Chapter 1

General Introduction

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1.1 Drug Repurposing

Drug repurposing, also known as drug repositioning, reprofiling, or retasking, is a method for discovering new applications for FDA approved or experimental medications that go beyond the limits of the original medical prescription [1]. Due to the high costs and slow pace of new drug discovery, there is a surge in drug repurposing for neuroprotection in brain dysfunction such as neurodegenerative disorder (as Alzheimer's disease, AD) and neurotropic infectious disease (as Japanese Encephalitis, JE and COVID19). The current increase in life expectancy, combined with the absence of a viable medicine for the treatment of AD, which grows with life expectancy, places an unnecessary strain on our medical system and raises an important public health concern. Similarly, there are currently not very effective treatment options for the critical contagious disease COVID19 as well as for JE virus infection. Some drugs are currently in the pipeline to be repurposed using basic knowledge of disease biology and drug pharmacodynamics, as well as computational in-vivo imaging tools.

1.1.1 Advantages of drug repurposing

- The risk of failure is reduced; because the repurposed drugs have previously been shown to be adequately safe in preclinical models and humans, and, if early-stage studies have been completed, then it is less likely to fail in later efficacy trials, at least from a safety standpoint.
- Since most preclinical research, safety evaluation, and in some cases formulation development may have been already be accomplished, the time frame for drug development can be shortened.

- Less investment is often required, and this can vary substantially depending on the repurposing candidate's stage and development process. The regulatory and phase III expenses for a repurposed medicine may be similar to those for a new treatment in the same indication, but there may be significant savings in preclinical and phase I and II expenditures.
- Repurposed medications might identify new targets and pathways that can be explored further.

1.1.2 Historical evidence of drug repurposing with the year of approval

- Zidovudine, anti-cancer drug repurposed for HIV/AIDS (1987)
- Minoxidil, anti-hypertensive drug repurposedfor hair loss (1988)
- Sildenafil, anti-hypertensive drug for repurposed erectile dysfunction (1998)
- Thalidomide, sedative repurposed for erythema nodosum leprosum (leprosy) and multiple myeloma (1998 and 2006)
- Celecoxib, anti-inflammatory drug repurposed for familial adenomatous polyps (2000)
- Atomoxetine, Parkinson disease drug repurposed for attention deficit disorder (2002)
- Duloxetine, anti-depressant drug repurposed for stress urinary incontinence (SUI) (2004)
- Rituximab, anti-cancer drug repurposed for rheumatoid arthritis (2006)
- Raloxifene, osteoporosis drug repurposed for breast cancer (2007)
- Fingolimod, transplant rejection drug repurposed for multiple sclerosis (2010)
- Dapoxetine, anti-depressant drug repurposed for premature ejaculation (2012)
- Topiramate, anti-epileptic drug for obesity (2012)
- Ketoconazole, anti-fungal drug repurposed for cushing syndrome (2014)
- Aspirin, analgesic drug repurposed for colorectal cancer (2015)

1.1.3 Drug repurposing approaches

The drug repurposing strategy consists of three steps:

1. Preparation of hypothesis: identification of candidate drug for given indication
2. Efficacy of drug in preclinical model and identification of their mechanism
3. Evaluation of safety efficacy in phase II clinical trial given there is adequate safety data from phase I studies conducted as part of the original indication.

1.1.3.1 Computational Approaches

The majority of computational techniques are data-driven, and they entail the methodical examination of data of any kind (such as gene expression, chemical structure, genotyping or proteomic data or electronic health records (EHRs)), which can subsequently lead to the formulation of repurposing hypotheses (Figure 1). The unique characteristics of a drug known as “signature” can be compared against another drug, disease and clinical trait [2]. Three main types of data could be utilized to determine a drug's signature: (i) transcriptomic (RNA), proteomic, or metabolomics data; (ii) chemical structures; and (iii) adverse event patterns [1].

The following deliberation will cover the computational methodologies that are utilized in our study for drug repurposing from a neurological perspective.

