Drug repositioning in Alzheimer's disease

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1. ABSTRACT

Drug repositioning offers an innovative approach to drug discovery with great potential in the field of Alzheimer's Disease and dementia therapeutics. Investigation of licensed compounds enables processing through the drug discovery pipeline in a rapid and cost-effective manner. A growing body of evidence supports the translation of priority compounds to be taken forward to clinical trials, based on established and proposed mechanisms of action. A number of drugs have already entered clinical trial following repositioning, and novel technologies have been created to enable high-throughput screening. This review discusses the novel approaches that build on transcriptional signature profiling to support repositioning in AD, and the novel candidate drugs that are emerging from this exciting new technique.

2. INTRODUCTION

Dementia is a worldwide public health issue, currently affecting 35 million people, with numbers expected to reach 115 million by 2050 (1). The condition has a devastating impact on individuals and their carers, in addition to exerting enormous financial burden on healthcare around the world. In 2010 dementia was estimated to incur a global cost of $604 billion (1). The impact of dementia is due not only to its prevalence but due to the complexity of the treatment and care needs of individuals with the condition, from diagnosis through to late-stage dementia. Despite this there are few treatments available, none of which confer any benefit to the underlying disease states. The four available treatments are licensed only for Alzheimer's Disease (AD), and provide symptomatic relief for an average of six months (2). Whilst this provides a valuable treatment option there is an urgent need to develop new, effective treatments to target disease pathologies and enable people to live well with dementia. In order to achieve this, a more comprehensive understanding of the disease pathologies are required, to enable more effective identification of potential treatment candidates.

The most common and best characterised cause of dementia is Alzheimer's Disease (AD), which accounts for over 60% of cases. The pathological hallmarks of AD are the development of neuritic plaques and neurofibrillary tangles (NFT). These structures, composed of β-amyloid (Aβ) and hyperphosphorylated tau proteins, are linked to loss of neuronal function and synaptic activity. The resulting characteristic progressive loss of brain volume is a diagnostic criterion for AD, which reflects the gradual symptomatic loss of cognition and function. The precise mechanism underlying AD pathology is not fully understood and represents some controversy in the field (3). Traditionally the amyloid cascade hypothesis, in which toxic Aβ peptides are thought to provide a catalyst for NFT formation and subsequent neurodegeneration, has been accepted as the primary mechanism in AD. However, the evidence for this is conflicting and the body of available literature indicates a more complex, multifactorial process involving inflammation, mitochondrial function and protective neuronal functions (4).

A number of critical factors have contributed to the failure of AD treatments in clinical trials in the last decade. Scrutiny of the literature highlights a trend of over-interpretation of earlier phase clinical trials and *in vitro* data which led to investment in large scale trials despite unsound preliminary data. This was the case for candidate treatments such as tarenflurbil (5, 6), dimebon (7) and semegestat (8) which have subsequently failed to show efficacy in phase III trials. A key factor is also the lack of targeted recruitment of trial participants, resulting in a vastly heterogenous cohort which likely confounds data

and prevents a clear measure of treatment efficacy. This is particularly the case in cohorts of people over 80 due to the prevalence of various micro- and macro-vascular pathologies and other comorbidities in these individuals. Importantly, cohorts have largely included individuals with moderate AD. Emerging evidence now suggests that the advanced nature of pathology in these individuals is likely to prevent a meaningful disease-modification effect due to the extensive neurological damage that has already occurred. Evaluation of novel treatments in early AD, or even pre-clinical subjects may provide a more promising and reliable measure of efficacy in individuals where disease-modification is more likely to impact on symptoms as well as pathology. Recent work supports the feasibility of using early diagnostic criteria to recruit a defined early AD cohort through the combination of validated neuropsychological testing with biomarker changes (9).

