Dienogest	Menorrhagia	-
Dinutuximab	Neuroblastoma	-
Docetaxel	Breast Neoplasms Carcinoma, Non-Small-Cell Lung Prostatic Neoplasms Squamous Cell Carcinoma of Head and Neck Stomach Neoplasms	-
Doxorubicin	Neoplasms Multiple Myeloma Carcinoma, Ovarian Epithelial Ovarian Neoplasms Leukemia, Lymphoid Breast Neoplasms Lymphoma, Follicular Thyroid Neoplasms Triple Negative Breast Neoplasms Glioma	47
Durvalumab	Carcinoma, Non-Small-Cell Lung Carcinoma, Transitional Cell	-
Dutasteride	Alcoholism Hyperplasia Hypertrophy Neoplasms Prostatic Hyperplasia	-
Duvelisib	Leukemia, Lymphocytic, Chronic, B-Cell Lymphoma, Follicular	-
Elotuzumab	Multiple Myeloma	-
Enasidenib	Leukemia, Myeloid, Acute	-
Encorafenib	Colorectal Neoplasms Melanoma	-
Enfortumab vedotin	Carcinoma, Transitional Cell Neoplasms	-
Entrectinib	Carcinoma, Non-Small-Cell Lung	-
Enzalutamide	Prostatic Neoplasms Prostatic Neoplasms, Castration-Resistant	-
Epirubicin	Breast Neoplasms	-
Erdafitinib	Urinary Bladder Neoplasms	-
Eribulin	Breast Neoplasms Drug-Related Side Effects and Adverse Reactions Neoplasms	-
Erlotinib	Carcinoma, Non-Small-Cell Lung Neoplasms Pancreatic Neoplasms	20
Erlotinib hydrochloride	Carcinoma, Non-Small-Cell Lung Gastrointestinal Stromal Tumors	-
Estramustine	Prostatic Neoplasms	-
Ethinyl Estradiol	Acne Vulgaris Neoplasms	6
Everolimus	Angiomyolipoma Arthrogryposis Astrocytoma Breast Neoplasms Carcinoma, Renal Cell Cysts Idiopathic Pulmonary Fibrosis Kidney Diseases, Cystic Kidney Failure, Chronic Lipoma Neuroendocrine Tumors Primary Graft Dysfunction Sclerosis Tuberous Sclerosis	-
Exemestane	Breast Neoplasms	57
Fedratinib	Primary Myelofibrosis	-
Finasteride	Hyperplasia Neoplasms Prostatic Hyperplasia	-
Flavopiridol	Leukemia, Lymphocytic, Chronic, B-Cell	-
Fluorouracil	Skin Neoplasms Neoplasms, Basal Cell Neoplasms, Second Primary Neoplasms, Squamous Cell Neoplasms Colorectal Neoplasms Pancreatic Neoplasms	53
Fluoxymesterone	Breast Neoplasms Hypogonadism Puberty, Delayed	-
Flutamide	Premenstrual Dysphoric Disorder Premenstrual Syndrome Prostatic Neoplasms	32
Fulvestrant	Breast Neoplasms	82
Gefitinib	Carcinoma, Non-Small-Cell Lung	-
Gemcitabine	Breast Neoplasms Carcinoma, Non-Small-Cell Lung Ovarian Neoplasms Pancreatic Neoplasms	-
Gemtuzumab ozogamicin	Leukemia, Myeloid, Acute	-
Gilteritinib	Leukemia, Myeloid, Acute	-
Glasdegib	Leukemia, Myeloid, Acute	-
Goserelin	Atrophy Breast Neoplasms Bulbo-Spinal Atrophy, X-Linked Endometriosis Muscular Atrophy Myoma Prostatic Neoplasms	-
Histrelin	Puberty, Precocious	-
Homoharringtonine	Leukemia, Myelogenous, Chronic, BCR-ABL Positive	-
Ibritumomab	Lymphoma, B-Cell Lymphoma, Follicular	-