1. Genetic Association

The differential gene expression signature which is the molecular signature of the drug, is derived by comparing the gene expression profile of a cell or tissue before and after treatment with the drug. This signature is then compared with a disease-associated expression profile obtained by differential expression analysis of disease versus healthy conditions. Drug efficacy can be inferred from the degree of the negative association between the drug's gene expression profile and that of the disease (i.e., the genes upregulated in the disease are downregulated with the medicine and vice versa). Regardless

of whether the chemical structures of two drugs are similar or distinct, the presence of a common transcriptome signature may suggest that they have a common therapeutic applicability [3]. Drug-disease and drug-drug similarity techniques rely significantly on publically available gene expression data since they both entail matching of transcriptome signatures. There are several important public repositories for transcriptomic data namely gene expression omnibus (http://www.ncbi.nlm.nih.gov/geo), array express (https://www.ebi.ac.uk/arrayexpress) and connectivity map (https://www.broadinstitute.org/connectivity-map-cmap).

2. Neuroimaging and Tractography

Computerized tomography (CT), positron emission tomography (PET), functional magnetic resonance imaging (fMRI), and diffusion tensor imaging (DTI) are all examples of neuroimaging methods used in neuroscience to examine brains in vivo and gain insight into how the nervous system functions [4]. The analysis of the human brain network (also called the human connectome) is a primary focus of this area of study since doing so will help researchers better comprehend the brain's structural and functional organization. Such an understanding is crucial to aid in the early diagnosis of neurological illnesses and to enhance treatments for these pathologies. Several neurological disease biomarkers have been identified thanks to connectome data analysis. Being complex, high-dimensional, and available in various spatial and temporal resolutions, the neuroimaging data need sophisticated analytic methods for accurate description. Analysing neuroimaging data after some therapeutic intervention can provide incisive information regarding the efficiency of the drugs in influencing the brain networks affected due to neurological disorders.

3. Pathway Mapping

It has been customary to use pathway-based or network-based methods to find drugs or therapeutic targets that may be suitable for repurposing. Network analysis involves the construction of drug or disease networks based on gene expression patterns, disease pathophysiology, and interactions between proteins in order to improve the discovery of repurposing drugs. Network analysis is used in several of the signature-matching research we have earlier discussed [5]. Moreover, various drug target databases such as DrugBank, Drug Gene Interaction Database, Therapeutic Target Database and PharmGKB are available to identify possibilities of drug reprofiling.

4. Molecular Docking

The preferred orientation of a ligand (drug) within a protein cavity (target receptor) can be determined by a structure-based computational strategy known as molecular docking [6]. Multiple drugs could be evaluated against a single receptor target (conventional docking: one target and multiple ligands) if that target is known to be involved in a disease. Inverse docking, in which many targets are docked to a single ligand, may be used to search drug libraries for new interactions that could be explored for repurposing.

5. Molecular Dynamics Simulation

Molecular dynamics (MD) is a computer simulation method for analysing the physical movements of atoms and molecules.MD simulation mimics the changes in the structures of biological molecules over a given period of time, giving us atomic insights about the change in structure [7]. It provides valuable information regarding the stability of the drug-receptor complex.

6. Retrospective Clinical Analysis

Electronic Health Records, post-marketing surveillance data, and clinical trial data can all be used to collect retrospective clinical data. EHRs contain massive amounts of

structured and unstructured data on patient outcomes. However, EHRs also contain considerable amounts of unstructured information, such as clinical descriptions of patient symptoms and signs (which are important in defining disease phenotype) and imaging data, in addition to the more structured diagnostic and pathophysiological data, such as laboratory test results and drug prescribing data.