There is also a severe lack in volume of trials in the field, with the number of trials falling far short of the critical mass required for successful novel drug development. Only 21 clinical trials in AD are currently registered on National Clinical Trial (NCT) and International Standard Randomised Controlled Trial Number (ISRCTN) databases, compared with over 1700 cancer treatment trials (10). In particular there is a consistent reluctance by the pharmaceutical industry to invest in AD drug development, leading to a dearth of trials. A critical factor in improving the landscape of clinical trials in AD and in galvanizing investment is the need to improve identification of targets and candidate treatments through better understanding of AD pathology and mechanisms. The vast majority of previous postulated treatments have focused on amyloid pathology, and this lack of breadth in treatment targets is widely considered to have been a driving force in the failure of these treatments at clinical trial (3). As evidence of alternative targets emerges, there are signs of increased interest in drug development with novel mechanistic targets such as noradrenergic and histaminergic systems, and immunotherapeutic approaches, being targeted by industry-funded studies (11). However, there remains an enormous lack of capacity and under-investment in the field, and a need for alternative approaches to drug development and discovery outside of the pharmaceutical industry.

3. DRUG REPOSITIONING IN ALZHEIMER'S DISEASE

Drug repositioning represents an important route to drug discovery in AD. It offers the opportunity to combine evidence-based identification of candidate treatments with high-throughput screening to provide a novel, efficient drug discovery pipeline. The process of repositioning involves the development of existing, licensed treatments that are currently in use for other conditions that have the potential to confer benefit in a new indication (12). The approach offers a rapid and cost-effective route from laboratory to clinic due to the existing safety and tolerability data that is available for these candidate drugs (13). The success of repositioning has been demonstrated in a number of clinical areas including psychosis, cancer and irritable bowel syndrome, as well as critical health areas such as obesity and smoking cessation (14). Repositioning methodology for AD is now an emerging avenue for research and a number of candidate treatments are already in phase II trials as a result. Trials that are currently underway in the field include a a phase III trial of the calcium channel blocker nivaldipine for people with sub-cortical ischaemic vascular dementia and phase II trials of the antibiotic minocycline, the Angiotensin Receptor Blocker losartan and two diabetes therapeutics, exendin and liraglutide in people with AD (13).

The methodology employed for drug repositioning has evolved in parallel with evidence of its success as an approach. The simplest route is to identify compounds with established mechanisms of action to the repurposed within the same function, for example the successful use of sildenafil for erectile dysfunction (14). Innovation in repositioning led to a more sophisticated approach involving the identification of novel targets for proposed mechanisms. This allows for a broader scope of target identification and increased novelty in the repositioning approach. This process has successfully led to the elucidation of the antithrombotic properties of aspirin, and also to the NMDA glutamate receptor antagonist activity of amantadine for use in Parkinson's Disease (15). These approaches were used in combination with a comprehensive systematic review of epidemiological, pre-clinical and clinical data alongside Delphi consensus with an expert panel to inform a recently published study which identified a number of novel AD candidate treatments, many of which are now entering clinical trial (16). Priority candidates with sufficient supporting evidence included antihypertensives, antibiotics, retinoid therapy and current treatments for diabetes (16). There is now a need to enhance the productivity and scope of repositioning in AD through combined methodology with high-throughput screening.

4. TRANSCRIPTIONAL SIGNATURE ANALYSIS FOR REPOSITIONING IN AD

There is a growing body of work that is identifying the genomic factors associated with development and progression of AD, as well as increasingly well-defined spectra of genetic pathways associated with different stages of the disease. Analysis of transcriptional signatures for disease is therefore an exciting new approach to target identification which could be directly applied to drug discovery in AD. The concept

of linking transcriptional changes with drug mechanisms was originally developed in yeast systems (17). The methodology has been extended in the connectivity map (CMAP) project, which made available a database of transcriptional profiles associated with 1,300 drug-like compounds in four different human cancer cell lines (18). In addition to this resource illuminating the transcriptional landscape of a large collection of drugs it enabled the systematic search for drugs with profiles anti-correlating with transcriptional signatures corresponding to diseases states. Here, drugs tending to reverse the transcriptional changes associated with disease are hypothesized to be potential therapeutics. This approach has been key in repositioning rapamycin as a drug for glucocorticoid resistant lymphoblastic leukaemia (19). The potential behind the CMAP approach is that it essentially considers the whole transcriptional landscape rather than a protein interaction hub or established pathway.

