From Department of Medical Biochemistry and Biophysics
Karolinska Institutet, Stockholm, Sweden

COMPUTATIONAL AND CHEMICAL APPROACHES TO DRUG REPURPOSING

Xuexin Li

Karolinska Institutet

Stockholm 2023

All previously published papers were reproduced with permission from the publisher.
Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2023.
© Xuexin Li, 2023
ISBN 978-91-8017-060-4
Cover illustration: the yin yang symbol embodies the concept of the wet and dry laboratories in drug repurposing.

Computational and chemical approaches to drug repurposing

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Xuexin Li

The thesis will be defended in public at the **Samuelssonsalen, Tomtebodavägen 6, Solna**, Karolinska Institutet on Friday, **September 27th, 2023, at 1:00 p.m. CET**.

Principal Supervisor:
Prof. Oscar Fernandez-Capetillo
Karolinska Institutet
Department of Medical Biochemistry and Biophysics

Co-supervisor(s):
Dr. Daniela Hühn
Karolinska Institutet
Department of Medical Biochemistry and Biophysics

Opponent:
Dr. Brinton Seashore-Ludlow
Karolinska Institutet
Department of Oncology-Pathology

Examination Board:
Prof. Per Svenningsson
Karolinska Institutet
Department of Clinical Neuroscience

Prof. Ernest Arenas
Karolinska Institutet
Department of Medical Biochemistry and Biophysics

Prof. Patrick Aloy
Institut de Recerca Biomèdica de Barcelona
Structural Systems Biology lab

ABSTRACT

Drug repurposing, which entails discovering novel therapeutic applications for already existing drugs, provides numerous benefits compared to conventional drug discovery methods. This strategy can be pursued through two primary approaches: computational and chemical. Computational methods involve the utilization of data mining and bioinformatics techniques to identify potential drug candidates, while chemical approaches involve experimental screens oriented to finding new potential treatments based on existing drugs. Both computational and chemical methods have proven successful in uncovering novel therapeutic uses for established drugs. During my PhD, I participated in several experimental drug repurposing screens based on high-throughput phenotypic approaches. Finally, attracted by the potential of computational drug repurposing pipelines, I decided to contribute and generate a web platform focused on the use of transcriptional signatures to identify potential new treatments for human disease. A summary of these studies follows:

In **Study I**, we utilized the tetracycline repressor (tetR)-regulated mechanism to create a human osteosarcoma cell line (U2OS) with the ability to express TAR DNA-binding protein 43 (TDP-43) upon induction. TDP-43 is a protein known for its association with several neurodegenerative diseases. We implemented a chemical screening with this system as part of our efforts to repurpose approved drugs. While the screening was unsuccessful to identify modulators of TDP-43 toxicity, it revealed compounds capable of inhibiting the doxycycline-dependent TDP-43 expression. Furthermore, a complementary CRISPR/Cas9 screening using the same cell system identified additional regulators of doxycycline-dependent TDP-43 expression. This investigation identifies new chemical and genetic modulators of the tetR system and highlights potential limitations of using this system for chemical or genetic screenings in mammalian cells.

In **Study II**, our objective was to reposition compounds that could potentially reduce the toxic effects of a fragment of the Huntingtin (HTT) protein containing a 94 amino acid long glutamine stretch (Htt-Q94), a feature of Huntington's disease (HD). To achieve this, we carried out a high-throughput chemical screening using a varied collection of 1,214 drugs, largely sourced from a drug repurposing library. Through our screening process, we singled out clofazimine, an FDA-approved anti-leprosy drug, as a potential therapeutic candidate. Its effectiveness was validated across several *in vitro* models as well as a zebrafish model of polyglutamine (polyQ) toxicity. Employing a combination of computational analysis of transcriptional signatures, molecular modeling, and biochemical assays, we deduced that clofazimine is an agonist for the peroxisome proliferator-activated receptor gamma (PPARγ), a receptor previously suggested to be a viable therapeutic target for HD due to its role in promoting mitochondrial biogenesis. Notably, clofazimine was successful in alleviating the mitochondrial dysfunction triggered by the expression of Htt-Q94. These findings lend substantial support to the potential of clofazimine as a viable candidate for drug repurposing in the treatment of polyQ diseases.

In **Study III**, we explored the molecular mechanism of a previously identified repurposing example, the use of diethyldithiocarbamate-copper complex (CuET), a disulfiram metabolite, for cancer treatment. We found CuET effectively inhibits cancer cell growth by targeting the NPL4 adapter of the p97VCP segregase, leading to translational arrest and stress in tumor cells. CuET also activates ribosomal biogenesis and autophagy in cancer cells, and its cytotoxicity can be enhanced by inhibiting these pathways. Thus, CuET shows promise as a cancer treatment, especially in combination therapies.

In **Study IV,** we capitalized on the Molecular Signatures Database (MSigDB), one of the largest signature repositories, and drug transcriptomic profiles from the Connectivity Map (CMap) to construct a comprehensive and interactive drug-repurposing database called the Drug Repurposing Encyclopedia (DRE). Housing over 39.7 million pre-computed drug-signature associations across 20 species, the DRE allows users to conduct real-time drug-repurposing analysis. This can involve comparing user-supplied gene signatures with existing ones in the DRE, carrying out drug-gene set enrichment analyses (drug-GSEA) using submitted drug transcriptomic profiles, or conducting similarity analyses across all database signatures using user-provided gene sets. Overall, the DRE is an exhaustive database aimed at promoting drug repurposing based on transcriptional signatures, offering deep-dive comparisons across molecular signatures and species.

Drug repurposing presents a valuable strategy for discovering fresh therapeutic applications for existing drugs, offering numerous benefits compared to conventional drug discovery methods. The studies conducted in this thesis underscore the potential of drug repurposing and highlight the complementary roles of computational and chemical approaches. These studies enhance our understanding of the mechanistic properties of repurposed drugs, such as clofazimine and disulfiram, and reveal novel mechanisms for targeting specific disease pathways. Additionally, the development of the DRE platform provides a comprehensive tool to support researchers in conducting drug-repositioning analyses, further facilitating the advancement of drug repurposing studies.

LIST OF SCIENTIFIC PAPERS

I. Colicchia V, Häggblad M, Sirozh O, Porebski B, Balan M, **Li X**, Lidemalm L, Carreras-Puigvert J, Hühn D, Fernandez-Capetillo O. New regulators of the tetracycline-inducible gene expression system identified by chemical and genetic screens. FEBS Open bio. 2022 Oct;12(10):1896-908.

II. **Li X**, Hernandez I, Haggblad M, Lidemalm L, Brautigam L, Lucas JJ, Carreras-Puigvert J, Huhn D, Fernandez-Capetillo O. The anti-leprosy drug clofazimine reduces polyQ toxicity through activation of PPARγ. bioRxiv. 2023:2023-02.
Preprint, manuscript in preparation.

III. Kanellis DC, Zisi A, Skrott Z, Lemmens B, Espinoza JA, Kosar M, Björkman A, **Li X**, Arampatzis S, Bartkova J, Andújar-Sánchez M. Actionable cancer vulnerability due to translational arrest, p53 aggregation and ribosome biogenesis stress evoked by the disulfiram metabolite CuET. Cell Death & Differentiation. 2023 May 4:1-3.

IV. **Li X**, Pan L, Sanchez-Burgos L, Huhn D, Fernandez-Capetillo O. The Drug Repurposing Encyclopedia (DRE): a web server for systematic drug repurposing across 20 organisms. bioRxiv. 2023:2023-03.
Preprint, manuscript in preparation.

CONTENTS

LIST OF ABBREVIATIONS

3-NP	3-nitropropionic acid
AMPK	AMP-activated protein kinase
ASOs	Antisense oligonucleotides
BBB	Blood-brain barrier
BDNF	Brain-derived neurotrophic factor
BH	Benjamini-Hochberg
Ca2+	Calcium ions
cAMP	Cyclic AMP
CFTR	Cystic fibrosis transmembrane conductance regulator
CFZ	Clofazimine
CK	Creatine kinase
CMap	Connectivity Map
CRE	Cyclic AMP-responsive element
CREB	Cyclic AMP response element-binding protein
CuET	Diethyldithiocarbamate-copper complex
DRE	Drug Repurposing Encyclopedia
DSEA	Drug-set enrichment analyses
DSF	Disulfiram
DTIs	Drug-target interactions
eIF2	Eukaryotic initiation factor 2
ER	Endoplasmic reticulum
ES	Enrichment scores
FDR	False discovery rate
FELASA	Federation of Laboratory Animal Science Associations
FOXP2	Forkhead box protein P2
GCN2	General control non-derepressible 2
GPCR	G-protein-coupled receptor
GSEA	Gene set enrichment analysis
GV-SOLAS	Society of Laboratory Animals
HCV	Hepatitis C virus
HD	Huntington’s disease
HRI	Heme-regulated inhibitor
HTS	High-throughput Screening
HTT	Huntingtin
Htt-Q94	Huntingtin harboring 94 glutamines
ISR	Integrated stress response
LINCS	Library of Network-Based Cellular Signatures
mHTT	Mutant Huntingtin
MoA	Mechanism of action
MOM	Mitochondrial outer membrane
MSigDB	Molecular Signatures Database
NES	Normalized enrichment scores
NF	Normalization factor

NRSF	Neuron-restrictive silencer factor
NUP62	Nucleoporin 62
PCr	Phosphocreatine
PDB	Protein Data Bank
PERK	Protein kinase RNA-like endoplasmic reticulum kinase
PIC	Pre-initiation complex
PKR	Protein kinase R
PolyP	Polyproline
PolyQ	Polyglutamine
PP1	Protein phosphatase 1
PP1c	Protein phosphatase 1 catalytic subunit
PPARγ	Peroxisome Proliferator-Activated Receptor Gamma
PPINs	Protein-Protein Interaction Networks
PRC	PGC-1 related co-activator
RanGAP1	Ran GTPase Activating Protein 1
REST	RE1-silencing transcription factor
RiBi	Ribosome biogenesis
RMSD	Root Mean Square Deviation
SMA	Spinal muscular atrophy
SMN	Survival of motor neuron
snRNP	Small nuclear ribonucleoprotein particle
TC	Ternary complex
TDP-43	TAR DNA-binding protein 43
tetO	Tet operator
tetR	Tetracycline repressor
tRNA	Transfer RNA
U2OS	Osteosarcoma cell line
uORFs	Upstream open reading frames
UPR	Unfolded protein response
UTRs	Untranslated regions
wt	Wild type

1 INTRODUCTION

1.1 DRUG REPURPOSING

Despite significant strides in scientific and technological fields, the conventional methods of new drug development continue to pose substantial challenges, notably in terms of time and resource investment. The strategy of drug repurposing, often referred to as drug repositioning or reprofiling, has emerged as an effective approach to these challenges, contributing to 30% of new drug authorizations in the United States (Plenge et al., 2013). The primary objective of drug repurposing is to identify alternative therapeutic uses for existing drugs, rather than developing entirely new drug compounds (**Fig. 1**).

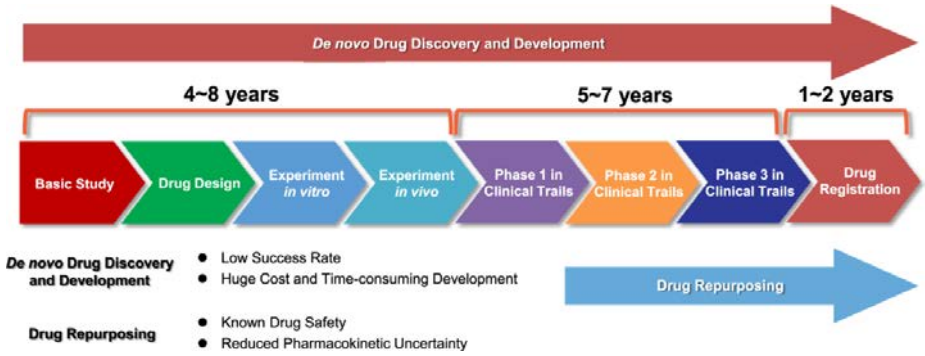


Figure 1. *The estimated time and main steps in de novo drug discovery and development and drug repurposing for cancer therapy. De novo drug discovery and development for cancer therapy takes 10–17 years and comprises basic discovery, drug design, in vitro and in vivo experimentation (including identifying safety and efficacy), clinical trials, and finally drug registration into the market. In contrast, drug repurposing for cancer therapy takes only 3–9 years as it can bypass several processes that have been completed for the original indication if the anticancer potential of the candidates is confirmed. Adapted from (Zhang et al., 2020), Copyright © 2020 by the author(s).*

Modern drug discovery has significantly evolved over time. It began with empirical methods in the 18th to early 20th century, primarily focusing on extracting active compounds from natural sources *(Wang et al., 2022)*. By the mid-to-late 20th century, the approach shifted towards rational drug design, utilizing an understanding of biochemical processes and molecular structures. High-throughput screening, which enables rapid testing of numerous compounds, further advanced the process by the late 20th and early 21st century. In the 21st century, genomics and bioinformatics revolutionized drug discovery by offering deeper genetic insights into diseases. Recently, the focus has shifted towards drug repurposing, which is a cost-effective method that leverages existing drugs for new uses. The latest advancements involve the use of artificial intelligence and machine learning to predict drug behavior, identify side effects, and suggest new drug targets, paving the way towards more efficient and personalized treatments (**Fig. 2**).

A wide range of experimental and computational approaches have gained traction in the drug repurposing field. Experimental techniques encompass various methods that address multiple aspects of drug discovery, including phenotypic screening, target-based screening, and drug combination screening (Menden et al., 2019; Moffat et al., 2014; Park, 2019; Plenge et al.,

2013; Singh et al., 2019; F. Vincent et al., 2022). These methods can offer valuable insights into potential new therapeutic applications by examining drug interactions with biological targets, observing drug effects on specific disease phenotypes, and evaluating drug synergy.

On the other hand, computational approaches take advantage of advanced technologies and algorithms to enable researchers to analyze large datasets and complex biological networks, predict drug-target interactions, and estimate the binding affinity of a drug to a specific target (Cui et al., 2020; Jarada et al., 2020; Tiwari & Singh, 2022). Such as network-based methods and molecular docking (Lin et al., 2020; Tiwari & Singh, 2022; Wu et al., 2020). By doing so, computational approaches can help to identify promising drug candidates for repurposing and provide a deeper understanding of the underlying mechanisms of action.

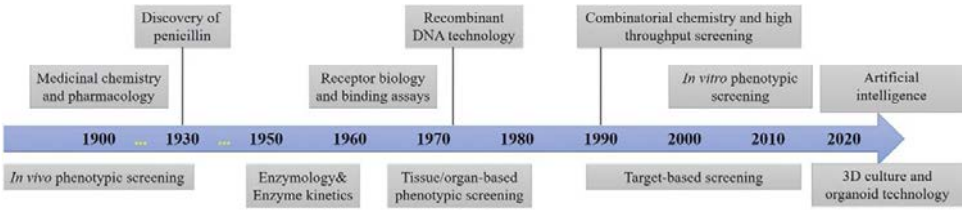


Figure 2. *The evolution of modern drug discovery. Adapted from (Wang et al., 2022), Copyright © 2022 Shou-bao Wang, Zihan Wang, Lianhua Fang, Yang Lv, Guanhua Du.*

The synergy between experimental and computational approaches has the potential to revolutionize the drug repurposing field, offering a more efficient and cost-effective pathway to discovering new therapeutic applications for existing drugs. By leveraging these complementary techniques, researchers can streamline the drug discovery process, accelerate the translation of scientific findings into clinical practice, and ultimately improve patient outcomes.

1.1.1 Experimental approaches for drug repurposing

Experimental approaches for drug repurposing involve using laboratory and clinical techniques to identify new therapeutic applications for existing drugs. These approaches can save time and resources compared to *de novo* drug discovery and often have a higher probability of success due to the already-established safety profiles of the drugs being tested. Some of the key experimental approaches for drug repurposing include phenotypic screening and knowledge and data-based screening.

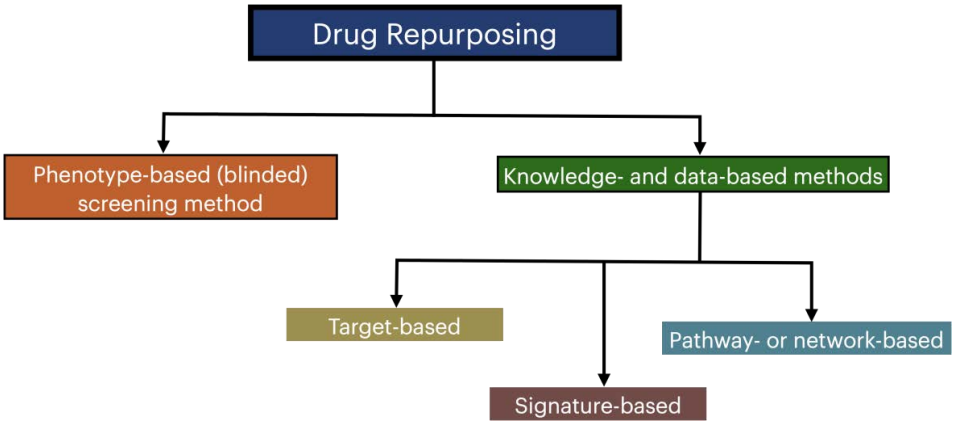


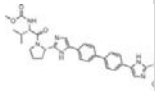
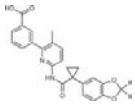
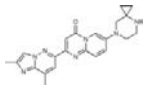
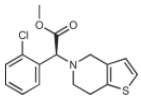
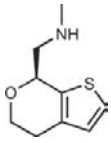
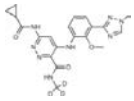
Figure 3. *Different approaches for drug repurposing. Adapted and modified from (Sarvagalla et al., 2019), Copyright © 2019 Elsevier Inc. All rights reserved. Reprinted and modifications with permission.*

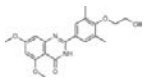
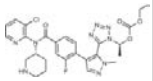
1.1.1.1 Phenotypic screening

Phenotypic screening is a powerful approach for drug repurposing that examines the biochemical or physical characteristics of individual cells or organisms in response to compounds (Aulner et al., 2019; Blay et al., 2020; Mithun et al., 2020). This screening method allows researchers to observe the effects of a compound on a complex whole-cell system rather than just an isolated component, providing a more holistic understanding of the drug's impact. It is particularly beneficial for identifying new therapeutic compounds in areas with intricate disease pathways or when the disease target is not yet known (Moffat et al., 2014; Swinney, 2013; Zheng et al., 2013). It is noteworthy that, as highlighted in the Moffat *et al.* 2014 report, a substantial portion (32%) of first-in-class drugs approved by the FDA between 1999 and 2008 were discovered via phenotypic screening (Moffat et al., 2014). Essentially, phenotypic screening has multiple benefits. It facilitates the detection of bioactive compounds that have a direct impact on disease-specific parameters and is streamlining the drug repurposing process (Mithun et al., 2020; Moffat et al., 2014; Zheng et al., 2013). Researchers can also discover novel biomarkers through phenotypic screening, aiding in disease diagnosis, progression monitoring, and treatment response (Kang et al., 2016; Warchal et al., 2020; Williams & McDermott, 2017). Moreover, it allows for the discovery of unknown drug targets and action mechanisms, which could pave the way for the development of innovative therapeutic methods (Ege et al., 2021; Moffat et al., 2017; Sandercock et al., 2015; Williams et al., 2016). Phenotypic screening is a precious resource in drug discovery that emphasizes the effects of drugs on cells, tissues, or organisms over specific molecular targets. This approach can facilitate the identification of drugs with beneficial impact on a specific disease phenotype, even if the exact action mechanism remains elusive.

Phenotypic screening has made significant contributions to the field of drug discovery. As stated by Berg in 2021, this methodology has led to the creation of numerous first-in-class drugs, which are those that represent a completely new unique mechanism of action or novel chemical structure. Such drugs often provide unique treatment options for various diseases, potentially offering therapeutic benefits over existing treatments. (**Table 1**).

Table 1. *Phenotypic origins of approved drugs and clinical-phase compounds. CFTR, cystic fibrosis transmembrane conductance regulator; GPCR, G-protein-coupled receptor; HCV, hepatitis C virus; MoA, mechanism of action; SMA, spinal muscular atrophy; SMN, survival of motor neuron; snRNP, small nuclear ribonucleoprotein particle. Adapted from (Fabien Vincent et al., 2022), Copyright © 2022, Springer Nature Limited. Reprinted with permission.*

Drug or clinical candidate	Structure	Indication	Phenotypic screening strategy	Mechanism of action	Development phase
Daclatasvir (modulators of NS5A are components of all anti-HCV drugs)		Hepatitis C infection	Target-agnostic viral replication screen (Lemm et al., 2010)	NS5A identified as molecular target; HCV replication inhibition; MoA unknown	Launched
Lumacaftor (component of Orkambi along with ivacaftor)		Cystic fibrosis	Mechanism-agnostic cellular screen to enhance CFTR function (Van Goor et al., 2011)	Correctors enhance the folding and plasma membrane insertion of CFTR; novel MoA (Van Goor et al., 2006)	Launched
Lenalidomide		Multiple myeloma and other haematological malignancies	Functional cellular assays and off-label observational studies in patients (Lindner & Krönke, 2016; Millrine & Kishimoto, 2017)	Alters protein substrate specificity of E3 ubiquitin ligase Cereblon; novel target class and MoA (Lu et al., 2014)	Launched
Risdiplam		SMA	Mechanism-agnostic cellular assay to correct *SMN2* pre-mRNA splicing (Naryshkin et al., 2014)	Engagement and stabilization of *SMN2* exon 7 and U1 snRNP complex; novel target class and MoA (Campagne et al., 2019; Naryshkin et al., 2014; Sivaramakrishnan et al., 2017)	Launched
Clopidogrel (prodrug of active metabolite responsible for activity)207		Cardiovascular disease	Anti-platelet activity identified using a battery of *in vivo* and ex vivo rodent models screened to explore anti-inflammatory activity (Maffrand, 2012)	Active metabolite selectively and irreversibly blocks platelet $P2Y_{12}$ ADP receptors (Savi et al., 2001)	Launched
SEP-363856		Schizophrenia, psychosis	Automated *in vivo* behavioural models, the 'SmartCube system (Alexandrov et al., 2015; Roberds et al., 2011; Shao et al., 2016)	Positive phase II results mediated by novel non-dopamine GPCR mechanism; novel MoA (Dedic et al., 2019)	Phase III (schizophrenia), phase II (psychosis)
Deucravacitinib		Psoriasis and other autoimmune conditions	Kinase biased compounds tested in cellular assay monitoring IL-23 signalling pathway (Tokarski et al., 2015)	Positive phase III results; novel MoA; allosteric inhibition of TYK2 kinase through catalytically inactive pseudo-kinase domain (Tokarski et al., 2015)	Phase III (psoriasis), phase II (other indications)

Drug or clinical candidate	Structure	Indication	Phenotypic screening strategy	Mechanism of action	Development phase
Compounds from multiple companies: apabetalone (RVX-208) shown as an example of the most advanced clinical candidates		Adverse cardiovascular events in type 2 diabetes; oncology, various tumour types	Initial compounds identified with mechanism-agnostic cellular assay, selective upregulation of ApoA1 (refs (C. W. Chung et al., 2011; Nicodeme et al., 2010))	Bromodomain proteins identified as novel drug target class for epigenetic gene regulation (C. W. Chung et al., 2011; Nicodeme et al., 2010)	Phase III (type 2 diabetes), phase II (oncology)
MLR-1023		Type 2 diabetes	Battery of *in vivo* models; effective with *in vivo* glucose tolerance test, oral delivery (Ochman et al., 2012; Saporito et al., 2012)	Positive phase II results; allosteric activation of Lyn kinase, novel MoA (Ochman et al., 2012; Saporito et al., 2012)	Phase II (type 2 diabetes)
PF-06815345		Dyslipidaemia	Mechanism-agnostic cellular assay for inhibition of PCSK9 secretion (Petersen et al., 2016)	Novel target and MoA; inhibition of PCSK9 translation via ternary complex of compound, PCSK9 amino terminus and ribosome (Lintner et al., 2017; Petersen et al., 2016)	Phase I (terminated)

1.1.1.2 Target-based screening

Target-based screening is a drug discovery approach that employs high-throughput methods to focus on well-defined molecular targets (Gilbert, 2013; M. Isgut et al., 2018; Paul, 2019). The goal is to find drugs that can effectively influence these targets. This method is also referred to as reverse pharmacology, as it proceeds in the opposite direction from traditional approaches, which typically involve identifying a genomic component after understanding its function (Moffat et al., 2014; Patwardhan et al., 2008; Zheng et al., 2013).

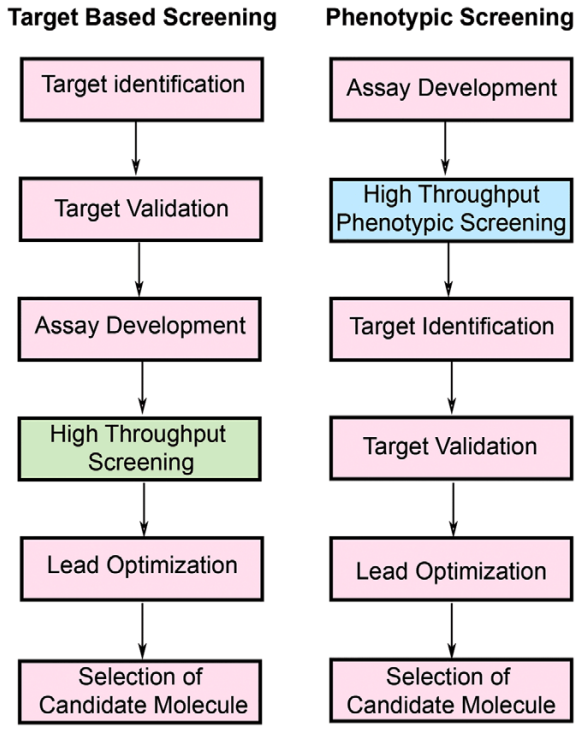


Figure 4. *Target-based screening versus phenotypic screening. Adapted from (Monica Isgut et al., 2018), © 2017 Wiley Periodicals, Inc.*

In target-based screening, pre-existing knowledge of the drug is taken into account, with studies and screening methods designed accordingly. This approach relies on a specific molecular hypothesis, often derived from previous knowledge or phenotypic screening. Target-based screening is extensively utilized in drug repurposing when the disease-causing molecule has been identified, and its mode of action is under examination (Brown, 2007; Croston, 2017). This technique is commonly used to pinpoint potential targets for new drugs aimed at addressing untreated diseases.