Ibrutinib	Graft vs Host Disease Leukemia, Lymphocytic, Chronic, B-Cell Lymphoma, B-Cell, Marginal Zone Lymphoma, Mantle-Cell Waldenstrom Macroglobulinemia	-
Idarubicin	Leukemia, Myeloid, Acute	-
Idelalisib	Leukemia, Lymphocytic, Chronic, B-Cell Lymphoma, Follicular	-
Ifosfamide	Neoplasms	66
Imatinib	Leukemia, Myelogenous, Chronic, BCR-ABL Positive Mastocytosis, Systemic Neoplasms	-
Inotuzumab ozogamicin	Precursor B-Cell Lymphoblastic Leukemia-Lymphoma	-
Ipilimumab	Carcinoma, Renal Cell Melanoma	-
Irinotecan	Colorectal Neoplasms	37
Ivosidenib	Leukemia, Myeloid, Acute	-
Ixabepilone	Breast Neoplasms	66
Ixazomib	Multiple Myeloma	-
Lapatinib	Breast Neoplasms	-
Larotrectinib	Neoplasm Metastasis	-
Lenalidomide	Brain Abscess Lupus Erythematosus, Cutaneous Myelodysplastic Syndromes Neoplasms, Plasma Cell	-
Lenvatinib	Carcinoma, Hepatocellular Carcinoma, Renal Cell Thyroid Neoplasms	-
Letrozole	Breast Neoplasms Cysts Fibroma Myofibroma Myoma Ovarian Cysts Syndrome	41
Leuprolide	Hot Flashes Ovarian Hyperstimulation Syndrome Prostatic Neoplasms Puberty, Precocious	-
Levamisole	Ascariasis Colonic Neoplasms Helminthiasis	-
Levonorgestrel	Epilepsy Hyperplasia Menorrhagia	-
Lomustine	Brain Neoplasms Hodgkin Disease	-
Lonafarnib	Breast Neoplasms Carcinoma, Non-Small-Cell Lung Central Nervous System Neoplasms Colorectal Neoplasms Gliosarcoma Head and Neck Neoplasms Leukemia, Myelomonocytic, Chronic  Liver Neoplasms Lymphoma Myelodysplastic Syndromes Ovarian Neoplasms Urethral Neoplasms Urinary Bladder Neoplasms	-
Lorlatinib	Carcinoma, Non-Small-Cell Lung	-
Masoprocol	Keratosi, Actinic	-
Medroxyprogesterone Acetate	Depression Depression, Postpartum Depressive Disorder Metrorrhagia Neoplasms Uterine Hemorrhage	21
Megestrol acetate	Acquired Immunodeficiency Syndrome Bites and Stings Breast Neoplasms Pain Wasting Syndrome	-
Methotrexate	Neoplasms Breast Neoplasms Head and Neck Neoplasms Ovarian Neoplasms Lymphoma, T-Cell, Peripheral Brain Neoplasms Colorectal Neoplasms Neuroblastoma Carcinoma, Squamous Cell	-
Methyltestosterone	Breast Neoplasms Hypogonadism Puberty, Delayed	70
Midostaurin	Leukemia, Mast-Cell Leukemia, Myeloid, Acute Mastocytosis, Systemic	-
Mitotane	Adrenocortical Carcinoma	59
Mitoxantrone	Autoimmune Diseases Autoimmune Diseases of the Nervous System Demyelinating Autoimmune Diseases, CNS Immune System Diseases Leukemia, Myeloid, Acute Multiple Sclerosis Myelitis Myelitis, Transverse Nervous System Diseases Neuromyelitis Optica Prostatic Neoplasms, Castration-Resistant	55
Mogamulizumab	Mycosis Fungoides Neoplasms Sezary Syndrome	-
Moxetumomab pasudotox	Leukemia, Hairy Cell Neoplasms	-
Necitumumab	Carcinoma, Non-Small-Cell Lung Neoplasms	-
Nelarabine	Precursor T-Cell Lymphoblastic Leukemia-Lymphoma	-
Neratinib	Breast Neoplasms	-
Nilotinib	Blast Crisis Leukemia, Myelogenous, Chronic, BCR-ABL	-



