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Worming Our Way to Alzheimer's Disease Drug Discovery

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“You may delay, but time will not.” Truer words were never said. For patients diagnosed with Alzheimer's disease (AD), this represents a cruel truth. Most AD patients are left with a few good years before neurodegeneration strips them away of their daily competence and, ultimately, their identity. Since its discovery more than 100 years ago, significant advancements have been made in understanding the pathology underlying AD. However, in terms of therapeutic strategies, we are no closer to curing it than we were decades ago. Even today, only five drugs are approved by the Food and Drug Administration (FDA) for the treatment of AD. These drugs treat AD only symptomatically and do not target the underlying neurodegenerative pathology. Currently approved therapeutics are successful in slowing the progression of AD but not in reversing or preventing the symptoms of AD.

Although numerous promising novel drugs remain in the pipeline, the time taken to establish safety profiles, approve these novel drug candidates, and get them out into the market runs nearly a decade. Unfortunately, time is not a luxury that patients with AD have. A promising approach is to revisit and screen FDA-approved drugs for a different disease. A very good example of such a candidate drug is bexarotene. Bexarotene has been approved by the FDA for treatment of cutaneous T-cell lymphoma. However recent work by Cramer *et al.* showed that bexarotene is quite effective in ameliorating symptoms of AD in a rodent model (1). Studies further characterizing the mechanism of action of bexarotene in AD are underway. But, because this drug is already FDA approved, its safety and toxicity profiles are already known. Repurposing of such drugs allows us to bypass cost and time hurdles in bringing the drug to the market.

Today at least 5 million people in the United States alone are diagnosed with AD. This number is projected to explode to 16 million by the year 2050, as the aging population continues to increase (2). This warrants a hard look at current drug discovery paradigms as well as therapeutic targets for AD. Most research groups in search of a cure for AD have mostly focused on the amyloid beta hypothesis (3). However, an equal if not more critical suspect in AD as well as other neurodegenerative disorders is aggregation of the microtubule-associated protein tau. Tauopathies and their hallmark neurofibrillary tangles, are found in a wide variety of neurodegenerative diseases such as frontotemporal dementia with Parkinsonism, Pick's disease, etc. (4). Yet they are relatively understudied as a target

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for therapeutic intervention in neurodegenerative disorders. One reason for this is the lack of robust, physiologically relevant in vivo models of tauopathies.

Current drug discovery and testing paradigms involve either simplistic in vitro assays or whole-animal rodent models. Although useful, both approaches have significant drawbacks. Until now, most drug-screening assays for tauopathies have focused on identifying compounds that prevent aggregation of tau fibrils, usually through fluorescence measurements. Although rapid, these studies have the limitation of inducing artificial tau aggregation and hence are not representative of the multiplicity of tau aggregate species in vivo cell-free screening assays. Such assays also do not take into account the cascade of effects triggered by protein aggregation or disaggregation. Therefore, information about drug action is often incomplete. Drugs discovered as hits in such paradigms often may not retain their usefulness when evaluated in whole-animal models. Screening of drugs in rodent models is also suboptimal because it is neither cost nor time effective. In rodent models, tau pathology develops relatively slowly over a period of several months to 2 years. Therefore, such models are not suitable for rapid robust screening of investigational drugs.

What is needed is a high throughput screening model that combines the speed of cell-based screening with the benefits of an in vivo holistic system that recapitulates the salient features of AD and other neurodegenerative disorders. Such a system should be genetically tractable so that individual targets can be systematically studied either by way of knockdown or genetic deletion. It should possess a simplistic nervous system that bears reasonable similarity to the human system so that disease-relevant pathologies can be well characterized. It should allow for multiple easily measurable endpoints that are qualitative as well as quantitative. To minimize time and labor associated with screening, a model system that allows for automated handling and measurements is also desirable. Finally, but most importantly, it should be both cost and time effective.