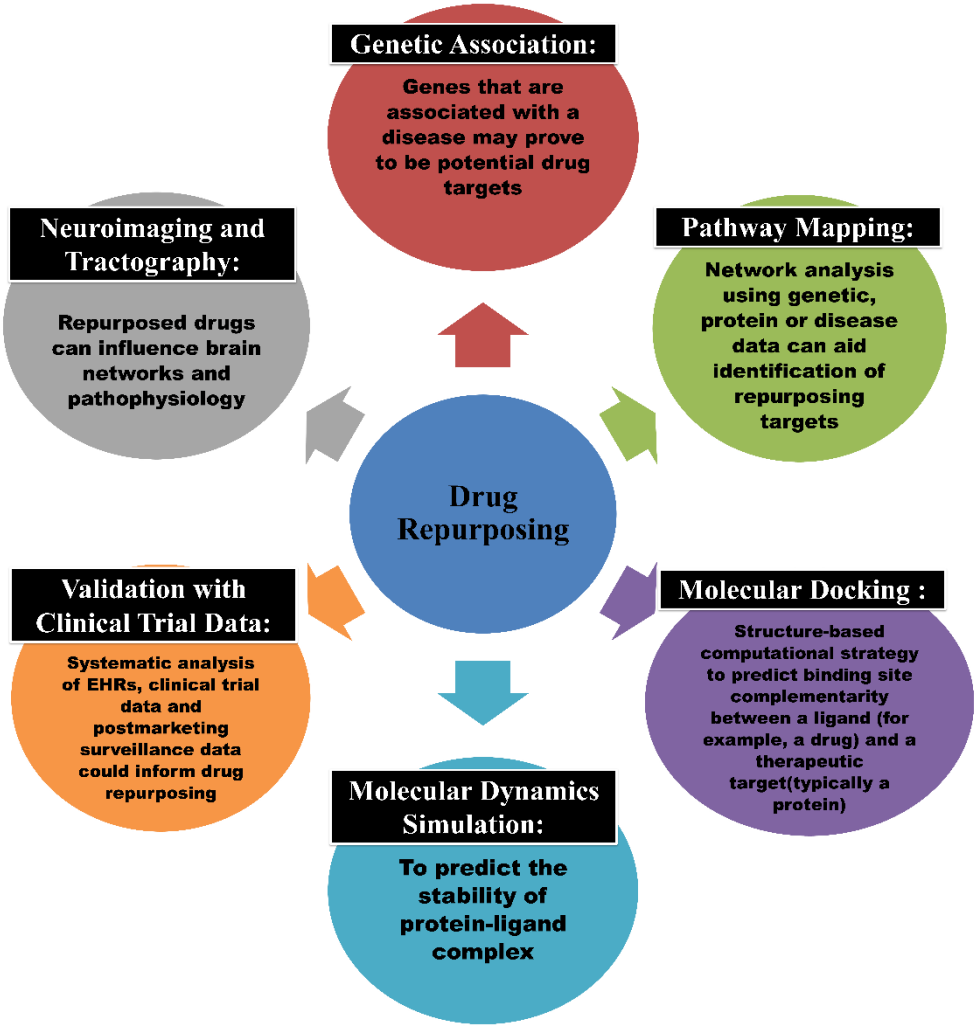

Figure 1: *Computational approaches used in drug repurposing: Different computational methods can be used alone or together to analyze different types of large-scale data in a structured way to get valuable insights for repurposing theories.*

1.2 Drug Repurposing in Alzheimer's disease

1.2.1 Alzheimer's disease statistics

The looming epidemic of age-induced neurodegenerative diseases globally, affecting 50 million people are mild cognitive impairment (MCI) and Alzheimer's disease (AD), which could triple by 2050. According to current statistics provided by WHO, dementia is the fifth leading cause of mortality costing $818 billion worldwide[8]. People of 85 years of age had a 30% prevalence of AD, and incidence rates increased from 0.5% per year for ages 65-75, similarly 6-8% per year for ages 85 and over. In the case of familial AD, the onset is from 50 years onwards, which comprises 5-10% of cases [9].

1.2.2 Alzheimer's disease pathophysiology

AD is the most common cause of dementia, accounting for 60 to 80 percent of cases. It is attributed to the accumulation of misfolded proteins, which include soluble and insoluble amyloid-β (Aβ42) plaques, tau tangles, and transactive response DNA binding protein 43 (TDP-43). Despite enormous investments in neurological research, the precise molecular basis of AD development remains unknown. Senile plaques and neurofibrillary tangles (NFTs) are hallmarks of Alzheimer's disease. Pathological effects on cell and organelle function have been linked to amyloid-β, a key component of senile plaques.

Current research has identified four genes that show promise as candidates for causing autosomal dominant or familial early onset Alzheimer's disease (FAD). Apolipoprotein E (ApoE), presenilin 1 (PS1), presenilin 2 (PS2), and amyloid precursor protein (APP) are the four genes involved. Increased synthesis of Aβ peptides, and in particular the more amyloidogenic form Aβ 42, may result from any mutation in either the APP or PS proteins. It has been hypothesized that Aβ overfills neurons with calcium ions by forming Ca^{2+} permeable pores, binding to, and modulating a wide variety of synaptic proteins, such as NMDAR, mGluR5, and VGCC. Thus, in AD, neuronal death, autophagy

impairments, mitochondrial abnormalities, poor neurotransmission, decreased synaptic plasticity, and neurodegeneration all result from cellular Ca^{2+} disturbances. Vulnerability to ER stress is caused by a mutation in PS1 that is associated with FAD (Figure S1: KEGG pathway-map05010).