	Positive Leukemia, Myeloid, Chronic-Phase	
Nilutamide	Prostatic Neoplasms	-
Nintedanib	Fibrosis Idiopathic Pulmonary Fibrosis	-
Niraparib	Carcinoma, Ovarian Epithelial Fallopian Tube Neoplasms Peritoneal Neoplasms	-
Nivolumab	Carcinoma, Non-Small-Cell Lung Kidney Neoplasms Neoplasms Lung Neoplasms Melanoma	-
Obinutuzumab	Leukemia, Lymphocytic, Chronic, B-Cell	-
Octreotide	Acromegaly Adenoma Ascites Carcinoid Tumor Fistula Pancreatic Fistula Pituitary Diseases Renal Insufficiency Vipoma	-
Ofatumumab	Leukemia, Lymphocytic, Chronic, B-Cell	-
Olaparib	Breast Neoplasms Carcinoma, Ovarian Epithelial Fallopian Tube Neoplasms Ovarian Neoplasms Pancreatic Neoplasms Peritoneal Neoplasms Prostatic Neoplasms, Castration-Resistant	-
Olaratumab	Sarcoma	-
Osimertinib	Carcinoma, Non-Small-Cell Lung	-
Oxaliplatin	Colonic Neoplasms Colorectal Neoplasms Neoplasms Rectal Neoplasms	-
Paclitaxel	Acute Coronary Syndrome Angina Pectoris Arteriosclerosis Breast Neoplasms Carcinoma, Non-Small-Cell Lung Cardiovascular Diseases Coronary Artery Disease Coronary Disease Coronary Stenosis Heart Diseases Myocardial Ischemia Ovarian Neoplasms Vascular Diseases	23
Palbociclib	Breast Neoplasms	-
Panitumumab	Colorectal Neoplasms	-
Panobinostat	Multiple Myeloma	-
Pazopanib	Carcinoma Carcinoma, Renal Cell Sarcoma	-
Pembrolizumab	Carcinoma, Hepatocellular Carcinoma, Merkel Cell Carcinoma, Non-Small-Cell Lung Carcinoma, Renal Cell Carcinoma, Transitional Cell Hodgkin Disease Melanoma Neoplasms Stomach Neoplasms	-
Pemetrexed	Carcinoma, Non-Small-Cell Lung Mesothelioma	-
Pentostatin	Leukemia, Hairy Cell	-
Pertuzumab	Breast Neoplasms	-
Pomalidomide	Multiple Myeloma	-
Ponatinib	Leukemia, Myelogenous, Chronic, BCR-ABL Positive Precursor Cell Lymphoblastic Leukemia-Lymphoma	-
Pralatrexate	Lymphoma, T-Cell, Peripheral	-
Radium Ra 223 Dichloride	Prostatic Neoplasms, Castration-Resistant	-
Ramucirumab	Stomach Neoplasms	-
Rasburicase	Hyperuricemia Leukemia Lymphoma Neoplasms Syndrome Tumor Lysis Syndrome	-
Regorafenib	Colorectal Neoplasms	-
Relugolix	Prostatic Neoplasms	-
Ribociclib	Breast Neoplasms	-
Rituximab	Arthritis Arthritis, Rheumatoid Granulomatosis with Polyangiitis Leukemia Leukemia, Lymphoid Lymphoma Lymphoma, B-Cell Lymphoma, Follicular Lymphoma, Non-Hodgkin Myelitis Neuromyelitis Optica Purpura Purpura, Thrombocytopenic Purpura, Thrombocytopenic, Idiopathic Thrombocytopenia	-
Romidepsin	Lymphoma, T-Cell, Cutaneous	-
Rucaparib	Carcinoma, Ovarian Epithelial Fallopian Tube Neoplasms Peritoneal Neoplasms Prostatic Neoplasms, Castration-Resistant	-
Ruxolitinib	Graft vs Host Disease Polycythemia Polycythemia Vera Primary Myelofibrosis Thrombocytosis	-
Selinexor	Multiple Myeloma	-
Selumetinib	Neurofibromatosis 1	-
Siltuximab	Giant Lymph Node Hyperplasia	-
Sirolimus	Angiomyolipoma Constriction, Pathologic Coronary Restenosis Eye	29