Approximately 70% of successful drug outcomes stem from target-based screening (Takenaka, 2001). One advantage of this method is its simplicity in comparison to phenotypic approaches, as the molecular mechanisms of the drug are usually known at an earlier stage. Once a molecular target has been identified, drug discovery can utilize techniques like mutational analysis, crystallography, and computational modeling to comprehend how a drug interacts with the target (Croston, 2017; Katsila et al., 2016; Lindh, 2017; Zheng et al., 2014). This understanding allows for the efficient development of structure-activity relationships, biomarker development, and the generation of future drugs that act on the same target (**Fig. 4**).

1.1.1.3 Drug combination screening

Drug combination screening is a common strategy used in drug repurposing to find synergistic effects between existing drugs (He et al., 2018). The aim is to discover drug combinations that offer enhanced therapeutic outcomes than individual drugs, particularly for complex diseases with multiple underlying mechanisms (Gu et al., 2022; Tseng, 2022). There are several advantages to this approach, including enhanced efficacy by targeting

multiple pathways or mechanisms simultaneously, reduced toxicity and side effects by combining drugs at lower doses, the ability to identify tailored treatments for individual patients through personalized medicine, cost and time efficiency by leveraging existing drugs with known safety profiles, and the ability to overcome drug resistance by targeting multiple pathways or mechanisms (Cokol-Cakmak et al., 2020; Lin et al., 2019; Nafshi & Lezon, 2021; Pemovska et al., 2018).

1.1.1.4 In vivo animal screening

Animal models are used to test the efficacy of repurposed drugs in a physiologically relevant context, providing valuable insights into safety, efficacy, and pharmacokinetics before advancing to clinical trials in humans (Romero & Vela, 2014; Salonee, 2020). Various animal models, such as mice, rats, zebrafish, fruit flies, nematodes, guinea pigs, rabbits, and non-human primates, are used in drug discovery, each with unique advantages and limitations (Bailey, 2005; Bryda, 2013; Elfawal et al., 2019; Kim et al., 2021; Lee, 2014; MacRae & Peterson, 2015; Mage et al., 2019; Nainu et al., 2023). The choice of animal model depends on the research question, the disease being studied, and desired outcome of the drug discovery process.

In vivo animal models are essential in the drug discovery process, as they provide insights into the safety and efficacy of potential therapeutic candidates in a physiologically relevant context. These models are especially useful in evaluating the effectiveness of repurposed drugs, which leverage existing drugs with known safety profiles. By testing these drugs in animal models, researchers can reduce drug development costs and accelerate the translation of novel therapies from the bench to the bedside (Ben-Yakar, 2019; Giacomotto & Ségalat, 2010). The combination of animal models and other methods can greatly enhance the efficiency and success rate of drug repurposing efforts, ultimately leading to better treatments and improved patient outcomes.

1.2 COMPUTATIONAL APPROACHES

Computational approaches for drug repurposing involve the use of a variety of computational techniques and tools to identify potential new uses for existing drugs (Baldi, 2010; Katsila et al., 2016; Ko, 2020). Various network methods and molecular docking techniques are employed in contemporary computational drug repurposing methodologies.

1.2.1 Network-based methods

Network methodologies are frequently utilized in the field of drug repurposing because of their remarkable capability to predict and illustrate interactions between proteins and compounds (**Fig. 5**). Such networks can further be enhanced by integrating quantitative data gathered from high-throughput experiments (Badkas et al., 2021; Xue et al., 2018). Interaction networks are fundamental in the field of biology, consisting of nodes representing genes, proteins, or complexes, and edges representing their interactions. These networks can incorporate various types of relationships and quantitative information obtained from high-throughput experiments (Charitou et al., 2016; Milano et al., 2022). DNA-Protein Interaction Networks are particularly valuable in network-based medicine, as disease conditions can systematically affect gene expression patterns (Radaeva et al., 2021). Differential gene expression analysis reveals significant variations in messenger RNA transcripts between healthy and disease samples, providing insights into potential drug targets, especially those functioning as transcription factors (Arndt, 2006; Koehler, 2010; Majmudar & Mapp, 2005).

In addition to this, Protein-Protein Interaction Networks (PPINs) are being extensively researched for their crucial role in drug repurposing (Adhami et al., 2021; Khojasteh et al., 2022; Safari-Alighiarloo et al., 2014; Xu et al., 2022). PPINs illustrate the connections between known drug targets and other proteins, as well as proteins that have indirect interactions with these targets. A central tenet underlying the use of PPINs to predict drug-target interactions is that proteins influenced by similar drugs are functionally interconnected and are 'neighboring' within the PPIN (Ozdemir et al., 2019; Park, 2019). Investigating the structure of PPINs provides an in-depth view of functional interactions within a cell, significantly enhancing the prediction of drug-target connections.

1.2.2 Molecular docking

Drug repurposing relies heavily on drug-target interactions (DTIs) (Amiri Souri et al., 2023; Middha et al., 2022). DTIs signify the interplay between drug molecules and their corresponding protein targets in the body. The 'target' generally refers to a critical molecule in a biological pathway or function associated with a disease. When the drug interacts or binds with its target, it can modulate the target's behavior, leading to potential therapeutic impacts. It is important to note that many drugs interact with targets beyond their primary ones, leading to off-target effects (Alberca & Talevi, 2020; Benek et al., 2020; Prati et al., 2014). Therefore, predicting drug targets can simplify the process of repurposing drugs. Experimental determination of DTIs is time-consuming and resource-intensive, which has led to the development of computational methods for predicting potential DTIs. interactions (Abbas et al., 2021). These interactions are derived from a combination of diverse pharmacologically and clinically relevant associations.

One common technique to study DTIs is molecular docking, a method used *in silico* molecular modeling. Molecular docking can predict how a drug (usually a small molecule) binds to its target (usually a protein). It does this by predicting the position and orientation (conformation) of the drug when it is most stably bound to its target. This technique gives insights into the potential strength and characteristics of the drug-target interaction, thus playing a critical role in the drug discovery and development process (Abdolmaleki et al., 2021; Torres et al., 2019).

The chief objective of ligand-protein docking is to deduce the most plausible interaction patterns between a ligand and a protein, given the known three-dimensional structure of the latter (Zhao et al., 2022). Robust docking methods are adept at effectively navigating high-dimensional spaces and employing a scoring function to precisely order possible ligand-protein interactions.

Essential elements for conducting docking studies, in addition to computational resources**,** are structural knowledge of both the target and the ligand. For proteins, these details can be obtained from X-ray crystallographic or NMR techniques when the structure is known. If the structure is unknown, homology modeling becomes crucial. Ligand structures can either be devised or a compound library can be utilized.

Docking methodologies vary depending on the rigidity or flexibility of both the ligand and receptor. For instance, some strategies involve rigid ligands and receptors, as in early versions of DOCK and FLOG, which prioritize robust binding and 3D complementarity. Alternatively, methods such as those used in Autodock and FlexX allow for ligand flexibility while keeping

the receptor rigid, balancing computational efficiency and accuracy. Other techniques facilitate both ligand and receptor flexibility, adhering to the induced fit docking principle and providing insights into protein-ligand binding, although these methods require extensive computational resources. In the context of drug repurposing, molecular docking serves the purpose of predicting both the structural and energetic aspects of molecular interactions. It enables the screening of large compound libraries to identify potential candidates that may exhibit efficacy against targets different from their originally intended use (De Ruyck et al., 2016; Rajkhowa & Deka, 2016). This approach allows researchers to leverage existing drug libraries and potentially uncover new therapeutic indications for known compounds.

Table 2. *Available Docking Software. Adapted from (Kumar & Kumar, 2019), Copyright © 2019 Elsevier Inc. All rights reserved. Reprinted with permission.*

S. No.	Docking Software	Published Year	Description	Licence/Web Service	References
1.	AADS	2011	Automated active site detection, docking, and score (AADS) used for protein having known structure based on Monte Carlo method	Free to use online	(Singh et al., 2011)
2.	AutoDock	1990	Automated docking of ligand to protein structure by Lamarckian Genetic algorithm and empirical free energy scoring function	Freeware, no web server available	(Goodsell et al., 1996)
3.	AutoDockVina	2010	New version of AutoDock	Open source, no web server available	(Morris et al., 2009)
4.	Blaster	2009	Combines DOCK with ZINC databases to find out ligand to target of interest	Freeware, no web server available	(Irwin et al., 2009)
5.	DOCK	1988	AMBER-type potential function and genetic algorithm	Academic licence is free, no web server available	(Ewing, 2001)
6.	DockingServer	2009	As the name suggest, it integrates a number of computational chemistry software	Commercial software, no web server available	(Bikadi & Hazai, 2009)
7.	DockVision	1992	Genetic algorithm, Monte Carlo based and for database screening	Commercial software, no web server available	(Hart & Read, 1992)
8.	eHITS	2006	Exhausted search algorithm	Commercial software, no web server available	(Zsoldos et al., 2007)
9.	FlexX	2001	Based on incremental build	Commercial software, no web server available	(Rarey et al., 1996)
10.	FLIPDock	2007	Docking program based on genetic algorithm represents ligand-protein complex using FlexTree data	Free for academic use, no web server available	(Zhao & Sanner, 2007)
11.	FLOG	1994	Rigid body docking using pregenerated conformation database	Academic licence, no web server available	(Kearsley et al., 1994)
12.	FRED	2003	Exhaustive, nonstochastic, systematic examination of all possible orientation with protein binding pocket combined with scoring function	Free for academic use, no web server available	(McGann, 2012)
13.	GEMDOCK	2004	Molecular docking uses generic evolutionary method	Freeware, no web server available	(Yang & Chen, 2004)
14.	Glide	2004	Docking based on exhaustive search	Commercial licence, no web server available	(Friesner et al., 2004)
15.	GOLD	1995	Partial flexibility for protein, flexible ligand, genetic algorithm based	Commercial licence, no web server available	(Jones et al., 1997)
16.	HADDOCK	2003	Mainly developed for protein-protein docking but can also be used for ligand-protein ligand	Freeware, web server available	(Dominguez et al., 2003)
17.	Hammerhead	1996	Fully automated docking of protein binding site to the flexible ligand	Academic licence, no web server available	(Welch et al., 1996)
18.	ICM	1994	Pseudo-Brownian sampling base docking program	Commercial licence, no web server available	(Abagyan et al., 1994)

19.	LigandFit	2003	Docking program based on CHARMm	Commercial licence, no web server available	(Venkatachalam et al., 2003)
20.	LigDockCSA	2011	Ligand-protein docking program using conformational space annealing	Academic licence, no web server available	(Shin et al., 2011)
21.	LIGIN	1996	Surface complementarity based docking software	Commercial licence, no web server available	(Sobolev et al., 1996)
22.	MCDOCK	1999	Nonconventional Monte Carlo simulation technique-based docking program	Freeware, no web server available	(Liu & Wang, 1999)
23.	MEDock	2005	Web server based on maximum-entropy docking at providing an efficient utility for prediction of binding site	Freeware, web server available	(Chang et al., 2005)
24.	Molecular operating environment (MOE)	2008	Docking application within MOE	Commercial licence, no web server available	(Vilar et al., 2008)
25.	MolDock	2006	Heuristic based search algorithm that combines differential evolution with pocket prediction algorithm	Academic licence, no web server available	(Thomsen & Christensen, 2006)
26.	MOLS 2.0	2016	Rigid small molecule-protein docking, flexible protein-peptide interaction	Open source, no web server available	(Paul & Gautham, 2016)
27.	MS-DOCK	2008	Multistage scoring/docking protocol	Academic licence, no web server available	(Sauton et al., 2008)
28.	ParDock	2007	Monte Carlo based all-atom energy, rigid protein docking	Freeware, web server available	(Gupta et al., 2007)
29.	PatchDock	2002	The algorithm carries out rigid docking, with surface flexibility/variability implicitly addressed through liberal intermolecular penetration	Freeware, web server available	(Schneidman-Duhovny et al., 2005)
30.	PLANTS	2006	Stochastic optimization algorithm based	Free for academic use, no web server available	(Korb et al., 2009)
31.	PRODOCK	1999	Monte Carlo-method based plus energy minimization	Academic licence, no web server available	(Trosset & Scheraga, 1999)
32.	PSI-DOCK	2006	Pose-sensitive inclined (PSI)-DOCK	Academic licence, no web server available	(Pei et al., 2006)
33.	PythDock	2011	Program is based on Heuristic docking program that utilizes Python programming language with a simple scoring function	Academic licence, no web server available	(J. Y. Chung et al., 2011)
34.	QXP	1997	Based on Monte Carlo perturbation with energy minimization	Academic licence, no web server available	(McMartin & Bohacek, 1997)
35.	SANDOCK	1998	Guided matching algorithm	Academic licence, no web server available	(Burkhard et al., 1999)
36.	Score	1998	It calculated different docking scores of receptor-ligand complexes	Freeware, web server is available	(Wang et al., 1998)
37.	SOFTDocking	1991	Molecular surface cubes are matched	Academic licence, no web server available	(Jiang & Kim, 1991)
38.	Surflex-Dock	2003	Idealized active site ligand based	Commercial licence, no web server available	(Jain, 2003)
39.	SwissDock	2011	Interactions between a small molecule and receptor are predicted	Free web server for academic use	(Grosdidier et al., 2011)
40.	YUCCA	2005	Rigid small molecule-receptor ligand interaction	Academic licence, no web server available	(Choi, 2005)

1.3 LIMITATIONS OF DRUG REPURPOSING

Although drug repurposing has exhibited considerable potential in discovering new therapeutic applications for existing drugs, it is not without its unique set of challenges.

One such challenge is the efficacy of the repurposed drug in its new role. A drug developed specifically for a certain condition might demonstrate optimal effectiveness and suitability for that ailment. However, a repurposed drug might not offer the same level of effectiveness for its new task. This could potentially be due to suboptimal pharmacokinetics or pharmacodynamics when applied to the new disease. Using the COVID-19 pandemic as a case in point, drug repurposing has been considered a promising approach for the swift application of drug discoveries from lab settings to actual patient care. Various repurposed drugs have been put through clinical trials, yet no efficacious repurposed antiviral drug has been identified. Notably, there has been no success in finding effective treatments for COVID-19, or any other viral diseases, through the repurposing of drugs discovered via unbiased, hypothesis-free screenings (**Table 3**).

Table 3. *Examples of antiviral drugs repurposed for COVID-19 that failed in the clinic. Adapted from (Martinez, 2022), © 2022 Elsevier Ltd. All rights reserved. Reprinted with permission.*

Repurposed drug	Original indication	Virus target	Refs
Favipiravir	Influenza virus	RNA polymerase	(Martinez, 2022)
Remdesivir	HCV, Ebola, MERS-CoV	RNA polymerase	(Martinez, 2020; Martinez, 2021; Yan & Muller, 2021)
Lopinavir-ritonavir	HIV-1	Protease	(Cao et al., 2020)
Darunavir/cobicistat	HIV-1	Protease	(Chen et al., 2020)
Hydroxychloroquine	Malaria	Cell entry	(S. M. Corsello et al., 2017; Martinez, 2020)
Azithromycin	Antibiotic	Not defined	(Butler et al., 2021)
Ivermectin	Intestinal strongyloidiasis and onchocerciasis	Not defined	(Popp et al., 2021)

Another concern is related to side effects. While a drug's side effect profile might be tolerable for its original indication, these effects may become more serious or unacceptable when the drug is used to treat a different condition. This could be particularly relevant if the patient demographics for the new indication vary significantly from the original one or if long-term use is required. Even though a repurposed drug has already undergone significant safety testing for its initial approval, it still needs to meet regulatory standards for the new indication. This involves conducting new clinical trials to demonstrate its safety and efficacy for the new use, which can be both costly and time-consuming (Krishnamurthy et al., 2022; Oprea et al., 2011).

Intellectual property issues also present a significant hurdle. When the original patent for a drug expires, obtaining a new patent for a repurposed use can be challenging. This lack of patent protection can reduce the commercial incentive for pharmaceutical companies to invest in the repurposing of existing drugs. Potential solutions include applying for secondary patents for new uses or formulations, which can be difficult to obtain and may offer narrower protection. Regulatory exclusivity, granted by authorities like the FDA or EMA, can provide a period of protection from generic competitors, as can data exclusivity, which prevents competitors from using the originator's data in their applications. Licensing may also be necessary if the drug is still under patent. Given the complexity of these IP issues, legal advice is often sought to develop a strategic approach that balances investment protection with the realities of the IP and healthcare landscapes (Halabi, 2019; Krishnamurthy et al., 2022).

In conclusion, despite these challenges, drug repurposing offers an innovative approach to drug development, with the potential to accelerate the delivery of effective therapies to

patients in need. However, it is crucial to recognize and navigate these challenges effectively to fully realize their potential.

1.4 HUNTINGTON'S DISEASE

HD is a prevalent neurodegenerative disorder that predominantly affects individuals of Caucasian descent, with an estimated incidence of approximately 3.6 to 5.7 cases per 100,000 individuals (Chaudhary & Mishra, 2016). Although the common age range for the appearance of HD symptoms is between 30 and 50 years, these symptoms can emerge as early as 2 years of age or as late as 80 years (Ohlmeier et al., 2019). The primary characteristics of HD include involuntary body movements, accompanied by a progressive decline in cognitive function and learning abilities, ultimately leading to death from complications such as pneumonia or other common underlying illnesses (G. P. Bates et al., 2015; R. H. Myers, 2004).

1.4.1 The biological background of Huntington's disease

HD is caused by an elongated trinucleotide (CAG) repeat in the HTT gene, which consists of 67 exons (Gillian P. Bates et al., 2015; Richard H. Myers, 2004). Exon-1 (aa 1-82) of HTT is found largely involved in HD pathology. Under normal conditions, the N-terminal of HTT comprises 17 highly conserved amino acids in vertebrates which are termed the HTT N17 domain. Subsequently, the polyQ region is directly followed by two proline-rich domains from the 18th amino acid, which consists of 11 and 10 prolines (Michalek et al., 2013). The N17 domain is highly conserved across vertebrate species, while the polyQ and polyproline (polyP) domains are not. For example, humans have the longest track, and the length of the track is found to gradually increase throughout the evolution of vertebrates (Mangiarini et al., 1996; Michalek et al., 2013). HTT carries both nuclear export signal and nuclear localization signals, which enables Huntingtin shuttling from the nucleus to the cytoplasm via nuclear transport (C. A. Ross et al., 2014; Tabrizi et al., 2020).

Despite the long-standing recognition of the connection of the HTT gene with HD, its precise functions remain incompletely understood. In 1995, Duyao *et al.* first proposed the involvement of HTT in neurons, and subsequent studies have highlighted its importance during embryonic development. Notably, experiments targeting exon 1 of HTT have demonstrated that its inactivation leads to lethality in mice at E7.5 (Duyao et al., 1995; White et al., 1997). HTT exhibits a widespread expression pattern in various tissues and is highly expressed in the brain and testicles (Li et al., 1993; Strong et al., 1993). Within cells, HTT participates in cell signaling, axonal transport, and protection against apoptosis. Furthermore, evidence suggests a role for HTT in DNA damage repair (Christopher A. Ross et al., 2014; Tabrizi et al., 2020).

Extensive research has focused on unraveling the pathogenic mechanisms associated with the mutant form of Huntingtin (mHTT) in comparison to its normal functions. Accumulating evidence suggests that mHTT contributes to a range of molecular changes, including dysregulation of transcription, impaired proteolysis, and post-translational modification, abnormal synaptic role and plasticity, and disrupted energy metabolism due to mitochondrial dysfunction (Cheng et al., 2018; Cui et al., 2006; A. Johri et al., 2013; Lin et al., 2005; Stephen J. McConoughey et al., 2010; Shimojo, 2008; Zuccato et al., 2003). The interaction of mHTT with numerous proteins is believed to be involved in the pathological mechanisms underlying HD. Additionally, the accumulation of large aggregates of mHTT in the cytosol can directly or indirectly trigger dysfunctional pathways (**Fig. 5**).

1.4.2 Pathologies driven by mHTT

1.4.2.1 Transcriptional dysregulation in HD

Emerging studies suggest that the dysregulation of gene transcription is a key factor in the neurodegenerative processes observed in HD. The HTT gene, associated with HD, has been found to interact with over 200 cellular proteins, several of which play crucial roles in gene transcription (Kaltenbach et al., 2007; van Hagen et al., 2017).

The mutant huntingtin significantly regulates the nuclear translocation of the RE1-silencing transcription factor (REST), also referred to as neuron-restrictive silencer factor (NRSF). REST is a protein that plays a key role in inhibiting neural genes in non-neuronal cells. Within the scope of HD cell and mouse models, it has been observed that mutant huntingtin engages directly with REST/NRSF, aiding its transport into the nucleus (Hwang & Zukin, 2018; Zuccato et al., 2003). Once positioned in the nucleus, REST serves to dampen the transcription of various genes, among which includes brain-derived neurotrophic factor (BDNF), a critical component for typical neuronal function (Shimojo, 2008).

A cluster of transcriptional coactivators, the peroxisome proliferator-activated receptor gamma coactivators 1 (PGC-1s), composed of PGC-1α, PGC-1β, and the PGC-1-related coactivators (PRC), are activated by several upstream molecules like the peroxisome proliferator-activated receptors (PPARs), the silent information regulator sirtuin 1(SIRT1), the AMP-activated protein kinase (AMPK), and the transducer of regulated 3'-5'-cyclic AMP (cAMP) response element-binding protein (CREB)-binding protein 1 (Lin et al., 2005). Regarding HD, the dysfunction of PGC-1α has emerged as a major factor contributing to mitochondrial dysfunction. Studies have shown that mice with PGC-1α knockout display mitochondrial dysfunction, aberrant movements, and the degeneration of striatal cells - all characteristic hallmarks of HD. Additionally, diminished function and levels of PGC-1α have been detected in HD mouse models and the postmortem examination of HD patients (Cui et al., 2006; A. Johri et al., 2013). On the other hand, the upregulation of PGC-1α in the striatum of R6/2 mice has demonstrated neuroprotective effects (S. J. McConoughey et al., 2010). Broadly speaking, PGC-1α regulates multiple downstream molecules involved in mitochondrial function and cellular survival.

Beyond REST and PGC-1, various other proteins have been associated with the pathology of HD due to transcriptional disruption. The forkhead box protein P2 (FOXP2) is one such example, shown to co-aggregate with mutant huntingtin in HD mouse models and human patients. Research indicates that reduced levels of FOXP2 in mice not carrying the HD mutation can mimic the behavioral deficits seen in HD. Contrastingly, overexpression of FOXP2 in HD model mice ameliorates these deficits (Hachigian et al., 2017). Moreover, modifications in the cAMP-responsive element (CRE), an early occurrence in HD pathology, have been detected. These changes may stem from the entrapment of CREB-binding protein (CBP) by mutant huntingtin or from interference with other crucial factors like TORC (Waxman & Lynch, 2005).

In summary, the growing body of evidence indicates that mutant huntingtin interferes with the regular control of gene transcription in neurons, implicating specific genes in the pathogenic processes of HD. These discoveries provide potential targets for the development of treatments for HD.

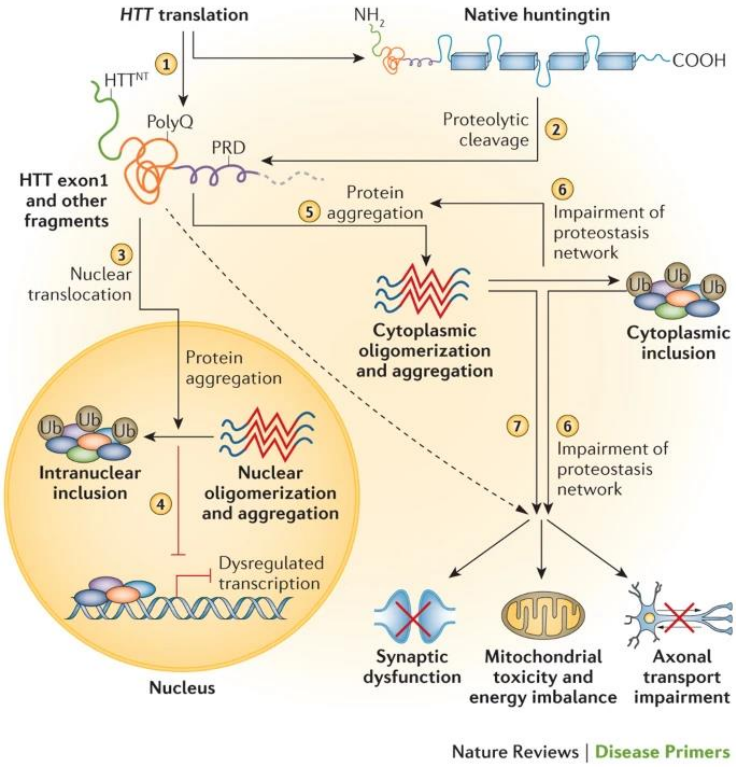


Figure 5. *Pathogenetic cellular mechanisms in Huntington's disease. (1) The full-length huntingtin protein is produced through HTT translation. Amino-terminal HTT exon1 fragment is produced as a result of aberrant splicing. (2) Through Proteolysis, full-length huntingtin is cleaved to produce additional protein fragments. (3) Nuclear translocation of Huntingtin. (4) In the nucleus, through self-association, oligomerization, and aggregation, Huntingtin forms inclusions, which promotes transcriptional dysregulation via various pathological processes. (5) In the cytoplasm, huntingtin fragments oligomerize and form aggregates. (6) Huntingtin aggregates in cytoplasm impair the proteostasis network. (7) Huntingtin aggregates further cause a broad range of cellular abnormalities, such as synaptic dysfunction, mitochondrial toxicity, and a decreased rate of axonal transport. PRD, proline-rich domain; Ub, ubiquitin. Adapted from (Gillian P. Bates et al., 2015), Copyright © 2015, Macmillan Publishers Limited. Preprinted with permission.*

1.4.2.2 Mitochondrial dysfunction in HD

Preserving the normal function of mitochondria is vital for the survival of cells and plays a critical role in maintaining cellular well-being. Extensive research in the field of mitochondrial biology/pathology has unveiled a correlation between the integrity of mitochondria and both the aging process and neurodegenerative conditions such as HD.

