

# Phenotypic Screening Strategies for Neurodegenerative Diseases: A Pathway to Discover Novel Drug Candidates and Potential Disease Targets or Mechanisms

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**Abstract:** Target-directed drug design, although a conceptually rational approach, is only one strategy for drug discovery. In the case of neurodegenerative diseases where molecular targets and disease mechanisms are unknown, even when specific genes are known to trigger the disease, phenotypic screening offers another approach. This review describes the establishment of phenotypic screening assays using primary neurons subjected to a disease-relevant pathophysiological stress and measuring the most important functional outcome, survival. Although a challenge both to screening teams to reproducibly produce the cells and chemists to interpret structure-activity relationships, such systems have historically identified or produced effective drugs. The primary screening assay is only the start; once hits are validated, they must be characterized using traditional target-directed or mechanism-based secondary assays to establish their selectivity, lack of side-effect liability, and eventually be shown to produce the desired effects in a preclinical animal model of the disease. These compounds then provide valuable pharmacological tools to identify neurodegenerative disease targets and mechanisms, whether or not they have all the properties required of a drug candidate.

**Keywords:** Motor neuron disease, Huntington's disease, cell-based assays, drug discovery, chemical genetics, phenotypic screening.

## INTRODUCTION

The obstacles to rational drug discovery for neurodegenerative diseases are multiple. Most cases of diseases like Alzheimer's disease (AD), Parkinson's disease (PD) or amyotrophic lateral sclerosis (ALS) are sporadic and the cause is totally unknown. Familial forms of these diseases account for 5-15% of the cases. However, as the genes identified code for ubiquitously expressed proteins, how or why they trigger the selective neuronal dysfunction and death characteristic of each disease remains a mystery. It is also unclear whether familial forms of the disease have mechanisms in common with sporadic cases of the disease. Huntington's disease (HD) is an exception: it is a dominant genetic disease resulting from inheritance of a single "mutant" copy of the huntingtin gene containing an extended CAG repeat in exon 1, which results in an enlarged polyglutamine domain near the N-terminus of the protein. Although huntingtin is expressed in every cell in the body and plays an essential role in early development [1, 2], mutant huntingtin exerts a selective toxicity for striatal medium spiny neurons that are important for controlling motor behavior. However, even in the case of HD and familial forms of AD, PD and ALS, symptoms of neurodegeneration usually only become apparent in adults over the age of 35, implying that the genetic disease mechanisms accelerate age-related neuronal dysfunction and death or vice versa. With age, the neuronal cell environment and multiple genetic factors contribute to the onset and progression of neurodegeneration, and it is clear that these diseases are all complex and multifactorial. Finally, since substantial neuronal cell loss occurs before symptoms become sufficiently apparent to seek medical advice, it will be a challenge to demonstrate the ability of a drug to slow or reverse neurodegeneration at this stage. Developing neuroprotective drugs to treat neurodegenerative diseases is further confounded by the need to test them as "add-on" to drugs already approved for their treatment, which mainly provide symptomatic improvement by targeting neurotransmitter dysfunction; this obscures detection of a possible slowing of disease progression. With all these unknowns and challenges it seems a Quixotic quest

to devise simple, target-directed, yet relevant experimental systems that can be used to identify drugs to prevent neurodegeneration.

Despite these ambiguities and challenges, ALS and HD present special opportunities for drug discovery and development. In the case of HD, the disease is an invariable consequence of inheriting a single copy of the mutant huntingtin gene. Therefore, it is possible to identify those at risk for HD and potentially begin treatment either before disease onset or at the earliest sign of the disease. In the case of ALS, because disease progression is rapid, leading almost invariably to death within 5 years of diagnosis, and because one drug, riluzole, has already been approved for the treatment of ALS, clinical trial design and regulatory approval criteria are well defined. Pharmaceutical companies, at least in the past, considered ALS the best or at least most rapid clinical indication to test a neuroprotective compound, aiming eventually at larger markets like AD or PD. HD may present similar opportunities because of the possibility of identifying patients prior to or at early stages of disease onset. Because of the inevitable, grave disease prognosis, ALS patients and those diagnosed or at risk for HD have been willing to participate in clinical trials. Currently, valuable natural history and biomarker studies (e.g., PREDICT-HD) as well as intervention clinical trials are ongoing in diagnosed or those at risk HD volunteers (see [clinicaltrials.gov](http://clinicaltrials.gov)). A large number of clinical trials for a variety of therapeutics have been conducted in ALS patients. Unfortunately, except in the case of riluzole, these have a dismal record of failure to affect function or survival rate in humans in spite of plausible targets and mechanisms of action or activity in relevant preclinical models [3-6]. ALS and HD are both orphan diseases; therefore, drug discovery and development can benefit from the assistance and incentives provided by various orphan drug acts in the European Union and United States. In addition, active patient organizations fund research and drug discovery for these diseases, either directly or by raising their visibility to encourage government support. And finally, should a drug prove to be effective and receive regulatory approval, the marketing costs are minimal since these diseases are treated by a small number of specialist neurologists.