	Diseases Immune System Diseases Kidney Failure, Chronic Lipoma Tuberous Sclerosis	
Sonidegib	Carcinoma, Basal Cell	-
Sorafenib	Carcinoma, Hepatocellular Carcinoma, Renal Cell Thyroid Neoplasms	-
Sunitinib	Adenoma Carcinoma, Renal Cell Digestive System Neoplasms Gastrointestinal Neoplasms Gastrointestinal Stromal Tumors Intestinal Neoplasms	-
Talazoparib	Breast Neoplasms	-
Tamoxifen	Breast Diseases Cystic Fibrosis Cysts Fibroadenoma Fibrocystic Breast Disease Hemorrhage Menorrhagia Menstruation Disturbances Metrorrhagia Neoplasms	1
Tamsulosin	Calculi Coronary Artery Disease Heart Diseases Hernia Hernia, Inguinal Inflammation Ischemia Lithiasis Lower Urinary Tract Symptoms Myocardial Ischemia Prostatic Hyperplasia Ureteral Calculi Urinary Calculi Urolithiasis Urologic Diseases	-
Temozolomide	Astrocytoma Nervous System Neoplasms	-
Temsirolimus	Carcinoma, Renal Cell	-
Teniposide	Precursor Cell Lymphoblastic Leukemia-Lymphoma	-
Thalidomide	Brain Abscess Immune System Diseases Multiple Myeloma Neoplasms, Plasma Cell	42
Tivozanib	Carcinoma, Renal Cell	-
Tocilizumab	Arthritis Arthritis, Juvenile Arthritis, Rheumatoid Behavior Cytokine Release Syndrome Giant Cell Arteritis Neurobehavioral Manifestations Oral Manifestations Psychotic Disorders Schizophrenia Tic Disorders	-
Topotecan	Small Cell Lung Carcinoma	-
Toremifene	Breast Neoplasms	59
Trabectedin	Leiomyosarcoma Liposarcoma	-
Trametinib	Carcinoma, Non-Small-Cell Lung Melanoma	-
Trastuzumab	Breast Neoplasms Neoplasms	-
Tretinoin	Lentigo	58
Triptorelin	Fatty Liver Hypogonadism Infertility, Female Prostatic Neoplasms	-
Tucatinib	Breast Neoplasms	-
Valrubicin	Urinary Bladder Neoplasms	-
Vandetanib	Thyroid Neoplasms	-
Vemurafenib	Melanoma	-
Venetoclax	Leukemia, Lymphocytic, Chronic, B-Cell Leukemia, Myeloid, Acute	-
Vinblastine	Glioma	-
Vincristine	Precursor Cell Lymphoblastic Leukemia-Lymphoma	-
Vinorelbine	Carcinoma, Non-Small-Cell Lung	-
Vismodegib	Carcinoma, Basal Cell	-
Vorinostat	Lymphoma, T-Cell, Cutaneous	-
Zoledronate	Arthritis Bone Marrow Diseases Brain Abscess Chronic Kidney Disease-Mineral and Bone Disorder Chronic Periodontitis HIV Infections Hypersensitivity Infections Kidney Diseases Metabolic Diseases Multiple Myeloma Neoplasms Neoplasms, Plasma Cell Neoplasms, Second Primary Osteitis Osteoarthritis Periodontitis Pleural Effusion, Malignant Prostatic Neoplasms Renal Insufficiency, Chronic Thalassemia Wounds and Injuries	-

## 6. Conclusion

We applied the software package "Genome Enhancer" to a data set that contains *metabolomics* data. The study is done in the context of *Lung Neoplasms*. The data were pre-processed, statistically analyzed and genes encoding enzymes metabolizing given metabolites were



identified. Also checked was the enrichment of GO or disease categories among the studied gene sets.

We propose the following drugs as most promising candidates for treating the pathology under study:



**tariquidar, Nitroarginine and UBIQUINONE-1**

These drugs were selected for acting on the following targets: CYP1A2 and NOS3, which were predicted to be involved in the molecular mechanism of the pathology under study.

The identified molecular mechanism of the studied pathology was predicted to be mainly based on the following key drug targets:



**eNOS, arginase-2, mitochondrial, amine oxidase (flavin-containing) A and eNOS**

These potential drug targets should be considered as a prospective research initiative for further drug repurposing and drug development purposes. The following drugs were predicted as, matching those drug targets: L-Arginine, Hesperetin, AZD2563, UBIQUINONE-1, Nitroarginine and L-Ornithine. These drugs should be considered with special caution for research purposes only.

In this study, we came up with a detailed signal transduction network regulating genes encoding enzymes metabolizing given metabolites in the studied pathology. In this network we have revealed the following top master regulators (signaling proteins and their complexes) that play a crucial role in the molecular mechanism of the studied pathology, which can be proposed as the most promising molecular targets for further drug repurposing and drug development initiatives.

- eNOS
- arginase-2, mitochondrial
- amine oxidase (flavin-containing) A
- eNOS

Potential drug compounds which can be affecting these targets can be found in the "Finding prospective drug targets" section.

## 7. Methods

### Databases used in the study

Transcription factor binding sites in promoters and enhancers of genes encoding enzymes metabolizing given metabolites were analyzed using known DNA-binding motifs described in the **TRANSFAC®** library, release 2023.1 (geneXplain GmbH, Wolfenbüttel, Germany) (<https://genexplain.com/transfac>).