In 1978, Goebel *et al.* made a significant discovery by identifying mitochondrial abnormalities in the cortical tissue of deceased individuals with HD, marking the earliest evidence of mitochondrial defects in HD (Goebel et al., 1978). Subsequent imaging studies have further demonstrated decreased glucose metabolism and increased lactate concentration in the brains of HD patients compared to healthy individuals, suggesting potential

mitochondrial modifications (Goebel et al., 1978). It is noteworthy that through the administration of 3-nitropropionic acid (3-NP), an irreversible inhibitor of mitochondrial complex II, the selective elimination of striatal medium spiny neurons resembling the situation in HD, has been accomplished in both rodent and non-human primate models (Borlongan et al., 1997), in which the condition mimics the most common phenotype of striatal medium spiny neurons being affected in HD.

There is evidence that mutant HTT directly interacts with various proteins associated with mitochondria. In the post-mortem brain biopsies of individuals with HD, mitochondrial proteins like Ran GTPase Activating Protein 1 (RanGAP1) and Nucleoporin 62 (NUP62) become trapped within huntingtin aggregates, disrupting the nuclear membrane and abnormal localization of these proteins. Furthermore, studies have revealed that mHTT directly interacts with the mitochondrial outer membrane (MOM), leading to the release of calcium ions (Ca^{2+}) and resulting in changes in the mitochondrial structure (Borlongan et al., 1997; Goebel et al., 1978).

Moreover, mutant HTT disrupts the function of the mitochondrial respiratory chain complexes. For example, mitochondria isolated from human lymphoblasts affected by HD exhibit a decrease in mitochondrial transmembrane potential, which is associated with an increase in the number of glutamine repeats (Sawa et al., 1999). Furthermore, HD pathology is connected to deficits in energy metabolism and oxidative regulation. In the brains of transgenic HD mice, there is a significant decrease in mitochondrial ATP levels coupled with an increased influx of calcium ions via N-methyl-D-aspartate receptors. Additionally, impaired creatine kinase (CK)/phosphocreatine (PCr) system in the brains of HD patients contributes to mitochondrial dysfunction (Goebel et al., 1978; Mochel et al., 2012).

Collectively, these findings indicate that the presence of mutant HTT disrupts the normal functioning of mitochondria, resulting in cellular demise, especially in high-energy-demanding cells like neurons.

2 RESEARCH AIMS

The field of drug repurposing presents a promising and innovative approach to identify new therapeutic applications for existing drugs, offering significant advantages over traditional drug development in terms of time, cost, and risks. The primary objective of this thesis was to explore and demonstrate various methodologies for drug repurposing, employing a combination of experimental and computational techniques.

Study I aimed to repurpose FDA-approved drugs to regulate TDP-43 expression in neurodegenerative diseases using a tetR-regulated system. Further experiments demonstrated chemicals counteracted doxycycline-dependent TDP-43 expression, though follow up validations suggesting they acted as inhibitors of the tetR system.

Study II aimed to repurpose drugs to decrease mutant HTT toxicity in HD and discovered the potential of clofazimine as a therapeutic intervention.

Study III aimed to evaluate the possibility of repurposing Disulfiram as a therapeutic agent for cancer by targeting the NPL4 adapter of the p97VCP segregase.

Study IV aimed to create the Drug Repurposing Encyclopedia, an online platform for *in silico* drug repurposing, providing a resource for transcriptional analysis-based drug repurposing.

Overall, these studies contribute to the growing body of knowledge in the field of drug repurposing and demonstrate the potential for this approach to uncover novel therapeutic uses for existing drugs.

3 METHODOLOGY

3.1 CELL-BASED HIGH-THROUGHPUT PHENOTYPIC SCREEN

Cell-based high-throughput phenotypic screening is a widely utilized technique in biology and drug discovery aimed at identifying compounds capable of altering specific cellular phenotypes. This method is known for its reliability and efficiency, enabling the screening of large compound libraries encompassing thousands to millions of compounds within a feasible timeframe. Cell-based high-throughput phenotypic screens have played a pivotal role in the discovery of novel drugs and drug targets, and they remain an indispensable tool in both drug development and biological research (An & Tolliday, 2010). The primary stages involved in a cell-based high-throughput phenotypic screen are outlined below.

3.1.1 Assay Development

At the outset, the first step entails the selection of an appropriate cell model and the establishment of a methodology to detect alterations in cellular phenotype. This may encompass various parameters such as cell growth, viability, morphology, protein levels, gene expression, or other quantifiable characteristics of the cells (Carettoni & Bader; Fawzi Faisal & Ashwag, 2021).

3.1.2 Compound Library Selection

Following that, the subsequent stage involves the choice of a compound library to be screened, comprising a vast array of thousands to millions of compounds. Such libraries can consist of commercially available compounds, natural products, or compounds synthesized within a laboratory setting. In the present thesis, the focus primarily revolved around the FDA-approved library, owing to its manageable size and potential for drug repurposing. Additionally, the Drug Repurposing Hub library and a library comprising natural compounds were employed. The careful selection of these libraries, along with considerations regarding concentration ranges and dosing schedules, holds significant influence over the outcomes derived from the screening process.

3.1.3 High-throughput Screening (HTS)

Through the utilization of automated machinery and robotics, the compound library is introduced to the cells, typically housed within multi-well plates. In the preparation of the screening process, the assay is meticulously configured and automated to facilitate high-throughput capabilities. This entails a systematic evaluation of various factors, including cell densities, fixation protocols, staining or labeling methods, as well as the establishment of image acquisition and analysis pipelines within 96-well plates. Once the assay is successfully established, it is further optimized for high-throughput screening within 384-well plates, employing liquid dispensing devices. Various strategies for introducing compounds to the cells are also explored, such as resuspending them in media and subsequently adding them to the cells or seeding the cells onto plates that are pre-spotted with compounds. These methodological considerations have been extensively examined to enhance the efficiency and effectiveness of the screening process (Macarron et al., 2011).

Furthermore, staining and labeling protocols that encompass multiple sequential steps, including the addition of reagents and subsequent washings, are automated and tailored to liquid handling devices. During this stage, the creation of suitable controls and the identification of an assay range are fundamentally important in evaluating the potential

effectiveness and practicality of the screen in pinpointing promising results. Should the need arise, further optimization may be implemented to enhance the robustness of the experiment. To streamline the imaging process and enable efficient statistical analysis, well-defined imaging, and analytical pipelines are devised. These pipelines aim to optimize image acquisition protocols, minimize computational and storage requirements, automate the compilation and representation of data, and ultimately expedite the overall screening process (Qiu et al., 2020; Szymański et al., 2012).

After establishing the assay, the procedure progresses to the initiation of the screening phase, which subsequently leads to subsequent assessments. During a typical screening procedure, compounds undergo testing in triplicate at a singular concentration, with the exposure duration fluctuating according to the screen's specific requirements. Nonetheless, during later validation stages or secondary screenings, a broader range of concentrations is implemented, and treatment durations are suitably modified.

3.1.4 Hit Identification

Following the primary screen, selected hits undergo validation using the same methodology employed in the initial screening, sometimes supplemented with an orthogonal assay. In these validation screens, cells are exposed to different concentrations of the compounds. Once validation is complete, the chosen hits are procured from vendors and subjected to further assays using identical methods to confirm their efficacy and determine appropriate dosing. It is essential to perform these additional assessments as there may be slight variations in the compounds obtained from vendors compared to those in the original libraries. This comprehensive characterization involves employing diverse readouts and techniques, as well as conducting functional testing in other relevant models of interest (Mayr & Bojanic, 2009; Mayr & Fuerst, 2008). Once hits are successfully validated, they undergo a comprehensive characterization process. This involves delving deeper into the compound's mechanism of action, optimizing its activity for improved efficacy or reduced toxicity, and conducting tests in different cell types or *in vivo* models to assess its performance in broader contexts (Moffat et al., 2017).

3.1.5 Image analysis techniques and statistical analysis

During the project's image analysis phase, we captured images using an IN Cell Analyzer 2200 (GE Healthcare) scanning microscope. These images were then examined using custom-built pipelines in CellProfiler (v.4.0). Primarily, we utilized a pipeline that identified nuclei through Hoechst staining. This pipeline distinguished nuclei based on nuclear shape and Hoechst signal intensity relative to the background. For the primary screen, we captured nine images per well at 10X magnification. By defining nuclei, we could evaluate cell viability based on nuclei count, followed by searching for drugs capable of mitigating the toxicity caused by mutant Huntingtin.

For the statistical analysis of high-throughput screening data, we used GraphPad Prism and the open-source modular KNIME Analytics Platform, creating custom pipelines grounded in the HTS-workflow. While different screening methods necessitated varying criteria, generally, we normalized the data to the negative DMSO control and selected hits that modulated the phenotype either above or below several standard deviations from the DMSO sample average. We carried out the screens in multiple replicates and accounted for variation by calculating the coefficient of variation (CV%), a measure of data dispersion around the mean. Furthermore, we ensured that hits were identified in several replicates. Establishing an

assay window is critical for running a screen, so we used control compounds to gauge potential changes affecting the study's phenotypes. Our screen analysis took into account the Z-prime factor (Z′) statistic, a measure of assay quality indicating the separation between positive and negative controls and the likelihood of false positives or negatives. Microsoft Excel and GraphPad Prism software facilitated additional statistical analyses.

In conclusion, the combination of image analysis and statistical methodologies was essential for accurately identifying and quantifying the cellular changes induced by various compound treatments. This method paved the way for the potential identification of compounds capable of modifying specific cellular processes or phenotypes. Such analyses are vital to the drug discovery and development process, aiding in identifying potential therapeutic compounds and enhancing our understanding of their mechanisms of action.

3.2 DATABASE CONSTRUCTION

3.2.1 Data collection and processing

3.2.1.1 Molecular Signatures

In the fourth study, a broad collection of 648,825 molecular signatures was gathered using the msigdbr R package containing MSigDB v7.5.1 (Dolgalev, 2020). This collection included gene sets from 20 diverse organisms (Dolgalev, 2020; A. Liberzon et al., 2015; Subramanian et al., 2005), averaging roughly 32,000 signatures for each organism. It is important to clarify that whereas human and mouse signatures obtained from this resource are based on primary RNA sequencing data, those for the rest of the organisms are inferred from the human data assigned to the corresponding orthologues. The research utilized all nine main molecular-signature categories found in MSigDB. These categories include hallmark gene sets, denoting clear biological states and pathways (H); gene sets based on chromosomal positions (C1); gene sets curated from scientific literature, encompassing pathways like KEGG (Kanehisa et al., 2022) and Reactome (Gillespie et al., 2022) (C2); target gene sets of regulatory nature (C3); computational gene sets with a cancer-focus (C4); gene sets from ontology, inclusive of Gene Ontology (GO) terms (Consortium, 2020) (C5); gene sets linked with oncogenesis (C6); gene sets of immunological relevance (C7); and gene sets signifying different cell types (C8) (A. Liberzon et al., 2015; Subramanian et al., 2005).

3.2.1.2 Drug Profiles collection

This phase involves the gathering of drug profiles. Specifically, we searched for and organized a collection of 4,690 consensus drug profiles. These were sourced from DREIMT, a specialized database for drug repurposing with a primary focus on immunomodulation. (Troulé et al., 2021). Following that, we procured drug profiles from the structured transcription data of drugs housed in the Library of Network-Based Cellular Signatures (LINCS) L1000, which is associated with the Connectivity Map (CMap) initiative. (A. Subramanian et al., 2017). We next sorted out the Level 3 data, containing gene expression counts for 978 key genes. These counts were normalized using consistent gene sets and standardized across experimental plates (A. Subramanian et al., 2017). Moreover, expression levels for an extra 11,350 genes were extrapolated from these normalized landmark gene counts. To validate the precision of these drug profiles, a differential expression analysis was carried out for each one. To account for potential biases caused by batch effects or specific cell line reactions to the drugs, an additive linear model was utilized (Troulé et al., 2021). The final drug profiles embody a consensus of the transcriptional alterations induced by the

drugs across diverse cell lines and under varying experimental conditions (Perales-Patón et al., 2019; Troulé et al., 2021).

3.3 WEB SERVER CONSTRUCTION

3.3.1 Association Analyses

To examine the resemblance between the transcriptional signatures linked with drugs from CMap and molecular signatures from MSigDB, we performed comprehensive enrichment analyses. This process entailed carrying out GSEA on each of the 648,825 molecular signatures derived from MSigDB in comparison with the 4,690 consensus drug profiles. The GSEA processes were conducted using the adaptive multilevel splitting Monte Carlo approach in order to conduct bootstrapping and estimation of the event probabilities (Cerou & Guyader, 2014; Korotkevich et al., 2021). To obtain preliminary p-values for the enrichment analyses, we performed 10,000 permutations for each analysis, resulting in a total of 3,042,989,250 associations across the 20 organisms. To adjust for multiple testing, we applied the Benjamini-Hochberg (BH) false discovery rate (FDR) correction (Benjamini & Hochberg, 1995). After applying the FDR correction, we retained a set of 198,648,641 associations that exhibited a significant FDR < 0.05.

3.3.2 Drug Prioritization Scores

To evaluate the specificity of drug associations with each molecular signature, we utilized a standardized drug prioritization Tau score (Aravind Subramanian et al., 2017), following the approach of LINCS L1000 (Aravind Subramanian et al., 2017) and DREIMT (Troulé et al., 2021). The GSEA yielded enrichment scores which were then utilized to determine Tau scores for each molecular signature. In order to standardize these associations, both the positive and negative enrichment scores were standardized independently by dividing them by the mean of the molecular signatures and drug profile enrichment scores (Troulé et al., 2021). A normalization factor (NF) was established using the formula,

$$NF_{k,l} = \frac{\sum_{i=1}^{n} ES_{i,j,k,l} \oplus \sum_{j=1}^{m} ES_{i,j,k,l} - ES_{i,j,k,l}}{\sum_{i=1}^{n} ES_{i,j,k,l} \oplus \sum_{j=1}^{m} ES_{i,j,k,l} - 1}$$

In this equation, NF stands for the normalization factor for organism k and association l, with l = 1 for positive ES and l = 2 for negative ES. The symbols i and j denote molecular signatures and drug profiles respectively. The normalization factors were then used to normalize the enrichment scores for each organism, resulting in the final normalized enrichment score (NES),

$$NES_{k,l} = \frac{ES_{k,l}}{|NF_{k,l}|}$$

With the purpose of determining Tau score for each molecular signature i and drug profile j, both positive and negative NES values were standardized together,

$$Tau_{i,j,k} = sgn(NES_{i,j,k}) \frac{100}{N} \sum_{l=1}^{N} [|NES_{l,j,k}| < |NES_{i,j,k}|]$$

Here, l represents the lth NES score in drug profile j. The resultant standardized Tau scores vary between -100 and 100 (Aravind Subramanian et al., 2017; Troulé et al., 2021). After

obtaining a total of 198,648,641 associations, the data was further refined based on an absolute Tau value exceeding 80. The resulting data, constituting 39,672,701 significant drug-signature associations, formed the final DRE database.

3.4 ZEBRAFISH TO VALIDATE MODELS OF TOXICITY

Zebrafish have become a widely accepted model organism for studying drug toxicity due to their genetic similarity to humans, rapid reproductive rate, and the transparent nature of their embryos, which allows for easy visualization of developmental processes.

In **Study II**, we generated transgenic zebrafish lines by employing a technique that involves the injection of a mixture containing transposase, a vector, and phenol red into zebrafish eggs at four cell stages. Transposases are enzymes capable of integrating or excising specific DNA sequences, suggesting that they were used to introduce a particular vector into the developing zebrafish genome. Phenol red, on the other hand, served as a pH indicator and facilitated the visualization of the injection process due to its red color.

Subsequently, the compound of interest, clofazimine (CFZ) at a concentration of 12.5 μM, was added to the E3 medium when eggs are seeded. E3 medium provides a suitable environment for the growth and development of zebrafish embryos. Control groups were also included in the study. One group consisted of fish that were injected but not treated with CFZ (referred to as the "naïve injected group"), while the other group consisted of fish that were not injected but were treated with DMSO (referred to as the "un-injected group"). DMSO is commonly used as a vehicle control in drug studies since many compounds are dissolved in it for delivery, despite lacking active therapeutic properties itself. After 24 hours of adding the compound, embryos were imaged and live versus dead embryos were counted. This experiment was repeated three times to ensure the reliability of the findings.

3.5 MOLECULAR DOCKING

Molecular docking is an essential tool in computational biology, serving to emulate molecular interactions. This potent methodology is commonly employed to anticipate the binding alignment of small entities like prospective drug molecules with their corresponding protein targets. By assessing the attraction and activity of these substances, molecular docking facilitates the appraisal of their prospective utility in diverse applications.

In **Study II**, we conducted a docking study focused on identifying potential ligands for PPARγ. The process began with obtaining the three-dimensional crystal structure of PPARγ from the Protein Data Bank (PDB, https://www.rcsb.org). The PDB serves as a comprehensive database containing a vast collection of 3D structural data for various macromolecules, including proteins and nucleic acids. The obtained protein structure was prepared using Autodock Vina (Huey et al., 2012), a software tool for molecular docking and virtual screening. This preparation involved the removal of water molecules and the addition of any missing side chains or residues. This step is critical to ensure the accuracy and reliability of the subsequent docking process.

We next acquired the chemical structures of potential ligands from the ZINC database (https://zinc.docking.org) (Irwin et al., 2020; Sterling & Irwin, 2015), a freely accessible database containing commercially available compounds suitable for virtual screening. Once the ligands were obtained, the actual docking process was carried out using Autodock Vina.

This software estimates the optimal orientation of the ligands when bound to the PPARγ protein, thereby forming stable complexes.

The effectiveness of the docking process was evaluated using two critical indicators: binding free energy and Root Mean Square Deviation (RMSD) values. The binding free energy provides insight into the stability and affinity of the ligand-protein interaction, while the RMSD indicates the deviation between the predicted and actual conformation of the ligand. Finally, we ranked the ligands based on their binding energies, with the top nine binding energies listed. Lower binding energy values signify stronger and more favorable interactions between the ligands and PPARγ. Consequently, ligands with the lowest binding energy values are considered potential candidates for further development in drug research, as they exhibit the most promising binding characteristics with PPARγ.

3.6 CRISPR SCREEN

In **Study I**, we utilized the CRISPR/Cas9 system, a groundbreaking gene-editing technology that enables precise modifications to genomic DNA. RISPR (clustered regularly interspaced short palindromic repeats)/Cas9 technology allows for site-specific using noncoding RNAs to guide the Cas9 nuclease to induce site-specific DNA cleavage (Ran et al., 2013). We utilized a Tet-ON cell model, to generate U2OS^{T43}, which allows for inducible TDP-43 expression. U2OS^{T43} were genetically modified to stably express the *Streptococcus pyogenes* Cas9 nuclease, followed by lentiviral transduction of the parental cells using pLenti-Cas9-T2A-Blast-BFP.

After blasticidin selection, a batch of cells showing high blue fluorescent protein (BFP) expression was sorted twice. These cells were then expanded and transduced with the Brunello sgRNA library, which comprises 77 441 sgRNAs (an average of 4 per gene) and 1000 nontargeting control sgRNAs, covering the entire genome (Doench et al., 2016). The CRISPR guide library was synthesized a second time to augment its performance, this time including Unique Molecular Identifiers (UMIs) (Schmierer et al., 2017).

The oligos synthesized by CustomArray, representing the guides, were pooled and subsequently cloned together. The resulting pool of guides was then packaged into a lentivirus named Brunello-UMI virus. For this purpose, the lentiviral backbone derived from lentiGuide-Puro (Addgene #52963) was utilized, incorporating AU-flip as described in the study conducted by (Cross et al., 2016). To determine the functional titer of the Brunello-UMI virus in U2OS^{T43} cells, a serial dilution of the virus was performed in 6-well plates, followed by the selection of cells using puromycin.

To introduce the Brunello-UMI virus into Cas9-expressing U2OS^{T43} cells, two replicates were transduced with the virus. The transduction process was conducted with an approximate multiplicity of infection (MOI) of 0.4 μgmL-1, using 1,000 cells per guide, and the addition of 2 μgmL-1 polybrene. Following transduction, the cells were subjected to puromycin selection (2 μgmL-1) from days two to seven after transduction. Subsequently, the cells were cultured for 10 days with or without doxycycline (10 ngmL-1). Throughout the experiment, the cell number per replicate remained above 63 x 10^6, and the cells were cultured in DMEM +10% tet-free FBS.

To analyze the genomic DNA, the QIAamp DNA Blood Maxi kit (Qiagen 51192) was used for isolation. The guide and UMI sequences were then amplified through PCR, following the protocol described in the study by (Schmierer et al., 2017). The obtained Next-Generation

Sequencing (NGS) data were analyzed using the MAGECK software developed by (Li et al., 2014). Additionally, UMI lineage dropout analysis, as described by (Schmierer et al., 2017), was conducted. Furthermore, the STRING database was used for further gene ontology analysis (Szklarczyk et al., 2021).

3.7 ETHICAL CONSIDERATIONS

The ethical considerations highlighted in the research play a crucial role in ensuring that our work is conducted with the utmost respect for the welfare of living organisms and in compliance with accepted ethical standards. In the case of commercially available cell lines, such as the ones from ATCC, are generally exempt from ethical clearance since they are widely accepted tools for research and are not directly obtained from humans or animals.

For studies involving animal research, it is important to note that the use of zebrafish embryos up to five days of age is typically exempt from specific ethical requirements. However, it remains essential for researchers to treat these organisms with respect and take measures to minimize any potential harm. The study adhered to the ethics guides introduced by the Stor Stockholm djuretiska ethics committee and complied with the EU directive 2010/63/EU, which establishes standards for the humane treatment of animals used for scientific purposes. Furthermore, the housing of the zebrafish at the Karolinska Institutet (Solna, Sweden) in the central facility was carried out in compliance with Swedish animal welfare legislation and the guidelines provided by the Society of Laboratory Animals (GV-SOLAS) as well as the Federation of Laboratory Animal Science Associations (FELASA). These organizations provide valuable guidance on the care and use of laboratory animals to ensure their humane treatment and prioritize their welfare.

Overall, the ethical considerations in our research demonstrate our commitment in conducting responsible research following the applicable guidelines and regulations to protect the welfare of the organisms involved.

4 SUMMARY OF RESEARCH PAPERS

4.1 PAPER I: NEW REGULATORS OF THE TETRACYCLINE-INDUCIBLE GENE EXPRESSION SYSTEM IDENTIFIED BY CHEMICAL AND GENETIC SCREENS

The tetracycline repressor (tetR)-controlled system is widely employed to regulate the expression of specific genes of interest (GOI) in eukaryotic cells in an inducible manner. This regulation is achieved by the addition or removal of tetracycline antibiotics. The fundamental mechanism of these systems relies on the interaction between the tetR protein and the tet operator (tetO) (Baron & Bujard, 2000), which was initially identified in the tetracycline resistance operon encoded by the Tn10 transposon of *Escherichia coli* (Hillen & Berens, 1994).

While tetR, when bound to tetracycline, acts as a transcriptional repressor, the Tet-ON system employs a tetR variant with four mutations that enable the inducible expression of the GOI in response to tetracycline antibiotics (Gossen et al., 1995). Since its initial application in eukaryotic cells (Gatz & Quail, 1988), the tetR-regulated system has undergone further refinement and has become a standard tool in molecular biology, widely used for controlling gene expression in both *in vitro* and *in vivo* experiments. However, it is important to note that using tetracycline antibiotics to induce GOI expression may have unintended effects, such as potential alterations in cell metabolism and gut microbiota, delays in plant growth, and inhibition of cell proliferation and mitochondrial protein translation. In this study, we describe the development of a Tet-ON cell model that enables the inducible expression of TDP-43. Utilizing this system, we conducted chemical and genome-wide CRISPR-Cas9-based forward genetic screens to identify novel regulators of TDP-43 toxicity.

In **Study I**, we generated a human osteosarcoma cell line using the tetR system, enabling the inducible expression of an EGFP fusion protein of TDP-43, a protein implicated in neurodegenerative diseases. The identification of mutations in the TARDBP gene, which encodes TDP-43, has provided compelling evidence linking TDP-43 dysfunction to amyotrophic lateral sclerosis (ALS) (Kabashi et al., 2008; Sreedharan et al., 2008). TDP-43 is recognized for its strong affinity for RNA and its involvement in various RNA-related processes such as translation, splicing, and transport (Portz et al., 2021). Despite considerable research, the exact mechanisms through which TDP-43 dysregulation contributes to neurodegeneration remain partially understood. Interestingly, both the loss and overexpression of TDP-43 can be toxic, resulting in the creation of experimental ALS models (Iguchi et al., 2013; Wu et al., 2012; Xu et al., 2010). These models have revealed mutations in proteins like ATXN-2 (Becker et al., 2017) and components of the autophagosome-lysosome pathway that alter TDP-43 toxicity. However, the quest for chemical therapies capable of significantly mitigating TDP-43 toxicity has so far proven unsuccessful.

Consistent with prior studies, in **Study I**, the overexpression of TDP-43 resulted in aggregate formation and reduced cell viability in U2OS cells. We performed a chemical screen using an FDA-approved drug library to seek potential therapeutic approaches. The initial screen identified several compounds that mitigated TDP-43 toxicity. However, subsequent analysis revealed that these compounds interfered with the doxycycline-induced expression of TDP-43. This counteractive effect was observed with both doxycycline and tetracycline and across different Tet-On cell lines expressing various genes, suggesting a widespread inhibitory effect of these compounds on the tetR system. We also performed a genome-wide CRISPR/Cas9 screen using the same cell line, identifying epigenetic regulators such as G9a methyltransferase and TRIM28 as potential modifiers of TDP-43 toxicity. Nevertheless,

additional tests indicated that G9a inhibition or TRIM28 loss impeded the doxycycline-dependent expression of TDP-43. Collectively, our research has revealed novel chemical and genetic regulators of the tetR system, highlighting the limitations of this technique for conducting chemical or genetic screening in mammalian cells. Our findings emphasize the complications posed by certain compounds that interfere with the inducible tetR system, both chemically and genetically. These insights contribute to a more profound understanding of the intricacies of using the tetR-regulated system and underline the necessity of considering its constraints when utilized for chemical or genetic screening in mammalian cells.