Moreover, AD is propelled by a complicated pathological cascade that includes an imbalance between amyloid-peptide synthesis and clearance. The following are the important reasons for reduction of amyloid clearance:

- Increased aggregation
- Inadequate breakdown
- An unbalanced transport across the blood-brain barrier
- Ineffective peripheral elimination of the peptide

1.2.3 Metabolism of amyloid-β

According to prior reports, the primary factor contributing to this build-up in elderly people is a decrease in the kidney's and liver's ability to remove Aβ [10]. The molecular weight of Aβ is 4 to 5 kd. Therefore more than 60% of peptides are metabolized by the liver, and the rest is excreted in the urine [11]. In elder patients, systemic clearance of Aβ across the liver is hampered, causing accumulation in the brain. Hepato-biliary excretion of Aβ shows a probable role for enterohepatic circulation (EHC) of Aβ metabolite from intestine to liver in triggering pathological Aβ accumulation in liver [12].

1.2.4 Cholesterol and amyloid-β

Aβ-peptide, Aβ precursor protein (APP), apolipoprotein E (apoE), and high cholesterol levels have all been connected to the pathogenesis of AD. Elevated cholesterol levels promote the expression of APP and apoE in human NT2 neuron progenitor cells. A cholesterol-rich environment also promotes APP processing, which results in the synthesis of Aβ and Aβ peptide fragments [13].

1.2.5 Hepatobilliary-Enterohepatic circulation of cholesterol and therapeutic intervention

Cholesterol is removed via production of bile acids (BAs). BAs are synthesized from cholesterol in the liver. Enterohepatic physiological circulation of BAs is very efficient and plays acrucial role in cholesterol homeostasis. For cholesterol elimination, combination therapy with two drugs, ursodiol and ezetimibe, has been reported to respectively increase hepato-biliary excretion of cholesterol into intestine, and to reduce cholesterol reabsorption from intestine [14].

The mechanism behind this phenomenon is increasing hepatic protein ABCG5 ABCG8 (G5G8) expression by ursodiol, promoting hepato-biliary cholesterol excretion. The protein G5G8 forms a sterol transporter, which prevents accumulation of dietary sterol in tissue, and mutation of these receptors can cause atherosclerosis. On the other hand, the second drug, ezetimibe prevents cholesterol reabsorption from intestine to blood by blocking the transmembrane protein Niemann-Pick C1-Like1 (NPC1L1), localized at apical membrane of enterocytes and canalicular membrane of hepatocytes [15].

1.2.6 Hepatobilliary-Enterohepatic circulation of amyloid-β and therapeutic intervention

Based upon a similar approach, we have identified some repurposed drugs which can promote hepatic Aβ clearance into intestine, and decrease Aβ reabsorption from intestine, and we enable these two complimentary processes by enhancing the expression of appropriate genes.

1.3 Drug Repurposing in Neurotropic Viral Infection

1.3.1 SARS-COV2 and JE statistics

Central Nervous System (CNS) infection due to neurorespiratory viruses is prevalent in Southeast Asia, including India. India suffers from diseases caused by neurotropic

viruses, such as seasonal Japanese Encephalitis virus (JE), measles virus (MV), herpes virus, human immunodeficiency virus (HIV), and this scenario was prevalent before the era of severe acute respiratory syndrome coronavirus 2 (SARS-COV2). Subsequently, the World Health Organization (WHO) reported about 760,360,956 confirmed cases of SARS-COV2, including 6,873,477 deaths globally. SARS-COV2 is another highly contagious infectious disease after Severe Acute Respiratory Syndrome Coronavirus (SARS-COV) and the Middle East Respiratory Syndrome Coronavirus (MERS-CoV). Respiratory distress is the most common characteristic symptom of COVID-19 (renamed by WHO).

On the other hand, Japanese Encephalitis Virus transmission is prevalent in 24 countries in the WHO South-East Asia and Western Pacific areas, putting the health of more than 3 billion people at risk. The yearly incidence of the clinical disease varies per endemic country, ranging from 1 to >10 per 100,000 population or even greater during outbreaks. According to a literature study, over 68,000 clinical cases of JEV occur worldwide each year, with 13600 to 20400 fatalities.

Children are the most vulnerable to JEV. Most adults in endemic nations have developed natural immunity after childhood infection. However, people of any age may be afflicted. In India, where JEV is widespread in 24 states, Uttar Pradesh accounted for more than 75% of recent cases. The seasonal pattern has evolved throughout time, moving the disease's epidemic peak forward by one month [16].