The master regulator search uses the **TRANSPATH®** database (BIOBASE), release 2023.1 (geneXplain GmbH, Wolfenbüttel, Germany) (<https://genexplain.com/transpath>). A comprehensive signal transduction network of human cells is built by the software on the basis of reactions annotated in **TRANSPATH®**.

The information about drugs corresponding to identified drug targets and clinical trials references were extracted from **HumanPSD™** database, release 2023.1 (<https://genexplain.com/humanpsd>).

The Ensembl database release Human104.38 (hg38) (<http://www.ensembl.org>) was used for gene IDs representation and Gene Ontology (GO) (<http://geneontology.org>) was used for functional classification of the studied gene set.

## **Methods for the analysis of enriched transcription factor binding sites and composite modules**

Transcription factor binding sites in promoters and enhancers of differentially expressed genes were analyzed using known DNA-binding motifs. The motifs are specified using position weight matrices (PWMs) that give weights to each nucleotide in each position of the DNA binding motif for a transcription factor or a group of them.

We search for transcription factor binding sites (TFBS) that are enriched in the promoters and enhancers under study as compared to a background sequence set such as promoters of genes that were not differentially regulated under the condition of the experiment. We denote study and background sets briefly as Yes and No sets. In the current work we used a workflow considering promoter sequences of a standard length of 1100 bp (-1000 to +100). The error rate in this part of the pipeline is controlled by estimating the adjusted p-value (using the Benjamini-Hochberg procedure) in comparison to the TFBS frequency found in randomly selected regions of the human genome (adj.p-value < 0.01).

We have applied the CMA algorithm (Composite Module Analyst) for searching composite modules [7] in the promoters and enhancers of the Yes and No sets. We searched for a composite module consisting of a cluster of 10 TFs in a sliding window of 200-300 bp that statistically significantly separates sequences in the Yes and No sets (minimizing Wilcoxon p-value).

## **Methods for finding master regulators in networks**

We searched for master regulator molecules in signal transduction pathways upstream of the identified transcription factors. The master regulator search uses a comprehensive signal transduction network of human cells. The main algorithm of the master regulator search has been described earlier [3,4]. The goal of the algorithm is to find nodes in the global signal transduction network that may potentially regulate the activity of a set of transcription factors found at the previous step of the analysis. Such nodes are considered as most promising drug targets, since any influence on such a node may switch the transcriptional programs of hundreds of genes that are regulated by the respective TFs. In our analysis, we have run the algorithm with a maximum radius of 12 steps upstream of each TF in the input set. The error rate of this algorithm is controlled by applying it 10000 times to randomly generated sets of input transcription factors of the same set-size. Z-score and FDR value of ranks are calculated then for each potential master regulator node on the basis of such random runs (see detailed description in [9]). We control the error rate by the FDR threshold 0.05.

## **Methods for analysis of pharmaceutical compounds**

We seek for the optimal combination of molecular targets (key elements of the regulatory network of the cell) that potentially interact with pharmaceutical compounds from a library of known drugs and biologically active chemical compounds, using information about known drugs from HumanPSD™ and predicting potential drugs using PASS program.

### ***Method for analysis of known pharmaceutical compounds***

We selected compounds from HumanPSD™ database that have at least one target. Next, we sort compounds using "Drug rank" that is the sum of the following ranks:

1. ranking by "Target activity score" ( $T\text{-score}_{PSD}$ ),
2. ranking by "Disease activity score" ( $D\text{-score}_{PSD}$ ),

3. ranking by "Clinical validity score".

"Target activity score" ( $T\text{-score}_{PSD}$ ) is calculated as follows:

$$T\text{-score}_{PSD} = -\frac{|T|}{|T| + w(|AT| - |T|)} \sum_{t \in T} \log_{10} \left( \frac{\text{rank}(t)}{1 + \maxRank(T)} \right),$$

where  $T$  is set of all targets related to the compound intersected with input list,  $|T|$  is number of elements in  $T$ ,  $AT$  and  $|AT|$  are set set of all targets related to the compound and number of elements in it,  $w$  is weight multiplier,  $\text{rank}(t)$  is rank of given target,  $\maxRank(T)$  equals  $\max(\text{rank}(t))$  for all targets  $t$  in  $T$ .