4.1.1 Discussion

ALS is characterized by diverse independent mutations, but the accumulation of TDP-43 aggregates is a common feature. Consequently, similar to efforts in finding a cure for neurological disorders like Alzheimer's disease, significant research has focused on identifying compounds capable of reducing these aggregates. Numerous chemical and genetic screens have been performed to investigate modulators of TDP-43 distribution. However, our study is the first to employ TDP-43-driven toxicity as a readout in the assay. To accomplish this, we chose to induce TDP-43EGFP expression using a widely used tet-regulated expression system that allows precise control of gene expression in mammalian cells. Despite screening over 4000 compounds, including the majority of approved drugs, none demonstrated a significant effect in alleviating the toxicity resulting from TDP-43 overexpression. This outcome raises doubts about the potential success of drug repurposing endeavors in this context.

Unfortunately, all primary hits identified in our screen were found to be antagonists of tetracycline antibiotics. We nevertheless pursued the characterization of these findings for two reasons. Firstly, it highlights the limitations of using the Tet-On/Tet-Off system for conducting chemical screens. Secondly, some of these compounds are approved for medical use, suggesting that their co-administration with antibiotics could potentially impact the efficacy of the antibiotics. Remarkably, one of the compounds identified in our screen, Lop, was independently identified in another study aiming to discover modulators of antibiotic efficacy when combined with non-antibiotic drugs (Ejim et al., 2011). In addition to the chemical findings, our study has unveiled the involvement of TRIM28 and the G9a histone methyltransferase in the regulation of transcriptional induction in the Tet-On system, underscoring the significance of considering the epigenetic regulation of this system. In conclusion, our findings provide valuable insights into the limited impact of medically approved drugs on modulating the toxicity associated with TDP-43 overexpression. Moreover, we have identified novel chemical and genetic regulators of the Tet-On system in mammalian cells, thereby enhancing our understanding of this widely used system.

4.2 PAPER II: THE ANTI-LEPROSY DRUG CLOFAZIMINE REDUCES POLYQ TOXICITY THROUGH THE ACTIVATION OF PPARG

PolyQ disorders are a group of nine genetic neurodegenerative diseases, united by an anomalous expansion of glutamine-encoding (Q) repeats in the exons of distinct genes (Lieberman et al., 2019). Among them, HD, one of the most common neurodegenerative diseases globally, affects 3-5 per 100,000 people (Rawlins et al., 2016). In HD, the pathology is linked with an extended CAG repeat in the first exon of the HTT gene. When the repetition length exceeds 35, it becomes pathogenic, and the disease severity escalates with longer repeat lengths (Kremer et al., 1994; Lee et al., 2012). Although abnormal HTT function has been implicated in HD (Dietrich et al., 2017; Zhang et al., 2006), an alternative theory

proposes that the disease results from the toxic gain-of-function of the mutant HTT protein carrying the polyQ expansion. Studies conducted by (Mangiarini et al., 1996; Schilling et al., 1999) demonstrated that mice expressing a fragment of the mHTT exon 1, which includes the expanded polyQ region, exhibit motor dysfunction and premature death. These findings are important as they highlight the causal role of polyQ toxicity in neurodegeneration and premature death. Additionally, (Ordway et al., 1997) revealed that the abnormal expression of polyQ expansions inserted into the HPRT gene, which is unaltered in patients, also leads to neurodegeneration and premature death, further emphasizing the detrimental effects of polyQ toxicity.

Our understanding of polyQ toxicity mechanisms is a work in progress. These polyQ expansions notably form insoluble aggregates, appearing as intraneuronal inclusions in mouse models and patients with various polyQ diseases, including HD (Davies et al., 1997; DiFiglia et al., 1997; Paulson et al., 1997). Regardless of their capacity to form inclusions, mHTT has been found to instigate several cellular changes, including disturbances in mRNA transcription (Conforti et al., 2013; Ryu et al., 2003; Steffan et al., 2001), impairments in protein degradation and post-translational modifications (Ortega et al., 2007), disruptions in synaptic function and plasticity (Milnerwood & Raymond, 2010; Murphy et al., 2000; Paraskevopoulou et al., 2021; Vezzoli et al., 2019; Wilkie et al., 2020), and disruptions in mitochondrial activity (Costa & Scorrano, 2012; Hayashida et al., 2010; Ashu Johri et al., 2013; Wang et al., 2021; Weydt et al., 2006).

Despite considerable progress in understanding the underpinnings of polyQ diseases, this knowledge has yet to yield clinical advances for HD treatment. Currently approved therapies for HD, such as tetrabenazine and deutetrabenazine, mainly mitigate involuntary movements (chorea) but do not cure the disease (Frank et al., 2016; Yero & Rey, 2008). Thus, there is a pressing need for the exploration and discovery of novel therapeutic approaches for polyQ diseases, an area of intense ongoing research. Various strategies are being explored, including interventions aimed at preventing the formation of mHTT aggregates or facilitating their clearance, and targeting the downstream pathological effects caused by these aggregates (reviewed in (Esteves et al., 2017)). Notably, several unbiased chemical screens have sought to identify compounds that reduce polyQ aggregates in biochemical assays. Yet, it is often observed that compounds showing efficacy in these assays may exhibit inherent toxicity when evaluated *in vivo* models (Heiser et al., 2002; Wang et al., 2005). Here, we present the results of our High-Throughput Imaging-based drug-repurposing screening, which aimed to identify compounds capable of reducing the toxicity associated with polyQ expansions. In summary, our screening process aimed to leverage the safety profile of FDA-approved drugs. As a result, we identified the anti-leprosy drug clofazimine as a promising candidate, which was subsequently confirmed through various *in vitro* models as well as a zebrafish model of polyQ toxicity. By conducting computational analyses of transcriptional signatures and employing molecular modeling and biochemical assays, we discovered that clofazimine acts as an agonist of the peroxisome proliferator-activated receptor gamma (PPARγ). Previous studies have suggested that PPARγ activation could be a potential therapeutic approach for HD by promoting mitochondrial biogenesis (Corona & Duchen, 2016; Jin et al., 2013). In line with this, our findings demonstrate that clofazimine effectively restored the mitochondrial dysfunction induced by Htt-Q94 expression. These results collectively support the repurposing of clofazimine as a potential treatment for polyQ-related disorders diseases.

4.2.1 Discussion

Despite significant progress in understanding the molecular mechanisms of polyQ diseases, effective treatments for these conditions remain elusive. Current research efforts are focused

on therapeutic strategies that aim to reduce the expression of polyQ-containing proteins, prevent polyQ aggregate formation, or enhance their clearance (Esteves et al., 2017). In our study, we aimed to identify compounds capable of mitigating the toxicity associated with polyQ expansions. A similar approach was undertaken by the Taylor laboratory, which searched for molecules that could reduce apoptosis induced by the expression of a truncated androgen receptor with a 112-glutamine repeat (Piccioni et al., 2004). In our screening model using U2OS cells, the expression of Htt-Q94 resulted in cell cycle arrest rather than apoptosis. Interestingly, this phenotype was more severe at lower cell densities, suggesting a potential enhancement of polyQ aggregate formation under sub-confluent conditions (Martín-Aparicio et al., 2002).

Our screening approach yielded promising results, including the identification of compounds such as TZD, which has previously demonstrated the ability to modulate polyQ pathology severity in preclinical models (Cho et al., 2013; Inestrosa et al., 2005; Moon et al., 2021; Weydt et al., 2006). However, despite its initial approval for diabetes treatment, TZD was later withdrawn from the market due to concerns about hepatic toxicity (Gottlieb, 2001). Nevertheless, accumulating evidence supporting the therapeutic potential of activating the PPARγ/PDC1a axis in neurodegenerative diseases (Jamwal et al., 2021) highlights the need for discovering new PPARγ agonists that can overcome the initial toxicities associated with TZD. In this context, our findings suggest that CFZ acts as a PPARγ agonist with a binding affinity comparable to TZD, while also being safe for the treatment of infectious diseases. However, the limited ability of CFZ to penetrate the blood-brain barrier (BBB) restricts its efficacy in treating CNS infections. Efforts are being made to address this limitation, such as the development of nanoparticle-based formulations of CFZ (de Castro et al., 2021). Nevertheless, our study findings suggest that CFZ holds promise as a potential alternative to thiazolidinediones (TZDs) in the treatment of non-central nervous system (CNS) pathologies. This highlights the potential of CFZ as a therapeutic option for addressing the severity of polyQ disease pathologies, particularly by restoring mitochondrial function. The results of our research further emphasize the potential of drug repurposing, utilizing already approved medications, in identifying new treatment options for neurodegenerative diseases.

While we recognize the existing limitations of CFZ, we believe that further preclinical investigations are warranted to explore the efficacy of CFZ or its derivatives specifically in polyQ diseases. These studies would provide valuable insights into the potential of CFZ as a targeted therapy for neurodegenerative conditions. By repurposing existing drugs, we can expedite the drug development process and potentially find effective treatments for these debilitating diseases.

4.3 PAPER III: ACTIONABLE CANCER VULNERABILITY DUE TO TRANSLATIONAL ARREST, P53 AGGREGATION, AND RIBOSOME BIOGENESIS STRESS EVOKED BY THE DISULFIRAM METABOLITE CUET

Cutting-edge progress in technology and innovative computational methods have significantly accelerated the identification of novel compounds with potential clinical impact (S. Pushpakom et al., 2019). In this study, our investigations have underscored that the anticancer effectiveness of DSF hinges on its, copper-containing metabolite (CuET), which instigates proteotoxic stress by confining NPL4. This entrapment interrupts the p97-dependent protein turnover pathway, resulting in the activation of the unfolded protein response (UPR) (Skrott et al., 2017).

In cancer cells, dysregulated protein homeostasis can result in the accumulation of aggregates, which have diverse implications for cancer cell fate and treatment strategies (Krastev et al., 2022; Majera et al., 2020). Importantly, aggregate formation is not limited to chemically treated cells; mutant p53 isoforms can also form aggregates that exert dominant-negative effects on wild-type p53 and its paralogs, potentially influencing tumor progression and treatment responses (Direito et al., 2021).

Both proteotoxic stress and the UPR trigger the integrated stress response (ISR), which regulates intracellular protein content through translational changes (Hurwitz et al., 2022). A key event in the ISR is the phosphorylation of eIF2α, resulting in a global translation halt while selectively translating mRNAs involved in resolving proteotoxic stress or promoting cell survival (Costa-Mattioli & Walter, 2020). Protein translation is also influenced by p53 through its control of ribosome biogenesis (RiBi), modulation of 4E-BP1 transcription (a central regulator of translation), and regulation of the assembly of translation initiation complexes (e.g., ternary and eIF4F complexes) (Kasteri et al., 2018; Tiu et al., 2021). Building upon our preliminary observation that both disulfiram and CuET increase p53 protein levels and activate UPR signaling, including the phosphorylation of eIF2 (Skrott et al., 2017), we conducted more extensive studies to unravel the mechanistic connections between CuET and protein translation, ribosome biogenesis, and p53. We also aimed to elucidate the chronological sequence of events in human cancer cells under the influence of CuET and their potential implications for tumor treatment.

4.3.1 Discussion

In our research, we have made significant discoveries regarding the cellular effects of CuET exposure. Firstly, we found that CuET inhibits ribosomal translation by activating ISR kinases and phosphorylating eIF2a, which is consistent with the ISR induced by thapsigargin. CuET also disrupts ubiquitination processes, resulting in translational abnormalities (Skrott et al., 2017). Additionally, we observed a unique event of nucleolar restructuring following translational pausing in CuET-treated cancer cells, distinct from the nucleolar stress response caused by pol I inhibition(Lindström et al., 2022).

An unexpected discovery was the entrapment and transcriptional deactivation of p53 by CuET. Our study revealed the confinement of p53 and MDM2 within NPL4-enriched regions, impeding p53 translocation and leading to p53 aggregate accumulation. This hindered p53 functionality and its ability to stimulate the expression of CDKN1A/p21. CuET-induced aggregates inhibited the post-translational modifications of p53, affecting its transcriptional activity. Despite this, CuET maintained its ability to induce cancer cell death, suggesting p53-independent mechanisms may be involved.

Our findings open possibilities for combination therapies using CuET and other drugs in clinical trials. We propose a novel concept of using active metabolites of FDA-/EMA-approved drugs for drug repurposing. This approach maximizes efficacy while minimizing potential adverse effects associated with other drug metabolites. Overall, our research provides insights into the cellular effects of CuET, potential combination therapies, and a novel approach to drug repurposing using active metabolites.

4.4 PAPER IV: THE DRUG REPURPOSING ENCYCLOPEDIA (DRE): A WEB SERVER FOR SYSTEMATIC DRUG REPURPOSING ACROSS 20 ORGANISMS

Drug repurposing, through either computational or experimental means, has a proven track record of success (Steven M. Corsello et al., 2017; Sudeep Pushpakom et al., 2019) Specific methodologies and databases dedicated to drug repurposing have surfaced (Steven M. Corsello et al., 2017; Janes et al., 2018; Sudeep Pushpakom et al., 2019; Aravind Subramanian et al., 2017; Tanoli et al., 2020). A standout example is CMap, an expansive repository containing over 30,000 transcriptional signatures derived from drug and genetic perturbations across diverse cell types. Researchers can harness CMap for myriad analyses, such as pinpointing drugs that evoke similar transcriptional changes, indicating a common mechanism of action (Aravind Subramanian et al., 2017). Furthermore, CMap can help identify drugs that generate transcriptional signatures as opposed to those associated with a specific disease, implying potential therapeutic utility for that condition (Ferguson et al., 2018; Manzotti et al., 2019). For instance, we recently applied this methodology to propose drugs that could ameliorate or intensify the severity of the cytokine storm observed in severe COVID-19 cases (Sanchez-Burgos et al., 2022). Notwithstanding these valuable resources, a comprehensive database systematically juxtaposing drug-related transcriptional signatures with those linked to diseases or specific signaling pathways is yet to be established.

To facilitate drug repurposing strategies based on transcriptional signatures, we have developed DRE, an interactive database accessible at https://www.drugrep.org. The DRE leverages transcriptional signatures from the MSigDB, the most extensive and widely utilized repository of its kind (Arthur Liberzon et al., 2015; Subramanian et al., 2005), along with drug transcriptomic profiles available in CMap. This comprehensive database houses an extensive collection of 198,648,641 associations between drugs and signatures across 20 different organisms. In addition to providing access to pre-calculated associations, the DRE web server enables real-time drug repurposing analysis. Users can compare their gene signatures with those in the DRE database, perform drug-set enrichment analyses (DSEA) using the available drug transcriptomic profiles, and conduct similarity analyses of gene sets across all signatures within the database. The DRE serves as a valuable resource for researchers, offering a comprehensive platform for investigating drug repurposing strategies based on transcriptional signatures across various molecular signatures and multiple species.

4.4.1 Discussion

DRE is a comprehensive platform specifically designed to facilitate drug repurposing initiatives through the utilization of transcriptional data. The development of DRE involved a meticulous exploration of two prominent molecular signature libraries: CMap and MSigDB. CMap provided a wealth of drug-associated transcriptional profiles, while the MSigDB offered an extensive collection of molecular signatures encompassing various pathways, diseases, and signaling routes across 20 different organisms.

This thorough exploration resulted in the assembly of over 198 million noteworthy associations, which are readily accessible for investigation on the DRE platform. The primary focus of DRE is to provide researchers engaged in drug repurposing endeavors with a user-friendly and efficient tool. It offers a streamlined pathway for examining specific drugs and formulating hypotheses regarding potential pathways relevant to their diseases of interest.

DRE was designed with simplicity and accessibility in mind, catering to researchers from both computational and non-computational backgrounds. The platform is engineered to handle high-volume data traffic, ensuring optimal performance even under heavy user load. Its user-friendly interface and efficient functionality aim to facilitate seamless exploration and analysis of drug-disease associations.

With its extensive database and user-friendly design, DRE is poised to become a valuable resource, supporting the scientific research community's efforts in the multifaceted field of drug repurposing. It is expected to serve as an invaluable instrument for researchers seeking to uncover new therapeutic possibilities and accelerate the development of effective treatments.

5 CONCLUSIONS AND POINTS OF PERSPECTIVE

Drug repurposing has become a notable and productive approach in drug discovery, providing a potential avenue to approval that is both quicker and more cost-efficient compared to the conventional process of creating new drugs from scratch. As mentioned previously, the existing knowledge about the pharmacological and safety profiles of approved drugs can significantly accelerate the process.

Each of the four studies presents a distinct approach to drug repurposing, highlighting its potential across various disease contexts. Drug repurposing can be achieved through two primary approaches, firstly in silico screening that employs computational techniques such as data mining, machine learning algorithms, and molecular modeling to predict potential new uses for drugs. And secondly, experimental screening involves laboratory investigations to assess the effects of existing drugs on proteins, cells, or animals. These approaches complement each other by enabling the screening of large datasets and validating hypotheses derived from these analyses. However, challenges arise concerning intellectual property rights and the possibility of unintended off-target effects. This thesis exemplifies a utilization of both *in silico* and experimental screening approaches in the field of drug repurposing.

In **Study I**, we employed the drug repurposing strategy to investigate the tetracycline repressor (tetR)-regulated system's ability to control gene expression in mammalian cells. A human osteosarcoma cell line was generated using this system, enabling the inducible expression of TAR DNA-binding protein 43 (TDP-43) fused with the enhanced green fluorescent protein (EGFP), which has been associated with neurodegenerative diseases. Consistent with previous research, TDP-43 overexpression resulted in aggregate accumulation and reduced viability in U2OS cells. To explore potential interventions, we conducted a chemical screen using a library containing FDA-approved drugs. While the primary screen identified several compounds that mitigated TDP-43 toxicity, subsequent experiments revealed that these chemicals interfered with the doxycycline-dependent expression of TDP-43. This antagonistic effect was observed with both doxycycline and tetracycline and in multiple Tet-On cell lines expressing different genes, highlighting the broad impact of these compounds as inhibitors of the tetR system. Utilizing the same cell line, a genome-wide CRISPR/Cas9 screen uncovered epigenetic regulators like the G9a methyltransferase and TRIM28 as potential modifiers of TDP-43 toxicity. Once again, further investigations demonstrated that inhibiting G9a or losing TRIM28 prevented the doxycycline-induced expression of TDP-43. In summary, these findings create exciting new avenues for drug repositioning and genetic investigations using the tetR-regulated system in mammalian cells. The discovered compounds exerted an antagonistic impact on the initiation of TDP-43 expression, providing an opportunity for further studies to decipher the underlying mechanics of this interaction. Assessing the specific pathways or molecular targets influenced by these compounds could yield crucial insights into gene expression regulation and prospective therapeutic strategies.

In **Study II**, our focus turned towards polyglutamine (polyQ) diseases - a category of neurodegenerative disorders marked by expanded CAG repeats. Currently, therapeutic alternatives for these conditions are woefully limited. To uncover potential remedies, we initiated a high-throughput chemical screening designed to identify medications capable of mitigating the toxic effects linked to the HTT protein with 94 glutamines (Htt-Q94), a variant found in the initial exon of the HTT protein. After testing numerous compounds, clofazimine, a drug typically used to combat leprosy, emerged as a promising candidate. To validate these preliminary results, we further tested the effects of clofazimine using an array of *in vitro* models and a zebrafish model that mirrors the toxicity profile of polyQ diseases. A

combination of computational analyses, molecular modeling, and biochemical tests unveiled clofazimine's role as an agonist for PPARγ - a receptor previously suggested as a potential therapeutic target for HD. The activation of PPARγ is linked with enhanced mitochondrial biogenesis, a process intrinsically associated with HD pathology. Crucially, clofazimine demonstrated the ability to rectify mitochondrial dysfunction provoked by Htt-Q94 expression. These compelling findings strongly advocate for the repurposing of clofazimine as a potential therapy for polyQ diseases, providing a glimmer of hope for the development of effective treatments for these debilitating neurodegenerative conditions.

Looking forward, these findings could spur a new wave of research and development in the treatment of polyQ diseases. The potential therapeutic role of clofazimine, a well-established anti-viral drug, can drastically cut down the time and resources needed for developing a new treatment from scratch, thereby potentially accelerating the delivery of a much-needed therapeutic solution to patients. Understanding the relationship between PPARγ activation and mitigation of disease symptoms could also provide new pathways for treating similar neurodegenerative disorders. Our study thus offers a promising foundation for future research, with the potential for substantial impact on our approach towards these challenging conditions.

In **Study III**, the focus was on repurposing disulfiram, a drug commonly used to treat alcohol dependency, for potential applications in oncology. We investigated the effects of a disulfiram metabolite called diethyldithiocarbamate, in combination with copper (CuET), on the growth of various cancer cell lines and xenograft models. They found that the combined agent, CuET, demonstrated significant suppression of cancer cell growth and exhibited genotoxic and proteotoxic effects. One intriguing discovery was that CuET induced an early translational arrest in cancer cells through a mechanism known as the ISR. Additionally, signs of nucleolar stress were observed at a later stage. Another noteworthy finding was that CuET led to the aggregation of the tumor-suppressing protein p53, specifically in NPL4-rich aggregates. This resulted in an elevation of p53 protein levels while functionally inhibiting its activity. Interestingly, this suggests that the cell death induced by CuET may not rely on the presence of functional p53, indicating a p53-independent mechanism. These findings shed light on the potential mechanisms underlying the anti-cancer effects of CuET and provide insights into its therapeutic implications beyond the traditional role of p53 in tumor suppression. The repurposing of disulfiram and its metabolite, diethyldithiocarbamate, in combination with copper, presents a promising avenue for further exploration in oncology research.

In **Study IV**, we made a significant advancement with the inauguration of DRE, a comprehensive online instrument designed to streamline drug repurposing research. Traditionally, the new drug development process, laden with protracted timelines and hefty costs, averages a span of over eight years from inception to clinical application. This prolonged course can become problematic in emergent scenarios, such as during the COVID-19 pandemic, where prompt resolutions are critical. Therefore, drug repurposing, which involves discovering new applications for existing drugs, has become popular due to its capacity to save both time and financial resources.

Computational and experimental methodologies are critical for drug repurposing, a field where many databases and techniques have been developed to streamline the process. However, a noticeable gap has existed in systematically juxtaposing transcriptional signatures of drugs with those associated with diseases or specific signaling pathways. In this study, we addressed this shortfall with the introduction of DRE. By conducting a comprehensive comparison between signatures drawn from MSigDB and drug

transcriptomic profiles from CMap, we have curated an interactive database. This vast compilation hosts almost 200 million associations spanning 20 different organisms, forging links between drugs and signatures.

The DRE web server acts not only as a repository for these pre-established associations but also enables users to conduct real-time drug repurposing analyses. Users are free to contrast their gene signatures with those in the DRE database, carry out Drug Set Enrichment Analysis (DSEA) using the available drug transcriptomic profiles, and perform cross-comparison of all gene sets encapsulated in the database's signatures. DRE serves as a groundbreaking web server, custom-built to enhance drug repurposing approaches reliant on transcriptional signatures. It provides a comprehensive toolbox for researchers, enabling extensive studies across diverse molecular signatures and species, and significantly propelling the progress of scientific endeavors.

Looking to the future, the DRE could revolutionize how we approach drug repurposing, potentially speeding up the process of identifying new therapeutic uses for existing drugs. As more and more transcriptional signatures are added to the database, the utility of the DRE will only continue to grow. It represents an important step forward in the field of drug repurposing and has the potential to make a significant impact on future medical advancements.

In conclusion, the discussed studies underscore the considerable potential of drug repurposing as a viable strategy for addressing a wide range of diseases. By repurposing approved drugs that are already established for other therapeutic uses, researchers can circumvent the time-consuming and costly aspects typically associated with traditional drug development, such as extensive safety testing and regulatory hurdles. This approach offers several notable advantages, including cost reduction, accelerated development timelines, and an increased likelihood of success.

Furthermore, these studies underscore the significance of integrating computational, chemical, and informatics approaches in the field of drug repurposing. Computational methods, such as virtual screening and molecular modeling, play a crucial role in identifying potential drug candidates that exhibit favorable characteristics for specific diseases. Chemical approaches, including high-throughput screening and medicinal chemistry, contribute to the refinement and validation of these candidates. Additionally, informatics tools and databases serve as invaluable resources for data mining, knowledge synthesis, and predictive modeling, aiding in the overall drug repurposing process.

Through the integration of these diverse disciplines, researchers can effectively explore a broad spectrum of drug candidates and uncover promising opportunities for repurposing. This collaborative and systematic approach greatly increases the likelihood of identifying effective treatments for diseases, particularly those that currently lack targeted therapies or have unmet medical needs.

In essence, the strength of drug repurposing stems from its capacity to harness existing knowledge, infrastructure, and resources to expedite the discovery of new therapeutic options. By synergizing computational, chemical, and informatics approaches, the efficiency and success rate of drug repurposing initiatives are enhanced, ultimately benefiting patients and driving advancements in the field of medical science.

6 ACKNOWLEDGEMENT

To **Oskar**, my main supervisor, your impact on my academic journey is immeasurable. Your unique blend of scholarly rigor, intellectual curiosity, and boundless patience has left an indelible mark on my professional development and personal growth. You have not merely been a guide, but also a mentor who has molded my research approach. Your thoughtful critiques have challenged my thinking, pushing me to delve deeper, question more, and strive for academic excellence. Your influence has fostered a culture of intellectual curiosity that extends far beyond the confines of our lab. You have instilled in me an insatiable desire for knowledge, a hunger to understand the world around us, and the courage to question established norms.

The lessons I have learned under your guidance have not only honed my skills as a scholar but also shaped my outlook on life. Your mentorship has taught me that the pursuit of knowledge is not just about understanding the world, but also about using that understanding to make a meaningful impact. Your unwavering belief in my potential has been a powerful motivator, pushing me to reach beyond my limitations and realize my full potential. You have shown me the transformative power of education and research, and for that, I am profoundly grateful.

In conclusion, Oskar, you have not only been an excellent supervisor but also a true mentor. Your influence extends far beyond the confines of our lab or academia. I am deeply grateful for your guidance, support, and the wisdom you have imparted. I look forward to carrying these invaluable lessons with me as I embark on the next chapter of my life.