1.3.2 SARS-COV2 and JE causing CNS infection

Several neurological signs like anosmia (loss of sense of smell) and ageusia (loss of sense of taste) are also reported in numerous countries as secondary manifestations of SARS-COV2 [17]. A cross-sectional study conducted by Printza et al. reported that, out of 182 patients, 38% reported gustatory and 41% olfactory impairment [18]. Furthermore, COVID-19 contributes to neurological complications, such as seizures, stroke,

encephalopathy, and even total paralysis [19]. To contrast, the hallmarks of JEV during viral infection of the CNS include reactive gliosis, an uncontrollable inflammatory response, and neuronal cell death [20].

1.3.3 Pathophysiology of SARS-COV2 and JE

It may be underscored that SARS-COV 1 and 2 have structural similarities, and they have a common binding site angiotensin converting enzyme 2 (ACE2) [21]. Current studies have revealed excessive expression of ACE2 receptors in alveolar epithelia cells, mucosa of oral cavity, the intestine, the kidney, and the heart. Recent investigations have also shown that maximum ACE2 receptors are expressed in tongue rather than buccal or gingival oral cavities, indicating vulnerability of oral mucosa toward COVID-19 infection [22].

Multiple studies on both human and animal models delineate that the central nervous system is a crucial target of SARS-COV [23], and the virus can enter the brain primarily via the olfactory lobe, with rapid spread inside the brain [24]. The angiotensin-converting enzyme 2 (ACE2) in neurons is the principal binding site for the COVID-19 virus. It was reported that, after intranasal administration of SARS-COV in transgenic mice that express hACE2, the virus spreads rapidly from airway epithelia to the brain, followed by the infiltration of macrophages and lymphocyte, thereby causing upregulation of cytokines and chemokines in both the lung and the brain [25].

In comparison, after being bitten by a mosquito carrying JEV, a person will develop primary asymptomatic viremia and infection of the dermal cells (dendritic cells, fibroblasts, endothelial cells, and pericytes) [26]. Secondary symptomatic viremia develops when the virus spreads throughout the body through the hematogenous pathway and the efferent lymphatic system, infecting vital organs such as the heart, liver, spleen, muscle, and brain. Ly6Chi monocytes, which originate from macrophage/dendritic cell precursors and exhibit

high levels of CCR2, are the primary cells in which JEV replicates in the periphery; these monocytes are capable of migrating to the central nervous system and contributing to the inflammatory response there [27].

1.3.4 Biological secondary metabolites as a therapeutic approach to viral infection

In earlier studies, the therapeutic potential of secondary metabolites of microorganisms, as minocycline was reported in treating several neurological viral diseases like Human Immunodeficiency Virus (HIV), Japanese Encephalitis (J.E.), and Simian Immunodeficiency Virus (SIV) [28, 29]. The tetracycline class of antibiotics (Minocycline, Doxycycline,Evracycline, Tigecycline) is highly lipophilic [30], and so may easily penetrate the lipophilic outer membrane of the SARS-COV2 virus and inhibit the viral RNA replication. These broad-spectrum secondary metabolites also exhibit anti-inflammatory and antiapoptotic activities [31].

The minocycline class of antibiotics modifies the functioning of the immune response [32] and exerts neuroprotective actions [33], which can promote its efficacy in treating COVID19. Moreover, Cephalosporins are also combined with antibiotics to treat viral diseases like influenza [34] and JE [29]. Furthermore, in Lyme disease, Cephalosporin is found to be efficacious as doxycycline [35].

However, antibiotics may possess some drawbacks, such as antibiotic resistance and side effects like fever, nausea, allergic reactions, and diarrhoea due to disruption in the normal balance of intestinal flora [36]. Globally, the prevalence of resistance with the tetracycline class of drugs is 8.7 and 24.3% for methicillin-resistant Staphylococcus aureus (MRSA) and Streptococcus pneumonia, respectively [37].

Therefore, a surge of interest of researchers in botanical secondary metabolites e.g. plant-based phytochemicals (as a possible alternative to antibiotics) is taking place to address these issues. Moreover, several reports suggested the noteworthy beneficial role of

phytochemicals in virus-mediated neuroinflammation. In the following chapters, the thesis reports the investigations performed on drug repurposing as a therapeutic approach to neuroprotection, with reference to AD and CNS viral infection as SARS-COV2 and JE.