In this issue, McCormick *et al.* (5) present a promising strategy using the tiny nematode *Caenorhabditis (C.) elegans* as a particularly useful model for high-throughput drug screens related to tauopathies. Many attributes of *C. elegans* make it a suitable model for studying human disease including direct orthologs for more than two thirds of human genes, facile genetics to generate transgenic models, or knockdown genes by ribonucleic acid interference in a single week, and a fully characterized nervous system in which the morphologic and functional integrity of identified neurons can be probed. Moreover, the short life span, high reproductive capacity, small size, and transparent body add to its desirability as a whole organism screening model. Numerous groups have already capitalized on this model system to screen chemical compounds against protein targets relevant to mammalian conditions [e.g., (6-8)]. Most of these systems rely on imaging systems to monitor endpoints of behavioral function such as swimming or survival of nematodes. In addition to these, with the use of fluorescent reporters, it is also possible to automate imaging or sorting devices that rely on fluorescence measurements. Finally, populations of *C. elegans* can be cultured in solid or liquid mediums to facilitate incorporation of automated liquid sorters that essentially treat worms as cells in flow cytometry for ultrahigh throughput assay designs. Such whole-organism screening also takes into account any ADMET (absorption, distribution, metabolism, excretion, and toxicity) concerns from the outset rather than

address it at a later stage of drug development because *C. elegans* shares toxicologic profile as humans (9).

In their paper, McCormick *et al.* (5) exploited the simple *C. elegans* model system to recapitulate features of tauopathies and screen a library of 1000 FDA-approved compounds (Figure 1). The use of an unbiased approach allowed McCormick *et al.* to isolate drug compounds solely on the basis-improved phenotypic endpoints. Such an approach has the benefits of discovering drug targets that have previously thought to be unrelated to the disease pathology (10).

Based on their findings, the authors show that the typical antipsychotic, azaperone, improved the survival, rescued tau pathology-induced immotility and prevented neurodegeneration in a worm model of tauopathy. First, using a behavioral screen alone, 16 candidates of a library of 1120 compounds were shown to be effective in rescuing tauopathy-induced functional defects. Of these 16, only two compounds azaperone and isoniazid were shown to reduce levels of insoluble tau, suggesting that these drugs were able to improve pathologic features because of a primary effect on insoluble tau. In addition to azaperone, other drugs belonging to the class of antipsychotics, such as flupenthixol, perphenazine, and zotepine, also improved the phenotypic features of tauopathy in worms.

To confirm that the beneficial effects of azaperone on tauopathy in nematode would translate to improvement in a mammalian model of tauopathy, different concentrations of the antipsychotic drugs were further tested for reduction of insoluble tau in a human embryonic kidney cell line overexpressing human 1N4R tau. Azaperone, flupenthixol, and zotepine were all found to cause significant reductions in levels of insoluble tau. Finally, taking advantage of the genetic tractability of *C. elegans* model system, the authors provided proof that dopaminergic D2 receptors may be responsible for the increase in insoluble tau in tauopathies. This finding was corroborated using small interfering ribonucleic acid knockdown studies in the human embryonic kidney/tau cell line as well.

The main finding of this study is that the D2 receptor antagonists such as azaperone reduce levels of insoluble tau and prevent the pathological features associated with tauopathies. This study is an exemplary instance of how a simple *C. elegans* model system may be used to rapidly screen drugs for diseases and evaluate mechanism of action. In addition, it also illustrates how repositioned drugs may be beneficial in highlighting a novel pathway to target diseases. It is important to acknowledge that D2 receptor antagonists are associated with numerous side effects, the most important one being induction of Parkinsonian symptoms. The authors themselves indicate that at higher doses, these drugs may have secondary effects promoting neurofibrillary tangles. Because FDA-approved drugs have established safety and toxicity profiles, one can draw from this information to predict and circumvent such problems. In this particular case, one can envision possible solutions in the form of dosing strategies or combination therapies. Lower doses of typical antipsychotics or combinations of two or more medications in optimal doses may be useful. Repurposing a drug with known side effects may certainly seem less than ideal; however, patients with Alzheimer's disease face a far more debilitating prospect. In the absence of newer, better

medications, multifaceted approaches, such as this one demonstrated by McCormick *et al.*, are demanded to tackle the crisis in untreatable neurodegenerative diseases.

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Figure 1.

Summary of tauopathy drug-screening paradigm with worms. Step 1. In the primary drug screen by McCormick *et al.* (5), a *C. elegans* model of tauopathy was exposed to different drugs in a multiwell plate format for 1 week. More than 1000 drugs were assessed in different batches with untreated controls. Most drugs had no effects on tauopathy-induced immobility (e.g., Drug A), whereas a few drugs rescued the defect (e.g., Drug B). Step 2. The dose responses were profiled for promising compounds within the second week. Step 3. An *in vitro* tauopathy model was used to test whether promising compounds altered

biochemical endpoints such as levels of soluble and insoluble tau protein in human cells.
Step 4. These promising compounds may be further subjected to in vivo testing in classical rodent models of tauopathy.

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