Dani, as a co-supervisor, your influence has been pivotal in my academic journey. Your comprehensive critiques, combined with your uplifting encouragement, have provided a balanced environment that fostered my growth and development. Your critiques were not just thought-provoking; they were a catalyst that sparked new ideas, encouraged in-depth exploration, and honed my analytical skills. You have been a guide, steering me towards greater clarity in my thinking and precision in my research. Your constant encouragement has been a beacon of positivity, bolstering my confidence and inspiring me to push the boundaries of my capabilities. You have shown me that every challenge is an opportunity for growth and every mistake is a lesson to be learned. Your belief in the power of collaboration has left a lasting impression. You have shown me that the confluence of different ideas, perspectives, and disciplines can lead to innovative solutions and groundbreaking research.

Maria, your presence in our lives has been a beacon of warmth and familiarity in an otherwise foreign land. You have offered us a sense of belonging that is so profound it has transformed our surroundings into a place we can truly call home.

Your care for Alba and me has been far more than just a friendly gesture. It's been a testament to your generous heart and boundless compassion. You have donned the role of a Swedish mother for us, offering comfort, guidance, and a nurturing touch that has eased our transition and enriched our experiences. You have shown us that despite cultural differences and geographical distances, the language of compassion and kindness is universal. You have embodied the truth that humanity knows no borders and that love transcends all boundaries. Your unconditional support has provided us with a strong foundation in this foreign land, enabling us to face challenges with confidence and pursue our dreams with determination. You have taught us that home isn't a physical place, but a feeling of warmth, safety, and acceptance.

Pelle, your ability to coordinate and balance all aspects of the division and your willingness to extend a helping hand have made a significant impact on our work environment. You have demonstrated that consideration for others and fostering a supportive atmosphere are critical components of a harmonious work environment. Your actions have not only benefited those you have directly assisted but have also inspired us to be more mindful and compassionate towards our colleagues.

You have shown us that a harmonious work environment is the result of both individual and collective efforts to support and uplift one another. I am deeply grateful for the invaluable lessons you have imparted, and I will strive to carry your spirit with me in all aspects of my life.

Alba, your infectious laughter, and unwavering positivity have been the very lifeblood of our lab. Your ever-present smile, your optimistic outlook, and your ability to find joy in the smallest of things have been a constant source of upliftment for all of us. You have shown us that positivity is not just a state of mind, but a choice that can transform the way we perceive and interact with the world around us. Your ability to spread happiness, uplift others with your optimism, and find joy in every situation is truly remarkable. I am certain that your positive spirit will continue to touch the lives of those around you, no matter where your journey takes you. Now, as you embark on your journey in the USA, I am confident that you will carry this infectious positivity with you. Even though you have been there for a year now, I am certain that the joy and positivity you have brought to our lab are resonating in your new environment.

Bartek, your comprehensive knowledge and the camaraderie we have shared, both within the lab and at the basketball court, have enriched my experience in ways beyond measure. Your deep well of understanding has been a constant source of enlightenment. Our engaging discussions and your insightful perspectives have broadened my horizons, contributing significantly to my academic growth and intellectual curiosity.

Beyond the lab, our shared passion for basketball has fostered a camaraderie that I deeply cherish. As you stand on the cusp of an exciting new chapter in your life, the journey into parenthood, I extend to you my heartfelt congratulations and best wishes. This journey will undoubtedly bring with it new challenges, joys, and learning experiences. I am confident that you will navigate this path with the same wisdom, patience, and kindness that you have shown us.

Mine, the time we've spent together in the lab, immersed in engaging discussions and shared experiences, has been both intellectually stimulating and immensely enjoyable. Our conversations, filled with diverse perspectives and thought-provoking insights, have significantly contributed to my academic growth and understanding.

Your intellectual curiosity, combined with your enthusiastic approach to our work, has not only made our shared experiences in the lab more enriching but also fostered a vibrant and collaborative atmosphere. These moments have not only shaped our academic journey but also left a lasting imprint on our personal growth.

As you embark on your future endeavors in Switzerland, I am confident that you will continue to thrive. Your dedication, intellectual prowess, and collaborative spirit are assets that will undoubtedly lead you to success in all your pursuits.

Oneka, your dedication and resilience, paired with your unwavering ambition, serve as an inspiration to us all. Your journey thus far has been a testament to the transformative power of dreams and the fruits of consistent hard work.

The way you pursue your goals, with an unyielding spirit and a steadfast resolve, is truly commendable. You have shown us that obstacles are merely stepping stones towards success and that resilience in the face of adversity is the hallmark of a true achiever.

Your unwavering ambition is a beacon that guides your journey, illuminating your path towards your dreams. You have demonstrated that having a clear vision, combined with relentless determination, can make the seemingly impossible, possible.

Oneka, I have no doubt that your journey will continue to inspire us and that your dreams will come to fruition. Your dedication, resilience, and ambition are not just your strengths, but also the keys that will unlock the door to your dreams.

Myriam, your friendship and unwavering support have been a source of comfort and strength throughout our journey together. Your uplifting presence and the camaraderie we share have not only made our shared experiences more enjoyable but have also fostered a sense of unity and mutual respect that I deeply cherish. As you continue your journey towards earning your PhD, I have no doubt that you will bring the same level of dedication, enthusiasm, and resilience to your future work. Your intellectual curiosity, combined with your tireless work ethic, makes you a formidable scholar and an asset to any research endeavor. Myriam, I wholeheartedly believe that your future in academia is bright. I look forward to witnessing your continued growth and success as you advance in your PhD journey. As you move forward, know that you carry with you not just my best wishes but also my admiration for your relentless pursuit of academic excellence.

Louise, your remarkable management skills and steadfast support have been a cornerstone in the efficient functioning of our lab. Your ability to organize, coordinate, and oversee our operations with a keen eye for detail and a deep understanding of our collective goals has been truly instrumental.

Your proficiency in managing the lab extends beyond mere administrative tasks. Your commitment to fostering a conducive work environment and your dedication to ensuring that each member of our team has the necessary resources and support to excel in their work have made a significant impact on our collective success.

Hendrik, as a young brother on the cusp of starting your PhD journey in the UK, I want to extend my heartfelt best wishes to you. This is an exciting, pivotal moment in your life, and I am confident that you will rise to the occasion with the same enthusiasm and dedication that you have consistently displayed. Hendrik, I wish you nothing but success as you embark on your PhD journey. May this new chapter of your life be filled with exciting discoveries, meaningful collaborations, and profound insights. May it challenge you, inspire you, and above all, lead you to a future that's as bright as the potential I see in you. Here's to your future, Hendrik - a future I am certain is filled with promise and excellence.

To **Jiri, Dimitris, and Martin**, I would like to express my heartfelt thanks for involving me in the project and for your constant encouragement. Your support during my graduation was crucial. You have shown the power of collaboration and academic solidarity. Working alongside such a dedicated and talented team has been an enriching journey that has significantly contributed to my professional development and academic growth.

Jiri, your guidance has been invaluable. Your mentorship, coupled with your deep expertise and wisdom, has not only shaped my understanding of our work but also fostered a culture of intellectual curiosity and academic rigor. Your leadership and mentorship have been a guiding light throughout my journey, and for that, I am deeply grateful.

Dimitris, your commitment to maintaining the quality of our research is truly admirable. Your meticulous attention to detail, your unwavering standards of excellence, and your tireless dedication to our project have significantly contributed to the success of our work. Your efforts have not only ensured the quality of our research but also instilled a sense of pride and responsibility in our team.

Martin, your support during my graduation has been instrumental. Your insights, your encouragement, and your unwavering faith in my abilities have provided a much-needed boost during this crucial phase of my academic journey. As you embark on your new journey as a PI at Zhejiang Edinburgh, I wish you all the success. I am confident that you will continue to make significant contributions to the field and inspire a new generation of researchers.

To Jiri, Dimitris, and Martin, thank you once again for your invaluable support and guidance. I look forward to following your future endeavors and witnessing the continued success of your research.

Finally, I would like to express my deep gratitude to **Jordi, Valeria, Jamie, Asimina, Ann-Sofie, Mikael, Johana, Mike, Abid,** and **Bennie**. Your collective contributions have created a nurturing and supportive environment that has been instrumental to my growth, both personally and professionally. Your friendships have enriched my experience and have been a source of great joy and camaraderie.

To my family,

Your unwavering faith, boundless love, and relentless support have been my anchors through this journey. You have stood by me in times of triumph and challenge, offering encouragement, wisdom, and comfort. Your belief in me, even in moments when I doubted myself, has been a source of strength that propelled me forward. Your love, encouragement, and pride in my accomplishments have meant the world to me. The roots you have provided have given me a sense of belonging, while the wings you have given me have inspired me to soar.

In this profound journey, each one of you has left an indelible mark on my academic and personal development. Every interaction, every shared experience, and every moment of collaboration has contributed significantly to shaping the scholar I am today.

To my **supervisors, colleagues, and friends**, I am deeply grateful for your unwavering support, invaluable guidance, and cherished friendship. The lessons I have learned from each of you extend beyond academia and have instilled in me the values of dedication, integrity, collaboration, and resilience.

I have learned the power of curiosity and the importance of intellectual rigor from our many scholarly discussions. I have learned the value of resilience and perseverance from the challenges we've tackled together. I have learned the strength of collaboration and the significance of mutual respect and support from our shared experiences.

Each one of you has been a pillar of support, a source of inspiration, and a guiding light. Your collective wisdom, shared knowledge, and friendship have enriched my journey in ways I could not have imagined.

As I prepare to embark on my future endeavors, I am excited to carry forward these lessons, apply the knowledge I have gained, and contribute to the world of academia in my own unique way. I am eager to explore new frontiers, face new challenges, and continue my journey of learning and growth.

In closing, let me express my deepest gratitude once again to each one of you. Your support, guidance, and friendship have been the greatest gifts of this journey. Thank you all.

7 REFERENCES

Abagyan, R., Totrov, M., & Kuznetsov, D. (1994). ICM—A new method for protein modeling and design: Applications to docking and structure prediction from the distorted native conformation. *Journal of Computational Chemistry*, *15*(5), 488-506. https://doi.org/https://doi.org/10.1002/jcc.540150503

Abbas, K., Abbasi, A., Dong, S., Niu, L., Yu, L., Chen, B., Cai, S. M., & Hasan, Q. (2021). Application of network link prediction in drug discovery. *BMC Bioinformatics*, *22*(1), 187. https://doi.org/10.1186/s12859-021-04082-y

Abdolmaleki, A., Shiri, F., & Ghasemi, J. B. (2021). Chapter 11 - Use of Molecular Docking as a Decision-Making Tool in Drug Discovery. In M. S. Coumar (Ed.), *Molecular Docking for Computer-Aided Drug Design* (pp. 229-243). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-822312-3.00010-2

Adhami, M., Sadeghi, B., Rezapour, A., Haghdoost, A. A., & MotieGhader, H. (2021). Repurposing novel therapeutic candidate drugs for coronavirus disease-19 based on protein-protein interaction network analysis. *BMC Biotechnology*, *21*(1), 22. https://doi.org/10.1186/s12896-021-00680-z

Alberca, L. N., & Talevi, A. (2020). The Efficiency of Multi-target Drugs: A Network Approach. In M. Bizzarri (Ed.), *Approaching Complex Diseases: Network-Based Pharmacology and Systems Approach in Bio-Medicine* (pp. 63-75). Springer International Publishing. https://doi.org/10.1007/978-3-030-32857-3_3

Alexandrov, V., Brunner, D., Hanania, T., & Leahy, E. (2015). High-throughput analysis of behavior for drug discovery. *Eur J Pharmacol*, *750*, 82-89. https://doi.org/10.1016/j.ejphar.2014.11.047

Amiri Souri, E., Chenoweth, A., Karagiannis, S. N., & Tsoka, S. (2023). Drug repurposing and prediction of multiple interaction types via graph embedding. *BMC Bioinformatics*, *24*(1), 202. https://doi.org/10.1186/s12859-023-05317-w

An, W. F., & Tolliday, N. (2010). Cell-based assays for high-throughput screening. *Mol Biotechnol*, *45*(2), 180-186. https://doi.org/10.1007/s12033-010-9251-z

Arndt, H.-D. (2006). Small Molecule Modulators of Transcription [https://doi.org/10.1002/anie.200600285]. *Angewandte Chemie International Edition*, *45*(28), 4552-4560. https://doi.org/https://doi.org/10.1002/anie.200600285

Aulner, N., Danckaert, A., Ihm, J., Shum, D., & Shorte, S. L. (2019). Next-Generation Phenotypic Screening in Early Drug Discovery for Infectious Diseases. *Trends Parasitol*, *35*(7), 559-570. https://doi.org/10.1016/j.pt.2019.05.004

Badkas, A., De Landtsheer, S., & Sauter, T. (2021). Topological network measures for drug repositioning. *Brief Bioinform*, *22*(4). https://doi.org/10.1093/bib/bbaa357

Bailey, J. (2005). Non-human primates in medical research and drug development: a critical review.

Baldi, A. (2010). Computational approaches for drug design and discovery: An overview. *Systematic reviews in Pharmacy*, *1*(1), 99.

Baron, U., & Bujard, H. (2000). Tet repressor-based system for regulated gene expression in eukaryotic cells: principles and advances. *Methods Enzymol*, *327*, 401-421. https://doi.org/10.1016/s0076-6879(00)27292-3

Bates, G. P., Dorsey, R., Gusella, J. F., Hayden, M. R., Kay, C., Leavitt, B. R., Nance, M., Ross, C. A., Scahill, R. I., Wetzel, R., Wild, E. J., & Tabrizi, S. J. (2015). Huntington disease. *Nat Rev Dis Primers*, *1*, 15005. https://doi.org/10.1038/nrdp.2015.5

Bates, G. P., Dorsey, R., Gusella, J. F., Hayden, M. R., Kay, C., Leavitt, B. R., Nance, M., Ross, C. A., Scahill, R. I., Wetzel, R., Wild, E. J., & Tabrizi, S. J. (2015). Huntington disease. *Nature Reviews Disease Primers*, *1*(1), 15005. https://doi.org/10.1038/nrdp.2015.5

Becker, L. A., Huang, B., Bieri, G., Ma, R., Knowles, D. A., Jafar-Nejad, P., Messing, J., Kim, H. J., Soriano, A., Auburger, G., Pulst, S. M., Taylor, J. P., Rigo, F., & Gitler, A. D. (2017). Therapeutic reduction of ataxin-2 extends lifespan and reduces pathology in TDP-43 mice. *Nature*, *544*(7650), 367-371. https://doi.org/10.1038/nature22038

Ben-Yakar, A. (2019). High-Content and High-Throughput In Vivo Drug Screening Platforms Using Microfluidics. *ASSAY and Drug Development Technologies*, *17*(1), 8-13. https://doi.org/10.1089/adt.2018.908

Benek, O., Korabecny, J., & Soukup, O. (2020). A Perspective on Multi-target Drugs for Alzheimer’s Disease. *Trends in Pharmacological Sciences*, *41*(7), 434-445. https://doi.org/10.1016/j.tips.2020.04.008

Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society. Series B (Methodological)*, *57*(1), 289-300. http://www.jstor.org/stable/2346101

Bikadi, Z., & Hazai, E. (2009). Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. *J Cheminform*, *1*, 15. https://doi.org/10.1186/1758-2946-1-15

Blay, V., Tolani, B., Ho, S. P., & Arkin, M. R. (2020). High-Throughput Screening: today's biochemical and cell-based approaches. *Drug Discov Today*, *25*(10), 1807-1821. https://doi.org/10.1016/j.drudis.2020.07.024

Borlongan, C. V., Koutouzis, T. K., & Sanberg, P. R. (1997). 3-Nitropropionic acid animal model and Huntington's disease. *Neurosci Biobehav Rev*, *21*(3), 289-293. https://doi.org/10.1016/s0149-7634(96)00027-9

Brown, D. (2007). Unfinished business: target-based drug discovery. *Drug Discovery Today*, *12*(23), 1007-1012. https://doi.org/https://doi.org/10.1016/j.drudis.2007.10.017

Bryda, E. C. (2013). The Mighty Mouse: the impact of rodents on advances in biomedical research. *Mo Med*, *110*(3), 207-211.

Burkhard, P., Hommel, U., Sanner, M., & Walkinshaw, M. D. (1999). The discovery of steroids and other novel FKBP inhibitors using a molecular docking program11Edited by F. E. Cohen. *Journal of Molecular Biology*, *287*(5), 853-858. https://doi.org/https://doi.org/10.1006/jmbi.1999.2621

Butler, C. C., Dorward, J., Yu, L.-M., Gbinigie, O., Hayward, G., Saville, B. R., Van Hecke, O., Berry, N., Detry, M., Saunders, C., Fitzgerald, M., Harris, V., Patel, M. G., de Lusignan, S., Ogburn, E., Evans, P. H., Thomas, N. P. B., & Hobbs, F. D. R. (2021). Azithromycin for community treatment of suspected COVID-19 in people at increased risk of an adverse clinical course in the UK (PRINCIPLE): a randomised, controlled, open-label, adaptive platform trial. *The Lancet*, *397*(10279), 1063-1074. https://doi.org/10.1016/S0140-6736(21)00461-X

Campagne, S., Boigner, S., Rüdisser, S., Moursy, A., Gillioz, L., Knörlein, A., Hall, J., Ratni, H., Cléry, A., & Allain, F. H. (2019). Structural basis of a small molecule targeting RNA for a specific splicing correction. *Nat Chem Biol*, *15*(12), 1191-1198. https://doi.org/10.1038/s41589-019-0384-5

Cao, B., Wang, Y., Wen, D., Liu, W., Wang, J., Fan, G., Ruan, L., Song, B., Cai, Y., Wei, M., Li, X., Xia, J., Chen, N., Xiang, J., Yu, T., Bai, T., Xie, X., Zhang, L., Li, C., . . . Wang, C. (2020). A Trial of Lopinavir–Ritonavir in Adults Hospitalized with Severe Covid-19. *New England Journal of Medicine*, *382*(19), 1787-1799. https://doi.org/10.1056/NEJMoa2001282

Carettoni, D., & Bader, B. Assay Development and High-Throughput Screening. In *Burger's Medicinal Chemistry and Drug Discovery* (pp. 1-42). https://doi.org/https://doi.org/10.1002/0471266949.bmc165.pub2

Cerou, F., & Guyader, A. (2014). Fluctuation Analysis of Adaptive Multilevel Splitting. arXiv:1408.6366. Retrieved August 01, 2014, from https://ui.adsabs.harvard.edu/abs/2014arXiv1408.6366C

Chang, D. T., Oyang, Y. J., & Lin, J. H. (2005). MEDock: a web server for efficient prediction of ligand binding sites based on a novel optimization algorithm. *Nucleic Acids Res*, *33*(Web Server issue), W233-238. https://doi.org/10.1093/nar/gki586

Charitou, T., Bryan, K., & Lynn, D. J. (2016). Using biological networks to integrate, visualize and analyze genomics data. *Genetics Selection Evolution*, *48*(1), 27. https://doi.org/10.1186/s12711-016-0205-1

Chaudhary, K. K., & Mishra, N. (2016). A review on molecular docking: novel tool for drug discovery. *databases*, *3*(4), 1029.

Chen, J., Xia, L., Liu, L., Xu, Q., Ling, Y., Huang, D., Huang, W., Song, S., Xu, S., Shen, Y., & Lu, H. (2020). Antiviral Activity and Safety of Darunavir/Cobicistat for the Treatment of COVID-19. *Open Forum Infect Dis*, *7*(7), ofaa241. https://doi.org/10.1093/ofid/ofaa241

Cheng, C. F., Ku, H. C., & Lin, H. (2018). PGC-1α as a Pivotal Factor in Lipid and Metabolic Regulation. *Int J Mol Sci*, *19*(11). https://doi.org/10.3390/ijms19113447

Cho, D. H., Lee, E. J., Kwon, K. J., Shin, C. Y., Song, K. H., Park, J. H., Jo, I., & Han, S. H. (2013). Troglitazone, a thiazolidinedione, decreases tau phosphorylation through the inhibition of cyclin-dependent kinase 5 activity in SH-SY5Y neuroblastoma cells and primary neurons. *J Neurochem*, *126*(5), 685-695. https://doi.org/10.1111/jnc.12264

Choi, V. (2005). Yucca: An Efficient Algorithm for Small-Molecule Docking. *Chemistry & Biodiversity*, *2*(11), 1517-1524. https://doi.org/https://doi.org/10.1002/cbdv.200590123

Chung, C. W., Coste, H., White, J. H., Mirguet, O., Wilde, J., Gosmini, R. L., Delves, C., Magny, S. M., Woodward, R., Hughes, S. A., Boursier, E. V., Flynn, H., Bouillot, A. M., Bamborough, P., Brusq, J. M., Gellibert, F. J., Jones, E. J., Riou, A. M., Homes, P., . . . Nicodeme, E. (2011). Discovery and characterization of small molecule inhibitors of the BET family bromodomains. *J Med Chem*, *54*(11), 3827-3838. https://doi.org/10.1021/jm200108t

Chung, J. Y., Cho, S. J., & Hah, J. M. (2011). A python-based docking program utilizing a receptor bound ligand shape: PythDock. *Arch Pharm Res*, *34*(9), 1451-1458. https://doi.org/10.1007/s12272-011-0906-5

Cokol-Cakmak, M., Cetiner, S., Erdem, N., Bakan, F., & Cokol, M. (2020). Guided screen for synergistic three-drug combinations. *PLOS ONE*, *15*(7), e0235929. https://doi.org/10.1371/journal.pone.0235929

Conforti, P., Mas Monteys, A., Zuccato, C., Buckley, N. J., Davidson, B., & Cattaneo, E. (2013). In vivo delivery of DN:REST improves transcriptional changes of REST-regulated genes in HD mice. *Gene Therapy*, *20*(6), 678-685. https://doi.org/10.1038/gt.2012.84

Consortium, T. G. O. (2020). The Gene Ontology resource: enriching a GOld mine. *Nucleic Acids Research*, *49*(D1), D325-D334. https://doi.org/10.1093/nar/gkaa1113

Corona, J. C., & Duchen, M. R. (2016). PPARγ as a therapeutic target to rescue mitochondrial function in neurological disease. *Free Radical Biology and Medicine*, *100*, 153-163. https://doi.org/https://doi.org/10.1016/j.freeradbiomed.2016.06.023

Corsello, S. M., Bittker, J. A., Liu, Z., Gould, J., McCarren, P., Hirschman, J. E., Johnston, S. E., Vrcic, A., Wong, B., Khan, M., Asiedu, J., Narayan, R., Mader, C. C., Subramanian, A., & Golub, T. R. (2017). The Drug Repurposing Hub: a next-generation drug library and information resource. *Nat Med*, *23*(4), 405-408. https://doi.org/10.1038/nm.4306

Corsello, S. M., Bittker, J. A., Liu, Z., Gould, J., McCarren, P., Hirschman, J. E., Johnston, S. E., Vrcic, A., Wong, B., Khan, M., Asiedu, J., Narayan, R., Mader, C. C., Subramanian, A., & Golub, T. R. (2017). The Drug Repurposing Hub: a next-generation drug library and information resource. *Nature Medicine*, *23*(4), 405-408. https://doi.org/10.1038/nm.4306

Costa, V., & Scorrano, L. (2012). Shaping the role of mitochondria in the pathogenesis of Huntington's disease. *Embo j*, *31*(8), 1853-1864. https://doi.org/10.1038/emboj.2012.65

Costa-Mattioli, M., & Walter, P. (2020). The integrated stress response: From mechanism to disease. *Science*, *368*(6489), eaat5314. https://doi.org/10.1126/science.aat5314

Cross, B. C. S., Lawo, S., Archer, C. R., Hunt, J. R., Yarker, J. L., Riccombeni, A., Little, A. S., McCarthy, N. J., & Moore, J. D. (2016). Increasing the performance of pooled CRISPR–Cas9 drop-out screening. *Scientific Reports*, *6*(1), 31782. https://doi.org/10.1038/srep31782

Croston, G. E. (2017). The utility of target-based discovery. *Expert Opin Drug Discov*, *12*(5), 427-429. https://doi.org/10.1080/17460441.2017.1308351

Cui, L., Jeong, H., Borovecki, F., Parkhurst, C. N., Tanese, N., & Krainc, D. (2006). Transcriptional repression of PGC-1alpha by mutant huntingtin leads to mitochondrial dysfunction and neurodegeneration. *Cell*, *127*(1), 59-69. https://doi.org/10.1016/j.cell.2006.09.015

Cui, W., Aouidate, A., Wang, S., Yu, Q., Li, Y., & Yuan, S. (2020). Discovering Anti-Cancer Drugs via Computational Methods. *Front Pharmacol*, *11*, 733. https://doi.org/10.3389/fphar.2020.00733

Davies, S. W., Turmaine, M., Cozens, B. A., DiFiglia, M., Sharp, A. H., Ross, C. A., Scherzinger, E., Wanker, E. E., Mangiarini, L., & Bates, G. P. (1997). Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. *Cell*, *90*(3), 537-548. https://doi.org/10.1016/s0092-8674(00)80513-9

de Castro, R. R., do Carmo, F. A., Martins, C., Simon, A., de Sousa, V. P., Rodrigues, C. R., Cabral, L. M., & Sarmento, B. (2021). Clofazimine functionalized polymeric nanoparticles for brain delivery in the tuberculosis treatment. *Int J Pharm*, *602*, 120655. https://doi.org/10.1016/j.ijpharm.2021.120655

De Ruyck, J., Brysbaert, G., Blossey, R., & Lensink, M. F. (2016). Molecular docking as a popular tool in drug design, an in silico travel. *Advances and Applications in Bioinformatics and Chemistry*, 1-11.