We use following formula to calculate "Disease activity score" ( $D\text{-score}_{PSD}$ ):

$$D\text{-score}_{PSD} = \begin{cases} \sum_{d \in D} \sum_{p \in P} \text{phase}(d, p) \\ 0, D = \emptyset \end{cases},$$

where  $D$  is the set of selected diseases, and if  $D$  is empty set,  $D\text{-score}_{PSD}=0$ .  $P$  is a set of all known phases for each disease,  $\text{phase}(p, d)$  equals to the phase number if there are known clinical trials for the selected disease on this phase and zero otherwise.

The clinical validity score reflects the number of the highest clinical trials phase (from 1 to 4) on which the drug was ever tested for any pathology.

### Method for prediction of pharmaceutical compounds

In this study, the focus was put on compounds with high pharmacological efficiency and low toxicity. For this purpose, comprehensive library of chemical compounds and drugs was subjected to a SAR/QSAR analysis. This library contains 13040 compounds along with their pre-calculated potential pharmacological activities of those substances, their possible side and toxic effects, as well as the possible mechanisms of action. All biological activities are expressed as probability values for a substance to exert this activity ( $Pa$ ).

We selected compounds that satisfied the following conditions:

1. Toxicity below a chosen toxicity threshold (defines as  $Pa$ , probability to be active as toxic substance).
2. For all predicted pharmacological effects that correspond to a set of user selected disease(s)  $Pa$  is greater than a chosen effect threshold.
3. There are at least 2 targets (corresponding to the predicted activity-mechanisms) with predicted  $Pa$  greater than a chosen target threshold.

The maximum  $Pa$  value for all toxicities corresponding to the given compound is selected as the "Toxicity score". The maximum  $Pa$  value for all activities corresponding to the selected diseases for the given compound is used as the "Disease activity score". "Target activity score" ( $T\text{-score}$ ) is calculated as follows:

$$T\text{-score}(s) = \frac{|T|}{|T| + w(|AT| - |T|)} \sum_{m \in M(s)} \left( pa(m) \sum_{g \in G(m)} IAP(g) \text{optWeight}(g) \right),$$

where  $M(s)$  is the set of activity-mechanisms for the given structure (which passed the chosen threshold for activity-mechanisms  $Pa$ );  $G(m)$  is the set of targets (converted to genes) that corresponds to the given activity-mechanism ( $m$ ) for the given compound;  $pa(m)$  is the probability to be active of the activity-mechanism ( $m$ ),  $IAP(g)$  is the invariant accuracy of prediction for gene from  $G(m)$ ;  $\text{optWeight}(g)$  is the additional weight multiplier for gene.  $T$  is set of all targets related to the compound intersected with input list,  $|T|$  is number of elements in  $T$ ,  $AT$  and  $|AT|$  are set set of all targets related to the compound and number of elements in it,  $w$  is weight multiplier.

"Druggability score" ( $D\text{-score}$ ) is calculated as follows:

$$D\text{-score}(g) = IAP(g) \sum_{s \in S(g)} \sum_{m \in M(s, g)} pa(m),$$

where  $S(g)$  is the set of structures for which target list contains given target,  $M(s,g)$  is the set of activity-mechanisms (for the given structure) that corresponds to the given gene,  $pa(m)$  is the probability to be active of the activity-mechanism (m),  $IAP(g)$  is the invariant accuracy of prediction for the given gene.

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**Thank you for using the Genome Enhancer!**

In case of any questions please contact us at [support@genexplain.com](mailto:support@genexplain.com)

## Supplementary material

1. [Supplementary table 1 - Detailed report. Composite modules and master regulators \(genes encoding enzymes, metabolising target metabolites in TNF vs NO\\_TNF 72h\).](#)
2. [Supplementary table 2 - Detailed report. Pharmaceutical compounds and drug targets.](#)

## Disclaimer

Decisions regarding care and treatment of patients should be fully made by attending doctors. The predicted chemical compounds listed in the report are given only for doctor's consideration and they cannot be treated as prescribed medication. It is the physician's responsibility to independently decide whether any, none or all of the predicted compounds can be used solely or in combination for patient treatment purposes, taking into account all applicable information regarding FDA prescribing recommendations for any therapeutic and the patient's condition, including, but not limited to, the patient's and family's medical history, physical examinations, information from various diagnostic tests, and patient preferences in accordance with the current standard of care. Whether or not a particular patient will benefit from a selected therapy is based on many factors and can vary significantly.

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