A compelling observation in the original CMAP study was the high degree of agreement between independently derived signatures and CMAP signatures sharing the same biological targets. This motivated the searchable platform-independent expression database (SPIED), which essentially applied the CMAP methodology to publicly available transcriptional data (20, 21). This platform allows for the comparison of disease state transcriptional profiles from independent sources and therefore can serve as a first pass validation of putative diseases signatures. In this context SPIED is populated with a variety of AD associated profiles derived from clinically-defined post-mortem human brain material and from various animal models of AD. SPIED has revealed conserved patterns of gene expression associated with neurodegenerative disease. In particular, AD associated gene expression changes were shown to be consistent across multiple independent studies and a core set of highly regulated genes showed a conspicuous anti-correlation with a set of drugs with established neuroprotective activity (20).This work enables combination of these expression sets into a robust AD signature that can be queried against CMAP for possible AD drug candidates.

Candidate compounds emerging from this analysis included licensed drugs such as the cholinesterase inhibitors galanthamine and tactine. Tacrine, the initial licensed cholinesterase inhibitor is now rarely used because of tolerability issues, but galanthamine is currently licensed and approved for the treatment of mild to moderate AD in most countries. Additional outputs include natural compounds with established neuroprotective properties such as the flavonides crysin, apigenin and luteolin (20). Other significant anti-correlating drugs were two kinase inhibitors H7 and GW8510, the alkaloid harmine, the dopamine reuptake inhibitor nomifensine and the acetylcholine receptor agonist carbachol. The initial

correlational analysis has also highlighted a number of classes of compound identified by the recent systematic review and Delphi consensus as key candidates drugs based upon pre-clinical and epidemiological evidence, including the tetracycline antibiotic metacycline and angiotensin converting enzyme (ACE) inhibitors (16). The search also identified several drugs that have already been developed an evaluated in clinical trials with some promise, including rosiglitazone and clioquinol (22, 23). The identification of overlapping candidates provides important candidate validation for this methodology, which will be further developed with new *in vitro* and *in vivo* testing of novel candidates.

5. FUTURE OUTLOOK FOR REPOSITIONING IN AD

Drug repositioning offers an exciting and potentially impactful route to drug development in AD. The appetite for this approach has increased in recent years, leading to larger-scale investment in high- and medium-throughput laboratory approaches. Combinations of transcriptomics and microarray techniques with clinical data and well-defined *in vitro* and *in vivo* models are increasingly becoming established as effective methods for identifying large numbers of potential treatment candidates. In addition, large libraries of drugs are now available, providing a source of candidates with existing safety data. Global gene expression offers a high content quantitative methodology to compare biological states, enabling transcriptional analysis to drive forward drug discovery. The output of the CMAP and SPIED analyses to date highlights the potential of disease-associated transcriptomes as a basis for drug repositioning in AD. A number of promising compounds identified through drug repositioning methods have already entered preliminary or large-scale clinical trials (16). Transcriptional analysis has further strengthened the rationale for investigating these compounds, which include nivaldipine (phase III trial) and losartan, minocycline, acicretin and two GLP-1 analogues liraglutide and exendin (phase II trials).

Studies are now underway to extend this work into pull-through of novel candidates to clinical trials. It will be imperative that this work continues to be informed by the most robust clinical data and transcriptomic methods, ensuring that the approach is both accurate and relevant to the complexities of AD in the clinic.

6. CONCLUSIONS

Drug repositioning is a promising and rapid approach to treatment development for AD, raising the potential for new, more effective disease-modifying treatments to be brought to clinic within a fraction of the time required for novel drug discovery approaches. The use of high-throughput genomic-based techniques such as the SPIED allows for rapid screening of large libraries of

Table 1. Advantages and disadvantages to CMAP for drug repositioning in AD

Advantages	Disadvantages
Time to clinic: 5-8 years v 15-20 years	Transcriptome approaches not yet delivered any successful therapies for CNS disorders
Tolerability: most compounds licensed with known tolerability profiles	Unclear how transcriptome changes map directly onto the disease biology for Alzheimer's disease
Does not depend upon specific mechanistic target	
Likely to identify novel target pathways for further drug development	
Proof of concept systematic review/Delphi consensus identified viable candidates for repositioning as AD treatments	
Drug repositioning has been successful approach to drug discovery in other areas, including CNS disorders (Parkinson's disease, psychiatry)	
CMAP successfully identified new treatments for cancer, and proof of concept study identified viable candidates as AD therapies	
Cost: £5-10 m to the clinic vs $1 billion, making it viable for academia and charities to contribute	

candidate compounds with which to populate the pipeline for clinical trials in the field. A number of priority candidates have been identified through this approach, emphasizing the importance of further investment in repositioning in this field.

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