Dedic, N., Jones, P. G., Hopkins, S. C., Lew, R., Shao, L., Campbell, J. E., Spear, K. L., Large, T. H., Campbell, U. C., Hanania, T., Leahy, E., & Koblan, K. S. (2019). SEP-363856, a Novel Psychotropic Agent with a Unique, Non-D(2) Receptor Mechanism of Action. *J Pharmacol Exp Ther*, *371*(1), 1-14. https://doi.org/10.1124/jpet.119.260281

Dietrich, P., Johnson, I. M., Alli, S., & Dragatsis, I. (2017). Elimination of huntingtin in the adult mouse leads to progressive behavioral deficits, bilateral thalamic calcification, and altered brain iron homeostasis. *PLOS Genetics*, *13*(7), e1006846. https://doi.org/10.1371/journal.pgen.1006846

DiFiglia, M., Sapp, E., Chase, K. O., Davies, S. W., Bates, G. P., Vonsattel, J. P., & Aronin, N. (1997). Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. *Science*, *277*(5334), 1990-1993. https://doi.org/10.1126/science.277.5334.1990

Direito, I., Monteiro, L., Melo, T., Figueira, D., Lobo, J., Enes, V., Moura, G., Henrique, R., Santos, M. A. S., Jerónimo, C., Amado, F., Fardilha, M., & Helguero, L. A. (2021). Protein Aggregation Patterns Inform about Breast Cancer Response to Antiestrogens and Reveal the RNA Ligase RTCB as Mediator of Acquired

Tamoxifen Resistance. *Cancers (Basel)*, *13*(13). https://doi.org/10.3390/cancers13133195

Doench, J. G., Fusi, N., Sullender, M., Hegde, M., Vaimberg, E. W., Donovan, K. F., Smith, I., Tothova, Z., Wilen, C., Orchard, R., Virgin, H. W., Listgarten, J., & Root, D. E. (2016). Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. *Nature Biotechnology*, *34*(2), 184-191. https://doi.org/10.1038/nbt.3437

Dolgalev, I. (2020). msigdbr: MSigDB gene sets for multiple organisms in a tidy data format. *R package version*, *7*(1).

Dominguez, C., Boelens, R., & Bonvin, A. M. (2003). HADDOCK: a protein-protein docking approach based on biochemical or biophysical information. *J Am Chem Soc*, *125*(7), 1731-1737. https://doi.org/10.1021/ja026939x

Duyao, M. P., Auerbach, A. B., Ryan, A., Persichetti, F., Barnes, G. T., McNeil, S. M., Ge, P., Vonsattel, J. P., Gusella, J. F., Joyner, A. L., & et al. (1995). Inactivation of the mouse Huntington's disease gene homolog Hdh. *Science*, *269*(5222), 407-410. https://doi.org/10.1126/science.7618107

Ege, N., Bouguenina, H., Tatari, M., & Chopra, R. (2021). Phenotypic screening with target identification and validation in the discovery and development of E3 ligase modulators. *Cell Chemical Biology*, *28*(3), 283-299. https://doi.org/https://doi.org/10.1016/j.chembiol.2021.02.011

Ejim, L., Farha, M. A., Falconer, S. B., Wildenhain, J., Coombes, B. K., Tyers, M., Brown, E. D., & Wright, G. D. (2011). Combinations of antibiotics and nonantibiotic drugs enhance antimicrobial efficacy. *Nat Chem Biol*, *7*(6), 348-350. https://doi.org/10.1038/nchembio.559

Elfawal, M. A., Savinov, S. N., & Aroian, R. V. (2019). Drug Screening for Discovery of Broad-spectrum Agents for Soil-transmitted Nematodes. *Scientific Reports*, *9*(1), 12347. https://doi.org/10.1038/s41598-019-48720-1

Esteves, S., Duarte-Silva, S., & Maciel, P. (2017). Discovery of Therapeutic Approaches for Polyglutamine Diseases: A Summary of Recent Efforts. *Med Res Rev*, *37*(4), 860-906. https://doi.org/10.1002/med.21425

Ewing, B. T. (2001). Monetary Policy and Stock Returns. *Bulletin of Economic Research*, *53*(1), 73-79. https://doi.org/https://doi.org/10.1111/1467-8586.00119

Fawzi Faisal, B., & Ashwag, A. (2021). Design and Implementation of High Throughput Screening Assays for Drug Discoveries. In K. S. Shailendra (Ed.), *High-Throughput Screening for Drug Discovery* (pp. Ch. 4). IntechOpen. https://doi.org/10.5772/intechopen.98733

Ferguson, L. B., Ozburn, A. R., Ponomarev, I., Metten, P., Reilly, M., Crabbe, J. C., Harris, R. A., & Mayfield, R. D. (2018). Genome-Wide Expression Profiles Drive Discovery of Novel Compounds that Reduce Binge Drinking in Mice. *Neuropsychopharmacology*, *43*(6), 1257-1266. https://doi.org/10.1038/npp.2017.301

Frank, S., Testa, C. M., Stamler, D., Kayson, E., Davis, C., Edmondson, M. C., Kinel, S., Leavitt, B., Oakes, D., O'Neill, C., Vaughan, C., Goldstein, J., Herzog, M., Snively, V., Whaley, J., Wong, C., Suter, G., Jankovic, J., Jimenez-Shahed, J., . . . Christopher, E. (2016). Effect of Deutetrabenazine on Chorea Among Patients With Huntington Disease: A Randomized Clinical Trial. *Jama*, *316*(1), 40-50. https://doi.org/10.1001/jama.2016.8655

Friesner, R. A., Banks, J. L., Murphy, R. B., Halgren, T. A., Klicic, J. J., Mainz, D. T., Repasky, M. P., Knoll, E. H., Shelley, M., Perry, J. K., Shaw, D. E., Francis, P., & Shenkin, P. S. (2004). Glide: a new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *J Med Chem*, *47*(7), 1739-1749. https://doi.org/10.1021/jm0306430

Gatz, C., & Quail, P. H. (1988). Tn10-encoded tet repressor can regulate an operator-containing plant promoter. *Proc Natl Acad Sci U S A*, *85*(5), 1394-1397. https://doi.org/10.1073/pnas.85.5.1394

Giacomotto, J., & Ségalat, L. (2010). High-throughput screening and small animal models, where are we? *Br J Pharmacol*, *160*(2), 204-216. https://doi.org/10.1111/j.1476-5381.2010.00725.x

Gilbert, I. H. (2013). Drug discovery for neglected diseases: molecular target-based and phenotypic approaches. *J Med Chem*, *56*(20), 7719-7726. https://doi.org/10.1021/jm400362b

Gillespie, M., Jassal, B., Stephan, R., Milacic, M., Rothfels, K., Senff-Ribeiro, A., Griss, J., Sevilla, C., Matthews, L., Gong, C., Deng, C., Varusai, T., Ragueneau, E., Haider, Y., May, B., Shamovsky, V., Weiser, J., Brunson, T., Sanati, N., . . . D'Eustachio, P. (2022). The reactome pathway knowledgebase 2022. *Nucleic Acids Res*, *50*(D1), D687-d692. https://doi.org/10.1093/nar/gkab1028

Goebel, H. H., Heipertz, R., Scholz, W., Iqbal, K., & Tellez-Nagel, I. (1978). Juvenile Huntington chorea: clinical, ultrastructural, and biochemical studies. *Neurology*, *28*(1), 23-31. https://doi.org/10.1212/wnl.28.1.23

Goodsell, D. S., Morris, G. M., & Olson, A. J. (1996). Automated docking of flexible ligands: applications of AutoDock. *J Mol Recognit*, *9*(1), 1-5. https://doi.org/10.1002/(sici)1099-1352(199601)9:1<1::aid-jmr241>3.0.co;2-6

Gossen, M., Freundlieb, S., Bender, G., Müller, G., Hillen, W., & Bujard, H. (1995). Transcriptional activation by tetracyclines in mammalian cells. *Science*, *268*(5218), 1766-1769. https://doi.org/10.1126/science.7792603

Gottlieb, S. (2001). Company played down drug's risks, report says. *Bmj*, *322*(7288), 696.

Grosdidier, A., Zoete, V., & Michielin, O. (2011). SwissDock, a protein-small molecule docking web service based on EADock DSS. *Nucleic Acids Res*, *39*(Web Server issue), W270-277. https://doi.org/10.1093/nar/gkr366

Gu, Z., Shi, C., Li, J., Han, Y., Sun, B., Zhang, W., Wu, J., Zhou, G., Ye, W., Li, J., Zhang, Z., & Zhou, R. (2022). Palbociclib-based high-throughput combination drug screening identifies synergistic therapeutic options in HPV-negative head and neck squamous cell carcinoma. *BMC Medicine*, *20*(1), 175. https://doi.org/10.1186/s12916-022-02373-6

Gupta, A., Gandhimathi, A., Sharma, P., & Jayaram, B. (2007). ParDOCK: an all atom energy based Monte Carlo docking protocol for protein-ligand complexes. *Protein Pept Lett*, *14*(7), 632-646. https://doi.org/10.2174/092986607781483831

Hachigian, L. J., Carmona, V., Fenster, R. J., Kulicke, R., Heilbut, A., Sittler, A., Pereira de Almeida, L., Mesirov, J. P., Gao, F., Kolaczyk, E. D., & Heiman, M. (2017). Control of Huntington's Disease-Associated Phenotypes by the Striatum-Enriched Transcription Factor Foxp2. *Cell Rep*, *21*(10), 2688-2695. https://doi.org/10.1016/j.celrep.2017.11.018

Halabi, S. F. (2019). The Drug Repurposing Ecosystem: Intellectual Property Incentives, Market Exclusivity, and the Future of "New" Medicines. In.

Hart, T. N., & Read, R. J. (1992). A multiple-start Monte Carlo docking method. *Proteins: Structure, Function, and Bioinformatics*, *13*(3), 206-222. https://doi.org/https://doi.org/10.1002/prot.340130304

Hayashida, N., Fujimoto, M., Tan, K., Prakasam, R., Shinkawa, T., Li, L., Ichikawa, H., Takii, R., & Nakai, A. (2010). Heat shock factor 1 ameliorates proteotoxicity in cooperation with the transcription factor NFAT. *The EMBO Journal*, *29*(20), 3459-3469. https://doi.org/https://doi.org/10.1038/emboj.2010.225

He, L., Kulesskiy, E., Saarela, J., Turunen, L., Wennerberg, K., Aittokallio, T., & Tang, J. (2018). Methods for High-throughput Drug Combination Screening and Synergy

Scoring. In L. von Stechow (Ed.), *Cancer Systems Biology: Methods and Protocols* (pp. 351-398). Springer New York. https://doi.org/10.1007/978-1-4939-7493-1_17
Heiser, V., Engemann, S., Bröcker, W., Dunkel, I., Boeddrich, A., Waelter, S., Nordhoff, E., Lurz, R., Schugardt, N., Rautenberg, S., Herhaus, C., Barnickel, G., Böttcher, H., Lehrach, H., & Wanker, E. E. (2002). Identification of benzothiazoles as potential polyglutamine aggregation inhibitors of Huntington's disease by using an automated filter retardation assay. *Proceedings of the National Academy of Sciences*, *99*(suppl_4), 16400-16406. https://doi.org/doi:10.1073/pnas.182426599
Hillen, W., & Berens, C. (1994). Mechanisms underlying expression of Tn10 encoded tetracycline resistance. *Annu Rev Microbiol*, *48*, 345-369. https://doi.org/10.1146/annurev.mi.48.100194.002021
Huey, R., Morris, G. M., & Forli, S. (2012). Using AutoDock 4 and AutoDock vina with AutoDockTools: a tutorial. *The Scripps Research Institute Molecular Graphics Laboratory*, *10550*(92037), 1000.
Hurwitz, B., Guzzi, N., Gola, A., Fiore, V. F., Sendoel, A., Nikolova, M., Barrows, D., Carroll, T. S., Pasolli, H. A., & Fuchs, E. (2022). The integrated stress response remodels the microtubule-organizing center to clear unfolded proteins following proteotoxic stress. *eLife*, *11*, e77780. https://doi.org/10.7554/eLife.77780
Hwang, J. Y., & Zukin, R. S. (2018). REST, a master transcriptional regulator in neurodegenerative disease. *Curr Opin Neurobiol*, *48*, 193-200. https://doi.org/10.1016/j.conb.2017.12.008
Iguchi, Y., Katsuno, M., Niwa, J., Takagi, S., Ishigaki, S., Ikenaka, K., Kawai, K., Watanabe, H., Yamanaka, K., Takahashi, R., Misawa, H., Sasaki, S., Tanaka, F., & Sobue, G. (2013). Loss of TDP-43 causes age-dependent progressive motor neuron degeneration. *Brain*, *136*(Pt 5), 1371-1382. https://doi.org/10.1093/brain/awt029
Inestrosa, N. C., Godoy, J. A., Quintanilla, R. A., Koenig, C. S., & Bronfman, M. (2005). Peroxisome proliferator-activated receptor gamma is expressed in hippocampal neurons and its activation prevents beta-amyloid neurodegeneration: role of Wnt signaling. *Exp Cell Res*, *304*(1), 91-104. https://doi.org/10.1016/j.yexcr.2004.09.032
Irwin, J. J., Tang, K. G., Young, J., Dandarchuluun, C., Wong, B. R., Khurelbaatar, M., Moroz, Y. S., Mayfield, J., & Sayle, R. A. (2020). ZINC20—A Free Ultralarge-Scale Chemical Database for Ligand Discovery. *Journal of Chemical Information and Modeling*, *60*(12), 6065-6073. https://doi.org/10.1021/acs.jcim.0c00675
Irwin, M. L., Varma, K., Alvarez-Reeves, M., Cadmus, L., Wiley, A., Chung, G. G., Dipietro, L., Mayne, S. T., & Yu, H. (2009). Randomized controlled trial of aerobic exercise on insulin and insulin-like growth factors in breast cancer survivors: the Yale Exercise and Survivorship study. *Cancer Epidemiol Biomarkers Prev*, *18*(1), 306-313. https://doi.org/10.1158/1055-9965.Epi-08-0531
Isgut, M., Rao, M., Yang, C., Subrahmanyam, V., Rida, P. C. G., & Aneja, R. (2018). Application of Combination High-Throughput Phenotypic Screening and Target Identification Methods for the Discovery of Natural Product-Based Combination Drugs. *Medicinal Research Reviews*, *38*(2), 504-524. https://doi.org/https://doi.org/10.1002/med.21444
Isgut, M., Rao, M., Yang, C., Subrahmanyam, V., Rida, P. C. G., & Aneja, R. (2018). Application of Combination High-Throughput Phenotypic Screening and Target Identification Methods for the Discovery of Natural Product-Based Combination Drugs. *Med Res Rev*, *38*(2), 504-524. https://doi.org/10.1002/med.21444
Jain, A. N. (2003). Surflex: fully automatic flexible molecular docking using a molecular similarity-based search engine. *J Med Chem*, *46*(4), 499-511. https://doi.org/10.1021/jm020406h
Jamwal, S., Blackburn, J. K., & Elsworth, J. D. (2021). PPARγ/PGC1α signaling as a potential therapeutic target for mitochondrial biogenesis in neurodegenerative

disorders. *Pharmacol Ther*, *219*, 107705. https://doi.org/10.1016/j.pharmthera.2020.107705

Janes, J., Young, M. E., Chen, E., Rogers, N. H., Burgstaller-Muehlbacher, S., Hughes, L. D., Love, M. S., Hull, M. V., Kuhen, K. L., Woods, A. K., Joseph, S. B., Petrassi, H. M., McNamara, C. W., Tremblay, M. S., Su, A. I., Schultz, P. G., & Chatterjee, A. K. (2018). The ReFRAME library as a comprehensive drug repurposing library and its application to the treatment of cryptosporidiosis. *Proceedings of the National Academy of Sciences*, *115*(42), 10750-10755. https://doi.org/doi:10.1073/pnas.1810137115

Jarada, T. N., Rokne, J. G., & Alhajj, R. (2020). A review of computational drug repositioning: strategies, approaches, opportunities, challenges, and directions. *Journal of Cheminformatics*, *12*(1), 46. https://doi.org/10.1186/s13321-020-00450-7

Jiang, F., & Kim, S. H. (1991). "Soft docking": matching of molecular surface cubes. *J Mol Biol*, *219*(1), 79-102. https://doi.org/10.1016/0022-2836(91)90859-5

Jin, J., Albertz, J., Guo, Z., Peng, Q., Rudow, G., Troncoso, J. C., Ross, C. A., & Duan, W. (2013). Neuroprotective effects of PPAR-γ agonist rosiglitazone in N171-82Q mouse model of Huntington's disease. *J Neurochem*, *125*(3), 410-419. https://doi.org/10.1111/jnc.12190

Johri, A., Chandra, A., & Flint Beal, M. (2013). PGC-1α, mitochondrial dysfunction, and Huntington's disease. *Free Radic Biol Med*, *62*, 37-46. https://doi.org/10.1016/j.freeradbiomed.2013.04.016

Johri, A., Chandra, A., & Flint Beal, M. (2013). PGC-1α, mitochondrial dysfunction, and Huntington's disease. *Free Radical Biology and Medicine*, *62*, 37-46. https://doi.org/https://doi.org/10.1016/j.freeradbiomed.2013.04.016

Jones, G., Willett, P., Glen, R. C., Leach, A. R., & Taylor, R. (1997). Development and validation of a genetic algorithm for flexible docking. *J Mol Biol*, *267*(3), 727-748. https://doi.org/10.1006/jmbi.1996.0897

Kabashi, E., Valdmanis, P. N., Dion, P., Spiegelman, D., McConkey, B. J., Vande Velde, C., Bouchard, J. P., Lacomblez, L., Pochigaeva, K., Salachas, F., Pradat, P. F., Camu, W., Meininger, V., Dupre, N., & Rouleau, G. A. (2008). TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. *Nat Genet*, *40*(5), 572-574. https://doi.org/10.1038/ng.132

Kaltenbach, L. S., Romero, E., Becklin, R. R., Chettier, R., Bell, R., Phansalkar, A., Strand, A., Torcassi, C., Savage, J., Hurlburt, A., Cha, G. H., Ukani, L., Chepanoske, C. L., Zhen, Y., Sahasrabudhe, S., Olson, J., Kurschner, C., Ellerby, L. M., Peltier, J. M., . . . Hughes, R. E. (2007). Huntingtin interacting proteins are genetic modifiers of neurodegeneration. *PLoS Genet*, *3*(5), e82. https://doi.org/10.1371/journal.pgen.0030082

Kanehisa, M., Furumichi, M., Sato, Y., Kawashima, M., & Ishiguro-Watanabe, M. (2022). KEGG for taxonomy-based analysis of pathways and genomes. *Nucleic Acids Research*, *51*(D1), D587-D592. https://doi.org/10.1093/nar/gkac963

Kang, J., Hsu, C.-H., Wu, Q., Liu, S., Coster, A. D., Posner, B. A., Altschuler, S. J., & Wu, L. F. (2016). Improving drug discovery with high-content phenotypic screens by systematic selection of reporter cell lines. *Nature Biotechnology*, *34*(1), 70-77. https://doi.org/10.1038/nbt.3419

Kasteri, J., Das, D., Zhong, X., Persaud, L., Francis, A., Muharam, H., & Sauane, M. (2018). Translation Control by p53. *Cancers (Basel)*, *10*(5). https://doi.org/10.3390/cancers10050133

Katsila, T., Spyroulias, G. A., Patrinos, G. P., & Matsoukas, M.-T. (2016). Computational approaches in target identification and drug discovery. *Computational and Structural Biotechnology Journal*, *14*, 177-184. https://doi.org/https://doi.org/10.1016/j.csbj.2016.04.004

Kearsley, S. K., Underwood, D. J., Sheridan, R. P., & Miller, M. D. (1994). Flexibases: a way to enhance the use of molecular docking methods. *J Comput Aided Mol Des*, *8*(5), 565-582. https://doi.org/10.1007/bf00123666

Khojasteh, H., Khanteymoori, A., & Olyaee, M. H. (2022). Comparing protein-protein interaction networks of SARS-CoV-2 and (H1N1) influenza using topological features. *Sci Rep*, *12*(1), 5867. https://doi.org/10.1038/s41598-022-08574-6

Kim, Y. M., Hussain, Z., Lee, Y. J., & Park, H. (2021). Altered Intestinal Permeability and Drug Repositioning in a Post-operative Ileus Guinea Pig Model. *J Neurogastroenterol Motil*, *27*(4), 639-649. https://doi.org/10.5056/jnm21018

Ko, Y. (2020). Computational Drug Repositioning: Current Progress and Challenges. *Applied Sciences*, *10*(15), 5076. https://www.mdpi.com/2076-3417/10/15/5076

Koehler, A. N. (2010). A complex task? Direct modulation of transcription factors with small molecules. *Current opinion in chemical biology*, *14*(3), 331-340.

Korb, O., Stützle, T., & Exner, T. E. (2009). Empirical scoring functions for advanced protein-ligand docking with PLANTS. *J Chem Inf Model*, *49*(1), 84-96. https://doi.org/10.1021/ci800298z

Korotkevich, G., Sukhov, V., Budin, N., Shpak, B., Artyomov, M. N., & Sergushichev, A. (2021). Fast gene set enrichment analysis. *bioRxiv*, 060012. https://doi.org/10.1101/060012

Krastev, D. B., Li, S., Sun, Y., Wicks, A. J., Hoslett, G., Weekes, D., Badder, L. M., Knight, E. G., Marlow, R., Pardo, M. C., Yu, L., Talele, T. T., Bartek, J., Choudhary, J. S., Pommier, Y., Pettitt, S. J., Tutt, A. N. J., Ramadan, K., & Lord, C. J. (2022). The ubiquitin-dependent ATPase p97 removes cytotoxic trapped PARP1 from chromatin. *Nat Cell Biol*, *24*(1), 62-73. https://doi.org/10.1038/s41556-021-00807-6

Kremer, B., Goldberg, P., Andrew, S. E., Theilmann, J., Telenius, H., Zeisler, J., Squitieri, F., Lin, B., Bassett, A., Almqvist, E., & et al. (1994). A worldwide study of the Huntington's disease mutation. The sensitivity and specificity of measuring CAG repeats. *N Engl J Med*, *330*(20), 1401-1406. https://doi.org/10.1056/nejm199405193302001

Krishnamurthy, N., Grimshaw, A. A., Axson, S. A., Choe, S. H., & Miller, J. E. (2022). Drug repurposing: a systematic review on root causes, barriers and facilitators. *BMC Health Services Research*, *22*(1), 970. https://doi.org/10.1186/s12913-022-08272-z

Kumar, S., & Kumar, S. (2019). Chapter 6 - Molecular Docking: A Structure-Based Approach for Drug Repurposing. In K. Roy (Ed.), *In Silico Drug Design* (pp. 161-189). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-816125-8.00006-7

Lee, H. (2014). Genetically engineered mouse models for drug development and preclinical trials. *Biomol Ther (Seoul)*, *22*(4), 267-274. https://doi.org/10.4062/biomolther.2014.074

Lee, J. M., Ramos, E. M., Lee, J. H., Gillis, T., Mysore, J. S., Hayden, M. R., Warby, S. C., Morrison, P., Nance, M., Ross, C. A., Margolis, R. L., Squitieri, F., Orobello, S., Di Donato, S., Gomez-Tortosa, E., Ayuso, C., Suchowersky, O., Trent, R. J., McCusker, E., . . . Gusella, J. F. (2012). CAG repeat expansion in Huntington disease determines age at onset in a fully dominant fashion. *Neurology*, *78*(10), 690-695. https://doi.org/10.1212/WNL.0b013e318249f683

Lemm, J. A., O'Boyle, D., 2nd, Liu, M., Nower, P. T., Colonno, R., Deshpande, M. S., Snyder, L. B., Martin, S. W., St Laurent, D. R., Serrano-Wu, M. H., Romine, J. L., Meanwell, N. A., & Gao, M. (2010). Identification of hepatitis C virus NS5A inhibitors. *J Virol*, *84*(1), 482-491. https://doi.org/10.1128/jvi.01360-09

Li, S. H., Schilling, G., Young, W. S., 3rd, Li, X. J., Margolis, R. L., Stine, O. C., Wagster, M. V., Abbott, M. H., Franz, M. L., Ranen, N. G., & et al. (1993). Huntington's

disease gene (IT15) is widely expressed in human and rat tissues. *Neuron*, *11*(5), 985-993. https://doi.org/10.1016/0896-6273(93)90127-d

Li, W., Xu, H., Xiao, T., Cong, L., Love, M. I., Zhang, F., Irizarry, R. A., Liu, J. S., Brown, M., & Liu, X. S. (2014). MAGeCK enables robust identification of essential genes from genome-scale CRISPR/Cas9 knockout screens. *Genome Biology*, *15*(12), 554. https://doi.org/10.1186/s13059-014-0554-4

Liberzon, A., Birger, C., Thorvaldsdóttir, H., Ghandi, M., Mesirov, J. P., & Tamayo, P. (2015). The Molecular Signatures Database (MSigDB) hallmark gene set collection. *Cell Syst*, *1*(6), 417-425. https://doi.org/10.1016/j.cels.2015.12.004

Liberzon, A., Birger, C., Thorvaldsdóttir, H., Ghandi, M., Mesirov, Jill P., & Tamayo, P. (2015). The Molecular Signatures Database Hallmark Gene Set Collection. *Cell Systems*, *1*(6), 417-425. https://doi.org/10.1016/j.cels.2015.12.004

Lieberman, A. P., Shakkottai, V. G., & Albin, R. L. (2019). Polyglutamine Repeats in Neurodegenerative Diseases. *Annu Rev Pathol*, *14*, 1-27. https://doi.org/10.1146/annurev-pathmechdis-012418-012857

Lin, G. L., Wilson, K. M., Ceribelli, M., Stanton, B. Z., Woo, P. J., Kreimer, S., Qin, E. Y., Zhang, X., Lennon, J., Nagaraja, S., Morris, P. J., Quezada, M., Gillespie, S. M., Duveau, D. Y., Michalowski, A. M., Shinn, P., Guha, R., Ferrer, M., Klumpp-Thomas, C., . . . Monje, M. (2019). Therapeutic strategies for diffuse midline glioma from high-throughput combination drug screening. *Science Translational Medicine*, *11*(519), eaaw0064. https://doi.org/doi:10.1126/scitranslmed.aaw0064

Lin, J., Handschin, C., & Spiegelman, B. M. (2005). Metabolic control through the PGC-1 family of transcription coactivators. *Cell Metab*, *1*(6), 361-370. https://doi.org/10.1016/j.cmet.2005.05.004

Lin, X., Li, X., & Lin, X. (2020). A Review on Applications of Computational Methods in Drug Screening and Design. *Molecules*, *25*(6). https://doi.org/10.3390/molecules25061375

Lindh, M. (2017). *Computational Modelling in Drug Discovery: Application of Structure-Based Drug Design, Conformal Prediction and Evaluation of Virtual Screening* Acta Universitatis Upsaliensis].

Lindner, S., & Krönke, J. (2016). The molecular mechanism of thalidomide analogs in hematologic malignancies. *J Mol Med (Berl)*, *94*(12), 1327-1334. https://doi.org/10.1007/s00109-016-1450-z

Lindström, M. S., Bartek, J., & Maya-Mendoza, A. (2022). p53 at the crossroad of DNA replication and ribosome biogenesis stress pathways. *Cell Death Differ*, *29*(5), 972-982. https://doi.org/10.1038/s41418-022-00999-w

Lintner, N. G., McClure, K. F., Petersen, D., Londregan, A. T., Piotrowski, D. W., Wei, L., Xiao, J., Bolt, M., Loria, P. M., Maguire, B., Geoghegan, K. F., Huang, A., Rolph, T., Liras, S., Doudna, J. A., Dullea, R. G., & Cate, J. H. (2017). Selective stalling of human translation through small-molecule engagement of the ribosome nascent chain. *PLoS Biol*, *15*(3), e2001882. https://doi.org/10.1371/journal.pbio.2001882

Liu, M., & Wang, S. (1999). MCDOCK: a Monte Carlo simulation approach to the molecular docking problem. *J Comput Aided Mol Des*, *13*(5), 435-451. https://doi.org/10.1023/a:1008005918983

Lu, G., Middleton, R. E., Sun, H., Naniong, M., Ott, C. J., Mitsiades, C. S., Wong, K. K., Bradner, J. E., & Kaelin, W. G., Jr. (2014). The myeloma drug lenalidomide promotes the cereblon-dependent destruction of Ikaros proteins. *Science*, *343*(6168), 305-309. https://doi.org/10.1126/science.1244917

Macarron, R., Banks, M. N., Bojanic, D., Burns, D. J., Cirovic, D. A., Garyantes, T., Green, D. V. S., Hertzberg, R. P., Janzen, W. P., Paslay, J. W., Schopfer, U., & Sittampalam, G. S. (2011). Impact of high-throughput screening in biomedical

research. *Nature Reviews Drug Discovery*, *10*(3), 188-195. https://doi.org/10.1038/nrd3368
MacRae, C. A., & Peterson, R. T. (2015). Zebrafish as tools for drug discovery. *Nature Reviews Drug Discovery*, *14*(10), 721-731. https://doi.org/10.1038/nrd4627
Maffrand, J.-P. (2012). The story of clopidogrel and its predecessor, ticlopidine: Could these major antiplatelet and antithrombotic drugs be discovered and developed today? *Comptes Rendus Chimie*, *15*(8), 737-743.
Mage, R. G., Esteves, P. J., & Rader, C. (2019). Rabbit models of human diseases for diagnostics and therapeutics development. *Developmental & Comparative Immunology*, *92*, 99-104. https://doi.org/https://doi.org/10.1016/j.dci.2018.10.003
Majera, D., Skrott, Z., Chroma, K., Merchut-Maya, J. M., Mistrik, M., & Bartek, J. (2020). Targeting the NPL4 Adaptor of p97/VCP Segregase by Disulfiram as an Emerging Cancer Vulnerability Evokes Replication Stress and DNA Damage while Silencing the ATR Pathway. *Cells*, *9*(2). https://doi.org/10.3390/cells9020469
Majmudar, C. Y., & Mapp, A. K. (2005). Chemical approaches to transcriptional regulation. *Current opinion in chemical biology*, *9*(5), 467-474.
Mangiarini, L., Sathasivam, K., Seller, M., Cozens, B., Harper, A., Hetherington, C., Lawton, M., Trottier, Y., Lehrach, H., Davies, S. W., & Bates, G. P. (1996). Exon 1 of the HD Gene with an Expanded CAG Repeat Is Sufficient to Cause a Progressive Neurological Phenotype in Transgenic Mice. *Cell*, *87*(3), 493-506. https://doi.org/https://doi.org/10.1016/S0092-8674(00)81369-0
Manzotti, G., Torricelli, F., Benedetta, D., Lococo, F., Sancisi, V., Rossi, G., Piana, S., & Ciarrocchi, A. (2019). An Epithelial-to-Mesenchymal Transcriptional Switch Triggers Evolution of Pulmonary Sarcomatoid Carcinoma (PSC) and Identifies Dasatinib as New Therapeutic Option. *Clin Cancer Res*, *25*(7), 2348-2360. https://doi.org/10.1158/1078-0432.CCR-18-2364
Martín-Aparicio, E., Avila, J., & Lucas, J. J. (2002). Nuclear localization of N-terminal mutant huntingtin is cell cycle dependent. *European Journal of Neuroscience*, *16*(2), 355-359. https://doi.org/https://doi.org/10.1046/j.1460-9568.2002.02075.x
Martinez, M. A. (2020). Clinical Trials of Repurposed Antivirals for SARS-CoV-2. *Antimicrob Agents Chemother*, *64*(9). https://doi.org/10.1128/aac.01101-20
Martinez, M. A. (2021). Lack of Effectiveness of Repurposed Drugs for COVID-19 Treatment [Opinion]. *Frontiers in Immunology*, *12*. https://doi.org/10.3389/fimmu.2021.635371
Martinez, M. A. (2022). Efficacy of repurposed antiviral drugs: Lessons from COVID-19. *Drug Discovery Today*, *27*(7), 1954-1960. https://doi.org/https://doi.org/10.1016/j.drudis.2022.02.012
Mayr, L. M., & Bojanic, D. (2009). Novel trends in high-throughput screening. *Current Opinion in Pharmacology*, *9*(5), 580-588. https://doi.org/https://doi.org/10.1016/j.coph.2009.08.004
Mayr, L. M., & Fuerst, P. (2008). The Future of High-Throughput Screening. *SLAS Discovery*, *13*(6), 443-448. https://doi.org/https://doi.org/10.1177/1087057108319644
McConoughey, S. J., Basso, M., Niatsetskaya, Z. V., Sleiman, S. F., Smirnova, N. A., Langley, B. C., Mahishi, L., Cooper, A. J., Antonyak, M. A., Cerione, R. A., Li, B., Starkov, A., Chaturvedi, R. K., Beal, M. F., Coppola, G., Geschwind, D. H., Ryu, H., Xia, L., Iismaa, S. E., . . . Ratan, R. R. (2010). Inhibition of transglutaminase 2 mitigates transcriptional dysregulation in models of Huntington disease. *EMBO Mol Med*, *2*(9), 349-370. https://doi.org/10.1002/emmm.201000084
McConoughey, S. J., Basso, M., Niatsetskaya, Z. V., Sleiman, S. F., Smirnova, N. A., Langley, B. C., Mahishi, L., Cooper, A. J. L., Antonyak, M. A., Cerione, R. A., Li, B., Starkov, A., Chaturvedi, R. K., Beal, M. F., Coppola, G., Geschwind, D. H.,

Ryu, H., Xia, L., Iismaa, S. E., . . . Ratan, R. R. (2010). Inhibition of transglutaminase 2 mitigates transcriptional dysregulation in models of Huntington disease. *EMBO Molecular Medicine*, *2*(9), 349-370. https://doi.org/https://doi.org/10.1002/emmm.201000084

McGann, M. (2012). FRED and HYBRID docking performance on standardized datasets. *J Comput Aided Mol Des*, *26*(8), 897-906. https://doi.org/10.1007/s10822-012-9584-8

McMartin, C., & Bohacek, R. S. (1997). QXP: powerful, rapid computer algorithms for structure-based drug design. *J Comput Aided Mol Des*, *11*(4), 333-344. https://doi.org/10.1023/a:1007907728892

Menden, M. P., Wang, D., Mason, M. J., Szalai, B., Bulusu, K. C., Guan, Y., Yu, T., Kang, J., Jeon, M., Wolfinger, R., Nguyen, T., Zaslavskiy, M., Abante, J., Abecassis, B. S., Aben, N., Aghamirzaie, D., Aittokallio, T., Akhtari, F. S., Al-lazikani, B., . . . AstraZeneca-Sanger Drug Combination, D. C. (2019). Community assessment to advance computational prediction of cancer drug combinations in a pharmacogenomic screen. *Nature Communications*, *10*(1), 2674. https://doi.org/10.1038/s41467-019-09799-2

Michalek, M., Salnikov, E. S., & Bechinger, B. (2013). Structure and topology of the huntingtin 1-17 membrane anchor by a combined solution and solid-state NMR approach. *Biophys J*, *105*(3), 699-710. https://doi.org/10.1016/j.bpj.2013.06.030

Middha, S. K., Usha, T., Sukhralia, S., Pareek, C., Yadav, R., Agnihotri, R., Tasneem, J., Goyal, A. K., & Babu, D. (2022). Chapter 23 - Prediction of drug–target interaction —a helping hand in drug repurposing. In A. Parihar, R. Khan, A. Kumar, A. K. Kaushik, & H. Gohel (Eds.), *Computational Approaches for Novel Therapeutic and Diagnostic Designing to Mitigate SARS-CoV-2 Infection* (pp. 519-536). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-323-91172-6.00006-6

Milano, M., Agapito, G., & Cannataro, M. (2022). Challenges and Limitations of Biological Network Analysis. *BioTech (Basel)*, *11*(3). https://doi.org/10.3390/biotech11030024

Millrine, D., & Kishimoto, T. (2017). A Brighter Side to Thalidomide: Its Potential Use in Immunological Disorders. *Trends Mol Med*, *23*(4), 348-361. https://doi.org/10.1016/j.molmed.2017.02.006

Milnerwood, A. J., & Raymond, L. A. (2010). Early synaptic pathophysiology in neurodegeneration: insights from Huntington's disease. *Trends Neurosci*, *33*(11), 513-523. https://doi.org/10.1016/j.tins.2010.08.002

Mithun, R., Shubham, J. K., & Anil, G. J. (2020). Drug Repurposing (DR): An Emerging Approach in Drug Discovery. In A. B. Farid (Ed.), *Drug Repurposing* (pp. Ch. 1). IntechOpen. https://doi.org/10.5772/intechopen.93193

Mochel, F., Durant, B., Meng, X., O'Callaghan, J., Yu, H., Brouillet, E., Wheeler, V. C., Humbert, S., Schiffmann, R., & Durr, A. (2012). Early alterations of brain cellular energy homeostasis in Huntington disease models. *J Biol Chem*, *287*(2), 1361-1370. https://doi.org/10.1074/jbc.M111.309849

Moffat, J. G., Rudolph, J., & Bailey, D. (2014). Phenotypic screening in cancer drug discovery - past, present and future. *Nat Rev Drug Discov*, *13*(8), 588-602. https://doi.org/10.1038/nrd4366

Moffat, J. G., Vincent, F., Lee, J. A., Eder, J., & Prunotto, M. (2017). Opportunities and challenges in phenotypic drug discovery: an industry perspective. *Nature Reviews Drug Discovery*, *16*(8), 531-543. https://doi.org/10.1038/nrd.2017.111

Moon, J. H., Hong, J. M., & Park, S. Y. (2021). The antidiabetic drug troglitazone protects against PrP (106-126)-induced neurotoxicity via the PPARγ-autophagy pathway in neuronal cells. *Mol Med Rep*, *23*(6). https://doi.org/10.3892/mmr.2021.12069

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with

selective receptor flexibility. *J Comput Chem*, *30*(16), 2785-2791. https://doi.org/10.1002/jcc.21256

Murphy, K. P. S. J., Carter, R. J., Lione, L. A., Mangiarini, L., Mahal, A., Bates, G. P., Dunnett, S. B., & Morton, A. J. (2000). Abnormal Synaptic Plasticity and Impaired Spatial Cognition in Mice Transgenic for Exon 1 of the Human Huntington's Disease Mutation. *The Journal of Neuroscience*, *20*(13), 5115-5123. https://doi.org/10.1523/jneurosci.20-13-05115.2000

Myers, R. H. (2004). Huntington's disease genetics. *NeuroRx*, *1*(2), 255-262. https://doi.org/10.1602/neurorx.1.2.255

Myers, R. H. (2004). Huntington's Disease Genetics. *NeuroRx*, *1*(2), 255-262. https://doi.org/https://doi.org/10.1602/neurorx.1.2.255

Nafshi, R., & Lezon, T. R. (2021). Predicting the Effects of Drug Combinations Using Probabilistic Matrix Factorization [Original Research]. *Frontiers in Bioinformatics*, *1*. https://doi.org/10.3389/fbinf.2021.708815

Nainu, F., Salim, E., As'ad, M. F., Chandran, D., Dhama, K., Rabaan, A. A., & Emran, T. B. (2023). Fruit fly for anticancer drug discovery and repurposing. *Ann Med Surg (Lond)*, *85*(2), 337-342. https://doi.org/10.1097/ms9.0000000000000222

Naryshkin, N. A., Weetall, M., Dakka, A., Narasimhan, J., Zhao, X., Feng, Z., Ling, K. K., Karp, G. M., Qi, H., Woll, M. G., Chen, G., Zhang, N., Gabbeta, V., Vazirani, P., Bhattacharyya, A., Furia, B., Risher, N., Sheedy, J., Kong, R., . . . Metzger, F. (2014). Motor neuron disease. SMN2 splicing modifiers improve motor function and longevity in mice with spinal muscular atrophy. *Science*, *345*(6197), 688-693. https://doi.org/10.1126/science.1250127

Nicodeme, E., Jeffrey, K. L., Schaefer, U., Beinke, S., Dewell, S., Chung, C. W., Chandwani, R., Marazzi, I., Wilson, P., Coste, H., White, J., Kirilovsky, J., Rice, C. M., Lora, J. M., Prinjha, R. K., Lee, K., & Tarakhovsky, A. (2010). Suppression of inflammation by a synthetic histone mimic. *Nature*, *468*(7327), 1119-1123. https://doi.org/10.1038/nature09589

Ochman, A. R., Lipinski, C. A., Handler, J. A., Reaume, A. G., & Saporito, M. S. (2012). The Lyn kinase activator MLR-1023 is a novel insulin receptor potentiator that elicits a rapid-onset and durable improvement in glucose homeostasis in animal models of type 2 diabetes. *J Pharmacol Exp Ther*, *342*(1), 23-32. https://doi.org/10.1124/jpet.112.192187

Ohlmeier, C., Saum, K.-U., Galetzka, W., Beier, D., & Gothe, H. (2019). Epidemiology and health care utilization of patients suffering from Huntington's disease in Germany: real world evidence based on German claims data. *BMC Neurology*, *19*(1), 318. https://doi.org/10.1186/s12883-019-1556-3

Oprea, T. I., Bauman, J. E., Bologa, C. G., Buranda, T., Chigaev, A., Edwards, B. S., Jarvik, J. W., Gresham, H. D., Haynes, M. K., Hjelle, B., Hromas, R., Hudson, L., Mackenzie, D. A., Muller, C. Y., Reed, J. C., Simons, P. C., Smagley, Y., Strouse, J., Surviladze, Z., . . . Sklar, L. A. (2011). Drug Repurposing from an Academic Perspective. *Drug Discov Today Ther Strateg*, *8*(3-4), 61-69. https://doi.org/10.1016/j.ddstr.2011.10.002

Ordway, J. M., Tallaksen-Greene, S., Gutekunst, C. A., Bernstein, E. M., Cearley, J. A., Wiener, H. W., Dure, L. S. t., Lindsey, R., Hersch, S. M., Jope, R. S., Albin, R. L., & Detloff, P. J. (1997). Ectopically expressed CAG repeats cause intranuclear inclusions and a progressive late onset neurological phenotype in the mouse. *Cell*, *91*(6), 753-763. https://doi.org/10.1016/s0092-8674(00)80464-x

Ortega, Z., Díaz-Hernández, M., & Lucas, J. J. (2007). Is the ubiquitin-proteasome system impaired in Huntington's disease? *Cellular and Molecular Life Sciences*, *64*(17), 2245-2257. https://doi.org/10.1007/s00018-007-7222-8

Ozdemir, E. S., Halakou, F., Nussinov, R., Gursoy, A., & Keskin, O. (2019). Methods for Discovering and Targeting Druggable Protein-Protein Interfaces and Their Application to Repurposing. In Q. Vanhaelen (Ed.), *Computational Methods for Drug Repurposing* (pp. 1-21). Springer New York. https://doi.org/10.1007/978-1-4939-8955-3_1

Paraskevopoulou, F., Parvizi, P., Senger, G., Tuncbag, N., Rosenmund, C., & Yildirim, F. (2021). Impaired inhibitory GABAergic synaptic transmission and transcription studied in single neurons by Patch-seq in Huntington's disease. *Proceedings of the National Academy of Sciences*, *118*(19), e2020293118. https://doi.org/doi:10.1073/pnas.2020293118

Park, K. (2019). A review of computational drug repurposing. *tcp*, *27*(2), 59-63. https://doi.org/10.12793/tcp.2019.27.2.59

Patwardhan, B., Vaidya, D. B. A., Chorghade, M., & Joshi, P. S. (2008). Reverse Pharmacology and Systems Approaches for Drug Discovery and Development. *Current Bioactive Compounds*, *4*(4), 201-212. https://doi.org/http://dx.doi.org/10.2174/157340708786847870

Paul, A. (2019). Translational and Reverse Pharmacology. In G. M. Raj & R. Raveendran (Eds.), *Introduction to Basics of Pharmacology and Toxicology: Volume 1: General and Molecular Pharmacology: Principles of Drug Action* (pp. 313-317). Springer Singapore. https://doi.org/10.1007/978-981-32-9779-1_22

Paul, D. S., & Gautham, N. (2016). MOLS 2.0: software package for peptide modeling and protein–ligand docking. *Journal of Molecular Modeling*, *22*(10), 239. https://doi.org/10.1007/s00894-016-3106-x

Paulson, H. L., Perez, M. K., Trottier, Y., Trojanowski, J. Q., Subramony, S. H., Das, S. S., Vig, P., Mandel, J. L., Fischbeck, K. H., & Pittman, R. N. (1997). Intranuclear inclusions of expanded polyglutamine protein in spinocerebellar ataxia type 3. *Neuron*, *19*(2), 333-344. https://doi.org/10.1016/s0896-6273(00)80943-5

Pei, J., Wang, Q., Liu, Z., Li, Q., Yang, K., & Lai, L. (2006). PSI-DOCK: towards highly efficient and accurate flexible ligand docking. *Proteins*, *62*(4), 934-946. https://doi.org/10.1002/prot.20790

Pemovska, T., Bigenzahn, J. W., & Superti-Furga, G. (2018). Recent advances in combinatorial drug screening and synergy scoring. *Current Opinion in Pharmacology*, *42*, 102-110. https://doi.org/https://doi.org/10.1016/j.coph.2018.07.008

Perales-Patón, J., Di Domenico, T., Fustero-Torre, C., Piñeiro-Yáñez, E., Carretero-Puche, C., Tejero, H., Valencia, A., Gómez-López, G., & Al-Shahrour, F. (2019). vulcanSpot: a tool to prioritize therapeutic vulnerabilities in cancer. *Bioinformatics*, *35*(22), 4846-4848. https://doi.org/10.1093/bioinformatics/btz465

Petersen, D. N., Hawkins, J., Ruangsiriluk, W., Stevens, K. A., Maguire, B. A., O'Connell, T. N., Rocke, B. N., Boehm, M., Ruggeri, R. B., Rolph, T., Hepworth, D., Loria, P. M., & Carpino, P. A. (2016). A Small-Molecule Anti-secretagogue of PCSK9 Targets the 80S Ribosome to Inhibit PCSK9 Protein Translation. *Cell Chem Biol*, *23*(11), 1362-1371. https://doi.org/10.1016/j.chembiol.2016.08.016

Piccioni, F., Roman, B. R., Fischbeck, K. H., & Taylor, J. P. (2004). A screen for drugs that protect against the cytotoxicity of polyglutamine-expanded androgen receptor. *Hum Mol Genet*, *13*(4), 437-446. https://doi.org/10.1093/hmg/ddh045

Plenge, R. M., Scolnick, E. M., & Altshuler, D. (2013). Validating therapeutic targets through human genetics. *Nat Rev Drug Discov*, *12*(8), 581-594. https://doi.org/10.1038/nrd4051

Popp, M., Stegemann, M., Metzendorf, M. I., Gould, S., Kranke, P., Meybohm, P., Skoetz, N., & Weibel, S. (2021). Ivermectin for preventing and treating COVID-19.

Cochrane Database Syst Rev, *7*(7), Cd015017. https://doi.org/10.1002/14651858.CD015017.pub2

Portz, B., Lee, B. L., & Shorter, J. (2021). FUS and TDP-43 Phases in Health and Disease. *Trends Biochem Sci*, *46*(7), 550-563. https://doi.org/10.1016/j.tibs.2020.12.005

Prati, F., Uliassi, E., & Bolognesi, M. L. (2014). Two diseases, one approach: multitarget drug discovery in Alzheimer's and neglected tropical diseases [10.1039/C4MD00069B]. *MedChemComm*, *5*(7), 853-861. https://doi.org/10.1039/C4MD00069B

Pushpakom, S., Iorio, F., Eyers, P. A., Escott, K. J., Hopper, S., Wells, A., Doig, A., Guilliams, T., Latimer, J., McNamee, C., Norris, A., Sanseau, P., Cavalla, D., & Pirmohamed, M. (2019). Drug repurposing: progress, challenges and recommendations. *Nature Reviews Drug Discovery*, *18*(1), 41-58. https://doi.org/10.1038/nrd.2018.168

Pushpakom, S., Iorio, F., Eyers, P. A., Escott, K. J., Hopper, S., Wells, A., Doig, A., Guilliams, T., Latimer, J., McNamee, C., Norris, A., Sanseau, P., Cavalla, D., & Pirmohamed, M. (2019). Drug repurposing: progress, challenges and recommendations. *Nat Rev Drug Discov*, *18*(1), 41-58. https://doi.org/10.1038/nrd.2018.168

Qiu, M., Zhou, B., Lo, F., Cook, S., Chyba, J., Quackenbush, D., Matzen, J., Li, Z., Mak, P. A., Chen, K., & Zhou, Y. (2020). A cell-level quality control workflow for high-throughput image analysis. *BMC Bioinformatics*, *21*(1), 280. https://doi.org/10.1186/s12859-020-03603-5

Radaeva, M., Ton, A.-T., Hsing, M., Ban, F., & Cherkasov, A. (2021). Drugging the 'undruggable'. Therapeutic targeting of protein–DNA interactions with the use of computer-aided drug discovery methods. *Drug Discovery Today*, *26*(11), 2660-2679. https://doi.org/https://doi.org/10.1016/j.drudis.2021.07.018

Rajkhowa, S., & Deka, R. C. (2016). Protein-Ligand Docking Methodologies and Its Application in Drug Discovery. In S. Dastmalchi, M. Hamzeh-Mivehroud, & B. Sokouti (Eds.), *Methods and Algorithms for Molecular Docking-Based Drug Design and Discovery* (pp. 196-219). IGI Global. https://doi.org/10.4018/978-1-5225-0115-2.ch008

Ran, F. A., Hsu, P. D., Wright, J., Agarwala, V., Scott, D. A., & Zhang, F. (2013). Genome engineering using the CRISPR-Cas9 system. *Nature Protocols*, *8*(11), 2281-2308. https://doi.org/10.1038/nprot.2013.143

Rarey, M., Kramer, B., Lengauer, T., & Klebe, G. (1996). A fast flexible docking method using an incremental construction algorithm. *J Mol Biol*, *261*(3), 470-489. https://doi.org/10.1006/jmbi.1996.0477

Rawlins, M. D., Wexler, N. S., Wexler, A. R., Tabrizi, S. J., Douglas, I., Evans, S. J., & Smeeth, L. (2016). The Prevalence of Huntington's Disease. *Neuroepidemiology*, *46*(2), 144-153. https://doi.org/10.1159/000443738

Roberds, S. L., Filippov, I., Alexandrov, V., Hanania, T., & Brunner, D. (2011). Rapid, computer vision-enabled murine screening system identifies neuropharmacological potential of two new mechanisms. *Front Neurosci*, *5*, 103. https://doi.org/10.3389/fnins.2011.00103

Romero, L., & Vela, J. M. (2014). Alternative Models in Drug Discovery and Development Part I: In Silico and In Vitro Models. In *In Vivo Models for Drug Discovery* (pp. 27-58). https://doi.org/https://doi.org/10.1002/9783527679348.ch02

Ross, C. A., Aylward, E. H., Wild, E. J., Langbehn, D. R., Long, J. D., Warner, J. H., Scahill, R. I., Leavitt, B. R., Stout, J. C., Paulsen, J. S., Reilmann, R., Unschuld, P. G., Wexler, A., Margolis, R. L., & Tabrizi, S. J. (2014). Huntington disease: natural history, biomarkers and prospects for therapeutics. *Nat Rev Neurol*, *10*(4), 204-216. https://doi.org/10.1038/nrneurol.2014.24

Ross, C. A., Aylward, E. H., Wild, E. J., Langbehn, D. R., Long, J. D., Warner, J. H., Scahill, R. I., Leavitt, B. R., Stout, J. C., Paulsen, J. S., Reilmann, R., Unschuld, P. G., Wexler, A., Margolis, R. L., & Tabrizi, S. J. (2014). Huntington disease: natural history, biomarkers and prospects for therapeutics. *Nature Reviews Neurology*, *10*(4), 204-216. https://doi.org/10.1038/nrneurol.2014.24

Ryu, H., Lee, J., Olofsson, B. A., Mwidau, A., Deodoglu, A., Escudero, M., Flemington, E., Azizkhan-Clifford, J., Ferrante, R. J., & Ratan, R. R. (2003). Histone deacetylase inhibitors prevent oxidative neuronal death independent of expanded polyglutamine repeats via an Sp1-dependent pathway. *Proceedings of the National Academy of Sciences*, *100*(7), 4281-4286. https://doi.org/doi:10.1073/pnas.0737363100

Safari-Alighiarloo, N., Taghizadeh, M., Rezaei-Tavirani, M., Goliaei, B., & Peyvandi, A. A. (2014). Protein-protein interaction networks (PPI) and complex diseases. *Gastroenterol Hepatol Bed Bench*, *7*(1), 17-31.

Salonee. (2020). *In vivo modelling within pharmacological research, its limitations and alternative methods of research*. Retrieved 2023-05-29 from https://ysjournal.com/ethics/in-vivo-modelling-within-pharmacological-research-its-limitations-and-alternative-methods-of-research/

Sanchez-Burgos, L., Gómez-López, G., Al-Shahrour, F., & Fernandez-Capetillo, O. (2022). An in silico analysis identifies drugs potentially modulating the cytokine storm triggered by SARS-CoV-2 infection. *Scientific Reports*, *12*(1), 1626. https://doi.org/10.1038/s41598-022-05597-x

Sandercock, A. M., Rust, S., Guillard, S., Sachsenmeier, K. F., Holoweckyj, N., Hay, C., Flynn, M., Huang, Q., Yan, K., Herpers, B., Price, L. S., Soden, J., Freeth, J., Jermutus, L., Hollingsworth, R., & Minter, R. (2015). Identification of anti-tumour biologics using primary tumour models, 3-D phenotypic screening and image-based multi-parametric profiling. *Molecular Cancer*, *14*(1), 147. https://doi.org/10.1186/s12943-015-0415-0

Saporito, M. S., Ochman, A. R., Lipinski, C. A., Handler, J. A., & Reaume, A. G. (2012). MLR-1023 is a potent and selective allosteric activator of Lyn kinase in vitro that improves glucose tolerance in vivo. *J Pharmacol Exp Ther*, *342*(1), 15-22. https://doi.org/10.1124/jpet.112.192096

Sarvagalla, S., Syed, S. B., & Coumar, M. S. (2019). Chapter 25 - An Overview of Computational Methods, Tools, Servers, and Databases for Drug Repurposing. In K. Roy (Ed.), *In Silico Drug Design* (pp. 743-780). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-816125-8.00025-0

Sauton, N., Lagorce, D., Villoutreix, B. O., & Miteva, M. A. (2008). MS-DOCK: accurate multiple conformation generator and rigid docking protocol for multi-step virtual ligand screening. *BMC Bioinformatics*, *9*, 184. https://doi.org/10.1186/1471-2105-9-184

Savi, P., Labouret, C., Delesque, N., Guette, F., Lupker, J., & Herbert, J. M. (2001). P2y(12), a new platelet ADP receptor, target of clopidogrel. *Biochem Biophys Res Commun*, *283*(2), 379-383. https://doi.org/10.1006/bbrc.2001.4816

Sawa, A., Wiegand, G. W., Cooper, J., Margolis, R. L., Sharp, A. H., Lawler, J. F., Jr., Greenamyre, J. T., Snyder, S. H., & Ross, C. A. (1999). Increased apoptosis of Huntington disease lymphoblasts associated with repeat length-dependent mitochondrial depolarization. *Nat Med*, *5*(10), 1194-1198. https://doi.org/10.1038/13518

Schilling, G., Becher, M. W., Sharp, A. H., Jinnah, H. A., Duan, K., Kotzuk, J. A., Slunt, H. H., Ratovitski, T., Cooper, J. K., Jenkins, N. A., Copeland, N. G., Price, D. L., Ross, C. A., & Borchelt, D. R. (1999). Intranuclear inclusions and neuritic aggregates in transgenic mice expressing a mutant N-terminal fragment of huntingtin. *Hum Mol Genet*, *8*(3), 397-407. https://doi.org/10.1093/hmg/8.3.397

Schmierer, B., Botla, S. K., Zhang, J., Turunen, M., Kivioja, T., & Taipale, J. (2017). CRISPR/Cas9 screening using unique molecular identifiers. *Mol Syst Biol*, *13*(10), 945. https://doi.org/10.15252/msb.20177834

Schneidman-Duhovny, D., Inbar, Y., Nussinov, R., & Wolfson, H. J. (2005). PatchDock and SymmDock: servers for rigid and symmetric docking. *Nucleic Acids Res*, *33*(Web Server issue), W363-367. https://doi.org/10.1093/nar/gki481

Shao, L., Campbell, U. C., Fang, Q. K., Powell, N. A., Campbell, J. E., Jones, P. G., Hanania, T., Alexandrov, V., Morganstern, I., & Sabath, E. (2016). In vivo phenotypic drug discovery: applying a behavioral assay to the discovery and optimization of novel antipsychotic agents. *MedChemComm*, *7*(6), 1093-1101.

Shimojo, M. (2008). Huntingtin regulates RE1-silencing transcription factor/neuron-restrictive silencer factor (REST/NRSF) nuclear trafficking indirectly through a complex with REST/NRSF-interacting LIM domain protein (RILP) and dynactin p150 Glued. *J Biol Chem*, *283*(50), 34880-34886. https://doi.org/10.1074/jbc.M804183200

Shin, W. H., Heo, L., Lee, J., Ko, J., Seok, C., & Lee, J. (2011). LigDockCSA: protein-ligand docking using conformational space annealing. *J Comput Chem*, *32*(15), 3226-3232. https://doi.org/10.1002/jcc.21905

Singh, T., Biswas, D., & Jayaram, B. (2011). AADS--an automated active site identification, docking, and scoring protocol for protein targets based on physicochemical descriptors. *J Chem Inf Model*, *51*(10), 2515-2527. https://doi.org/10.1021/ci200193z

Singh, V. K., Seed, T. M., & Olabisi, A. O. (2019). Drug discovery strategies for acute radiation syndrome. *Expert Opinion on Drug Discovery*, *14*(7), 701-715. https://doi.org/10.1080/17460441.2019.1604674

Sivaramakrishnan, M., McCarthy, K. D., Campagne, S., Huber, S., Meier, S., Augustin, A., Heckel, T., Meistermann, H., Hug, M. N., Birrer, P., Moursy, A., Khawaja, S., Schmucki, R., Berntenis, N., Giroud, N., Golling, S., Tzouros, M., Banfai, B., Duran-Pacheco, G., . . . Metzger, F. (2017). Binding to SMN2 pre-mRNA-protein complex elicits specificity for small molecule splicing modifiers. *Nat Commun*, *8*(1), 1476. https://doi.org/10.1038/s41467-017-01559-4

Skrott, Z., Mistrik, M., Andersen, K. K., Friis, S., Majera, D., Gursky, J., Ozdian, T., Bartkova, J., Turi, Z., Moudry, P., Kraus, M., Michalova, M., Vaclavkova, J., Dzubak, P., Vrobel, I., Pouckova, P., Sedlacek, J., Miklovicova, A., Kutt, A., . . . Bartek, J. (2017). Alcohol-abuse drug disulfiram targets cancer via p97 segregase adaptor NPL4. *Nature*, *552*(7684), 194-199. https://doi.org/10.1038/nature25016

Sobolev, V., Wade, R. C., Vriend, G., & Edelman, M. (1996). Molecular docking using surface complementarity. *Proteins: Structure, Function, and Bioinformatics*, *25*(1), 120-129. https://doi.org/https://doi.org/10.1002/(SICI)1097-0134(199605)25:1<120::AID-PROT10>3.0.CO;2-M

Sreedharan, J., Blair, I. P., Tripathi, V. B., Hu, X., Vance, C., Rogelj, B., Ackerley, S., Durnall, J. C., Williams, K. L., Buratti, E., Baralle, F., de Belleroche, J., Mitchell, J. D., Leigh, P. N., Al-Chalabi, A., Miller, C. C., Nicholson, G., & Shaw, C. E. (2008). TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science*, *319*(5870), 1668-1672. https://doi.org/10.1126/science.1154584

Steffan, J. S., Bodai, L., Pallos, J., Poelman, M., McCampbell, A., Apostol, B. L., Kazantsev, A., Schmidt, E., Zhu, Y. Z., Greenwald, M., Kurokawa, R., Housman, D. E., Jackson, G. R., Marsh, J. L., & Thompson, L. M. (2001). Histone deacetylase inhibitors arrest polyglutamine-dependent neurodegeneration in Drosophila. *Nature*, *413*(6857), 739-743. https://doi.org/10.1038/35099568

Sterling, T., & Irwin, J. J. (2015). ZINC 15--Ligand Discovery for Everyone. *J Chem Inf Model*, *55*(11), 2324-2337. https://doi.org/10.1021/acs.jcim.5b00559

Strong, T. V., Tagle, D. A., Valdes, J. M., Elmer, L. W., Boehm, K., Swaroop, M., Kaatz, K. W., Collins, F. S., & Albin, R. L. (1993). Widespread expression of the human and rat Huntington's disease gene in brain and nonneural tissues. *Nat Genet*, *5*(3), 259-265. https://doi.org/10.1038/ng1193-259

Subramanian, A., Narayan, R., Corsello, S. M., Peck, D. D., Natoli, T. E., Lu, X., Gould, J., Davis, J. F., Tubelli, A. A., Asiedu, J. K., Lahr, D. L., Hirschman, J. E., Liu, Z., Donahue, M., Julian, B., Khan, M., Wadden, D., Smith, I. C., Lam, D., . . . Golub, T. R. (2017). A Next Generation Connectivity Map: L1000 Platform and the First 1,000,000 Profiles. *Cell*, *171*(6), 1437-1452.e1417. https://doi.org/10.1016/j.cell.2017.10.049

Subramanian, A., Narayan, R., Corsello, S. M., Peck, D. D., Natoli, T. E., Lu, X., Gould, J., Davis, J. F., Tubelli, A. A., Asiedu, J. K., Lahr, D. L., Hirschman, J. E., Liu, Z., Donahue, M., Julian, B., Khan, M., Wadden, D., Smith, I. C., Lam, D., . . . Golub, T. R. (2017). A Next Generation Connectivity Map: L1000 Platform and the First 1,000,000 Profiles. *Cell*, *171*(6), 1437-1452.e1417. https://doi.org/10.1016/j.cell.2017.10.049

Subramanian, A., Tamayo, P., Mootha, V. K., Mukherjee, S., Ebert, B. L., Gillette, M. A., Paulovich, A., Pomeroy, S. L., Golub, T. R., Lander, E. S., & Mesirov, J. P. (2005). Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences*, *102*(43), 15545-15550. https://doi.org/doi:10.1073/pnas.0506580102

Swinney, D. C. (2013). Phenotypic vs. target-based drug discovery for first-in-class medicines. *Clin Pharmacol Ther*, *93*(4), 299-301. https://doi.org/10.1038/clpt.2012.236

Szklarczyk, D., Gable, A. L., Nastou, K. C., Lyon, D., Kirsch, R., Pyysalo, S., Doncheva, N. T., Legeay, M., Fang, T., Bork, P., Jensen, L. J., & von Mering, C. (2021). The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. *Nucleic Acids Res*, *49*(D1), D605-d612. https://doi.org/10.1093/nar/gkaa1074

Szymański, P., Markowicz, M., & Mikiciuk-Olasik, E. (2012). Adaptation of high-throughput screening in drug discovery-toxicological screening tests. *Int J Mol Sci*, *13*(1), 427-452. https://doi.org/10.3390/ijms13010427

Tabrizi, S. J., Flower, M. D., Ross, C. A., & Wild, E. J. (2020). Huntington disease: new insights into molecular pathogenesis and therapeutic opportunities. *Nat Rev Neurol*, *16*(10), 529-546. https://doi.org/10.1038/s41582-020-0389-4

Takenaka, T. (2001). Classical vs reverse pharmacology in drug discovery. *BJU Int*, *88 Suppl 2*, 7-10; discussion 49-50. https://doi.org/10.1111/j.1464-410x.2001.00112.x

Tanoli, Z., Seemab, U., Scherer, A., Wennerberg, K., Tang, J., & Vähä-Koskela, M. (2020). Exploration of databases and methods supporting drug repurposing: a comprehensive survey. *Briefings in Bioinformatics*, *22*(2), 1656-1678. https://doi.org/10.1093/bib/bbaa003

Thomsen, R., & Christensen, M. H. (2006). MolDock: a new technique for high-accuracy molecular docking. *J Med Chem*, *49*(11), 3315-3321. https://doi.org/10.1021/jm051197e

Tiu, G. C., Kerr, C. H., Forester, C. M., Krishnarao, P. S., Rosenblatt, H. D., Raj, N., Lantz, T. C., Zhulyn, O., Bowen, M. E., Shokat, L., Attardi, L. D., Ruggero, D., & Barna, M. (2021). A p53-dependent translational program directs tissue-selective phenotypes in a model of ribosomopathies. *Dev Cell*, *56*(14), 2089-2102.e2011. https://doi.org/10.1016/j.devcel.2021.06.013

Tiwari, A., & Singh, S. (2022). Chapter 13 - Computational approaches in drug designing. In D. B. Singh & R. K. Pathak (Eds.), *Bioinformatics* (pp. 207-217). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-323-89775-4.00010-9

Tokarski, J. S., Zupa-Fernandez, A., Tredup, J. A., Pike, K., Chang, C., Xie, D., Cheng, L., Pedicord, D., Muckelbauer, J., Johnson, S. R., Wu, S., Edavettal, S. C., Hong, Y., Witmer, M. R., Elkin, L. L., Blat, Y., Pitts, W. J., Weinstein, D. S., & Burke, J. R. (2015). Tyrosine Kinase 2-mediated Signal Transduction in T Lymphocytes Is Blocked by Pharmacological Stabilization of Its Pseudokinase Domain. *J Biol Chem*, *290*(17), 11061-11074. https://doi.org/10.1074/jbc.M114.619502

Torres, P. H. M., Sodero, A. C. R., Jofily, P., & Silva-Jr, F. P. (2019). Key Topics in Molecular Docking for Drug Design. *Int J Mol Sci*, *20*(18). https://doi.org/10.3390/ijms20184574

Trosset, J.-Y., & Scheraga, H. A. (1999). Prodock: Software package for protein modeling and docking. *Journal of Computational Chemistry*, *20*(4), 412-427. https://doi.org/https://doi.org/10.1002/(SICI)1096-987X(199903)20:4<412::AID-JCC3>3.0.CO;2-N

Troulé, K., López-Fernández, H., García-Martín, S., Reboiro-Jato, M., Carretero-Puche, C., Martorell-Marugán, J., Martín-Serrano, G., Carmona-Sáez, P., Glez-Peña, D., Al-Shahrour, F., & Gómez-López, G. (2021). DREIMT: a drug repositioning database and prioritization tool for immunomodulation. *Bioinformatics*, *37*(4), 578-579. https://doi.org/10.1093/bioinformatics/btaa727

Tseng, P.-J. (2022). *Repurposed drug combination via high-throughput screening and dual drug delivery system development for the treatment of retinoblastoma*.

Van Goor, F., Hadida, S., Grootenhuis, P. D. J., Burton, B., Stack, J. H., Straley, K. S., Decker, C. J., Miller, M., McCartney, J., Olson, E. R., Wine, J. J., Frizzell, R. A., Ashlock, M., & Negulescu, P. A. (2011). Correction of the F508del-CFTR protein processing defect in vitro by the investigational drug VX-809. *Proceedings of the National Academy of Sciences*, *108*(46), 18843-18848. https://doi.org/doi:10.1073/pnas.1105787108

Van Goor, F., Straley, K. S., Cao, D., González, J., Hadida, S., Hazlewood, A., Joubran, J., Knapp, T., Makings, L. R., Miller, M., Neuberger, T., Olson, E., Panchenko, V., Rader, J., Singh, A., Stack, J. H., Tung, R., Grootenhuis, P. D., & Negulescu, P. (2006). Rescue of DeltaF508-CFTR trafficking and gating in human cystic fibrosis airway primary cultures by small molecules. *Am J Physiol Lung Cell Mol Physiol*, *290*(6), L1117-1130. https://doi.org/10.1152/ajplung.00169.2005

van Hagen, M., Piebes, D. G. E., de Leeuw, W. C., Vuist, I. M., van Roon-Mom, W. M. C., Moerland, P. D., & Verschure, P. J. (2017). The dynamics of early-state transcriptional changes and aggregate formation in a Huntington's disease cell model. *BMC Genomics*, *18*(1), 373. https://doi.org/10.1186/s12864-017-3745-z

Venkatachalam, C. M., Jiang, X., Oldfield, T., & Waldman, M. (2003). LigandFit: a novel method for the shape-directed rapid docking of ligands to protein active sites. *J Mol Graph Model*, *21*(4), 289-307. https://doi.org/10.1016/s1093-3263(02)00164-x

Vezzoli, E., Caron, I., Talpo, F., Besusso, D., Conforti, P., Battaglia, E., Sogne, E., Falqui, A., Petricca, L., Verani, M., Martufi, P., Caricasole, A., Bresciani, A., Cecchetti, O., Rivetti di Val Cervo, P., Sancini, G., Riess, O., Nguyen, H., Seipold, L., . . . Zuccato, C. (2019). Inhibiting pathologically active ADAM10 rescues synaptic and cognitive decline in Huntington's disease. *J Clin Invest*, *129*(6), 2390-2403. https://doi.org/10.1172/jci120616

Vilar, S., Cozza, G., & Moro, S. (2008). Medicinal chemistry and the molecular operating environment (MOE): application of QSAR and molecular docking to drug discovery. *Curr Top Med Chem*, *8*(18), 1555-1572. https://doi.org/10.2174/156802608786786624

Vincent, F., Nueda, A., Lee, J., Schenone, M., Prunotto, M., & Mercola, M. (2022). Phenotypic drug discovery: recent successes, lessons learned and new directions.

Nat Rev Drug Discov, *21*(12), 899-914. https://doi.org/10.1038/s41573-022-00472-w

Vincent, F., Nueda, A., Lee, J., Schenone, M., Prunotto, M., & Mercola, M. (2022). Phenotypic drug discovery: recent successes, lessons learned and new directions. *Nature Reviews Drug Discovery*, *21*(12), 899-914. https://doi.org/10.1038/s41573-022-00472-w

Wang, J., Gines, S., MacDonald, M. E., & Gusella, J. F. (2005). Reversal of a full-length mutant huntingtin neuronal cell phenotype by chemical inhibitors of polyglutamine-mediated aggregation. *BMC Neurosci*, *6*, 1. https://doi.org/10.1186/1471-2202-6-1

Wang, R., Liu, L., Lai, L., & Tang, Y. (1998). SCORE: A New Empirical Method for Estimating the Binding Affinity of a Protein-Ligand Complex. *Molecular modeling annual*, *4*(12), 379-394. https://doi.org/10.1007/s008940050096

Wang, S., Wang, Z., Fang, L., Lv, Y., & Du, G. (2022). Advances of the target-based and phenotypic screenings and strategies in drug discovery. *International Journal of Drug Discovery and Pharmacology*, 2-2.

Wang, Y., Guo, X., Ye, K., Orth, M., & Gu, Z. (2021). Accelerated expansion of pathogenic mitochondrial DNA heteroplasmies in Huntington's disease. *Proceedings of the National Academy of Sciences*, *118*(30), e2014610118. https://doi.org/doi:10.1073/pnas.2014610118

Warchal, S. J., Dawson, J. C., Shepherd, E., Munro, A. F., Hughes, R. E., Makda, A., & Carragher, N. O. (2020). High content phenotypic screening identifies serotonin receptor modulators with selective activity upon breast cancer cell cycle and cytokine signaling pathways. *Bioorganic & Medicinal Chemistry*, *28*(1), 115209. https://doi.org/https://doi.org/10.1016/j.bmc.2019.115209

Waxman, E. A., & Lynch, D. R. (2005). N-methyl-D-aspartate receptor subtypes: multiple roles in excitotoxicity and neurological disease. *Neuroscientist*, *11*(1), 37-49. https://doi.org/10.1177/1073858404269012

Welch, W., Ruppert, J., & Jain, A. N. (1996). Hammerhead: fast, fully automated docking of flexible ligands to protein binding sites. *Chem Biol*, *3*(6), 449-462. https://doi.org/10.1016/s1074-5521(96)90093-9

Weydt, P., Pineda, V. V., Torrence, A. E., Libby, R. T., Satterfield, T. F., Lazarowski, E. R., Gilbert, M. L., Morton, G. J., Bammler, T. K., Strand, A. D., Cui, L., Beyer, R. P., Easley, C. N., Smith, A. C., Krainc, D., Luquet, S., Sweet, I. R., Schwartz, M. W., & La Spada, A. R. (2006). Thermoregulatory and metabolic defects in Huntington's disease transgenic mice implicate PGC-1alpha in Huntington's disease neurodegeneration. *Cell Metab*, *4*(5), 349-362. https://doi.org/10.1016/j.cmet.2006.10.004

White, J. K., Auerbach, W., Duyao, M. P., Vonsattel, J. P., Gusella, J. F., Joyner, A. L., & MacDonald, M. E. (1997). Huntingtin is required for neurogenesis and is not impaired by the Huntington's disease CAG expansion. *Nat Genet*, *17*(4), 404-410. https://doi.org/10.1038/ng1297-404

Wilkie, C. M., Barnes, J. R., Benson, C. M., Brymer, K. J., Nafar, F., & Parsons, M. P. (2020). Hippocampal Synaptic Dysfunction in a Mouse Model of Huntington Disease Is Not Alleviated by Ceftriaxone Treatment. *eNeuro*, *7*(3). https://doi.org/10.1523/eneuro.0440-19.2020

Williams, G. S., Mistry, B., Guillard, S., Ulrichsen, J. C., Sandercock, A. M., Wang, J., González-Muñoz, A., Parmentier, J., Black, C., Soden, J., Freeth, J., Jovanović, J., Leyland, R., Al-Lamki, R. S., Leishman, A. J., Rust, S. J., Stewart, R., Jermutus, L., Bradley, J. R., . . . Wilkinson, R. W. (2016). Phenotypic screening reveals TNFR2 as a promising target for cancer immunotherapy. *Oncotarget*, *7*(42), 68278-68291. https://doi.org/10.18632/oncotarget.11943

Williams, S. P., & McDermott, U. (2017). The Pursuit of Therapeutic Biomarkers with High-Throughput Cancer Cell Drug Screens. *Cell Chemical Biology*, *24*(9), 1066-1074. https://doi.org/https://doi.org/10.1016/j.chembiol.2017.06.011

Wu, F., Zhou, Y., Li, L., Shen, X., Chen, G., Wang, X., Liang, X., Tan, M., & Huang, Z. (2020). Computational Approaches in Preclinical Studies on Drug Discovery and Development. *Front Chem*, *8*, 726. https://doi.org/10.3389/fchem.2020.00726

Wu, L. S., Cheng, W. C., & Shen, C. K. (2012). Targeted depletion of TDP-43 expression in the spinal cord motor neurons leads to the development of amyotrophic lateral sclerosis-like phenotypes in mice. *J Biol Chem*, *287*(33), 27335-27344. https://doi.org/10.1074/jbc.M112.359000

Xu, J., Mao, C., Hou, Y., Luo, Y., Binder, J. L., Zhou, Y., Bekris, L. M., Shin, J., Hu, M., Wang, F., Eng, C., Oprea, T. I., Flanagan, M. E., Pieper, A. A., Cummings, J., Leverenz, J. B., & Cheng, F. (2022). Interpretable deep learning translation of GWAS and multi-omics findings to identify pathobiology and drug repurposing in Alzheimer's disease. *Cell Reports*, *41*(9), 111717. https://doi.org/https://doi.org/10.1016/j.celrep.2022.111717

Xu, Y. F., Gendron, T. F., Zhang, Y. J., Lin, W. L., D'Alton, S., Sheng, H., Casey, M. C., Tong, J., Knight, J., Yu, X., Rademakers, R., Boylan, K., Hutton, M., McGowan, E., Dickson, D. W., Lewis, J., & Petrucelli, L. (2010). Wild-type human TDP-43 expression causes TDP-43 phosphorylation, mitochondrial aggregation, motor deficits, and early mortality in transgenic mice. *J Neurosci*, *30*(32), 10851-10859. https://doi.org/10.1523/jneurosci.1630-10.2010

Xue, H., Li, J., Xie, H., & Wang, Y. (2018). Review of Drug Repositioning Approaches and Resources. *Int J Biol Sci*, *14*(10), 1232-1244. https://doi.org/10.7150/ijbs.24612

Yan, V. C., & Muller, F. L. (2021). Why Remdesivir Failed: Preclinical Assumptions Overestimate the Clinical Efficacy of Remdesivir for COVID-19 and Ebola. *Antimicrob Agents Chemother*, *65*(10), e0111721. https://doi.org/10.1128/aac.01117-21

Yang, J. M., & Chen, C. C. (2004). GEMDOCK: a generic evolutionary method for molecular docking. *Proteins*, *55*(2), 288-304. https://doi.org/10.1002/prot.20035

Yero, T., & Rey, J. A. (2008). Tetrabenazine (Xenazine), An FDA-Approved Treatment Option For Huntington's Disease-Related Chorea. *P t*, *33*(12), 690-694.

Zhang, Y., Leavitt, B. R., van Raamsdonk, J. M., Dragatsis, I., Goldowitz, D., MacDonald, M. E., Hayden, M. R., & Friedlander, R. M. (2006). Huntingtin inhibits caspase-3 activation. *The EMBO Journal*, *25*(24), 5896-5906. https://doi.org/https://doi.org/10.1038/sj.emboj.7601445

Zhang, Z., Zhou, L., Xie, N., Nice, E. C., Zhang, T., Cui, Y., & Huang, C. (2020). Overcoming cancer therapeutic bottleneck by drug repurposing. *Signal Transduction and Targeted Therapy*, *5*(1), 113. https://doi.org/10.1038/s41392-020-00213-8

Zhao, L., Zhu, Y., Wang, J., Wen, N., Wang, C., & Cheng, L. (2022). A brief review of protein–ligand interaction prediction. *Computational and Structural Biotechnology Journal*, *20*, 2831-2838. https://doi.org/https://doi.org/10.1016/j.csbj.2022.06.004

Zhao, Y., & Sanner, M. F. (2007). FLIPDock: Docking flexible ligands into flexible receptors. *Proteins: Structure, Function, and Bioinformatics*, *68*(3), 726-737. https://doi.org/https://doi.org/10.1002/prot.21423

Zheng, H., Hou, J., Zimmerman, M. D., Wlodawer, A., & Minor, W. (2014). The future of crystallography in drug discovery. *Expert Opinion on Drug Discovery*, *9*(2), 125-137. https://doi.org/10.1517/17460441.2014.872623

Zheng, W., Thorne, N., & McKew, J. C. (2013). Phenotypic screens as a renewed approach for drug discovery. *Drug Discov Today*, *18*(21-22), 1067-1073. https://doi.org/10.1016/j.drudis.2013.07.001

Zsoldos, Z., Reid, D., Simon, A., Sadjad, S. B., & Johnson, A. P. (2007). eHiTS: a new fast, exhaustive flexible ligand docking system. *J Mol Graph Model*, *26*(1), 198-212. https://doi.org/10.1016/j.jmgm.2006.06.002

Zuccato, C., Tartari, M., Crotti, A., Goffredo, D., Valenza, M., Conti, L., Cataudella, T., Leavitt, B. R., Hayden, M. R., Timmusk, T., Rigamonti, D., & Cattaneo, E. (2003). Huntingtin interacts with REST/NRSF to modulate the transcription of NRSE-controlled neuronal genes. *Nat Genet*, *35*(1), 76-83. https://doi.org/10.1038/ng1219