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## DRUG DISCOVERY USING THE TARGET-DIRECTED APPROACH

In the twenty-first century target-directed drug discovery is often taken for granted as the method of choice, although the ability

**Table 1. Chicken and Egg in Drug Discovery: What Came First the Drug or the Target?**

This table provides some references to a non-exhaustive list of drugs that were discovered before their molecular target. There are also cases of drugs on the market that are still looking for their target or therapeutic mechanism (e.g. riluzole, metformin)

Clinical Indication & Marketed Drugs	Target Discovery Using Drugs or Pharmacological Tools
Dyslipidemia: statins	3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors lower cholesterol by increasing low density lipoprotein receptor expression: clearance may be more important than decreased cholesterol synthesis [43, 44].
Analgesia: non-steroidal anti-inflammatory drugs	Ibuprofen was discovered in 1960 using a screen based on UV-erythema in guinea pigs since analgesic effects of aspirin could not be demonstrated in animal models; mechanism of action elucidated in 1972 [45].
Diabetes: PPAR $\gamma$ agonists & metformin	Glitazones (PPAR $\gamma$ agonists) were identified by the associating peroxisomes with fat-lowering activity. Biguanidines in medicinal plants used to treat diabetes since medieval times: lead to the discovery of metformin (mechanism of action still under investigation) [46].
Anticoagulants	Anti-thrombotic drugs like clopidogrel and ticlopidine were marketed before their target, the G-protein coupled receptor, P2Y <sub>12</sub> , was discovered [47].
Cardiovascular disease: calcium channel antagonists	Anti-hypertensive effects of verapamil, diltiazem and nifedipine aided in the discovery of voltage-operated calcium channels [48].
CNS - epilepsy, anxiety & sleep disorders: benzodiazepines	Benzodiazepines were first marketed in the 1960s (valium); their anti-convulsive and anxiolytic effects were found to be due to allosteric modulation of cloned and recombinantly expressed $\gamma$ -aminobutyric acid-a receptors in the 1980s [49]. Similar studies revealed the mechanism for the selective hypnotic effect of zolpidem [50].

PPAR, peroxisome proliferator-activated receptor.

to identify and select “druggable” targets is a “post-genomic” phenomenon. The use of natural products for medicinal purposes is as old as mankind (Fig. 1). Most drugs have as their origin the active principle found in natural products derived from plants, mushrooms, venoms, etc. that were almost certainly discovered by bioassay. Organic chemistry developed in the mid 19<sup>th</sup> century in order to identify and synthesize the active ingredients found in natural medicinal products (e.g., heroin, quinine, aspirin) giving rise to the chemical and pharmaceutical industries [7]. It was only in the 1960's that specific plasma membrane receptors were discovered to transmit signals from insulin and other hormones as well as the few then-known neurotransmitters (acetylcholine, norepinephrine, dopamine). Disease-relevant drug targets and pathways have, for the most part, only been identified after a drug has been found effective (Table 1). It should also be kept in mind that the drug, even if designed to act on a specific molecular target, may eventually be found to have other applications than the original clinical target (Eflornithine, Rogain and Viagra are some examples). Target-directed drug discovery first became fashionable in the mid 1980s when high throughput screening could exploit the large-scale availability of selected targets by exploiting recombinant DNA technology developed in the 1970s. So while targeting receptors, transporters, ion channels or enzymes is considered the rational approach to drug discovery, it is still a gamble until clinically validated.

#### DRUG DISCOVERY WHEN THERE ARE NO VALIDATED TARGETS OR BIOMARKERS

Discovering drugs for neurodegenerative diseases having unknown and possibly multiple mechanisms is further confounded by the lack of short-term effects or biomarkers predictive of clinical efficacy. To tackle these problems, “disease relevant” phenotypic screening assays are a possible alternative. To be pathophysiologically relevant these assays should use primary neurons derived from the region of the central nervous system (CNS) affected in a particular neurodegenerative disease. For example, motor neuron cultures to screen for drugs to treat motor neuron diseases or primary striatal neurons containing mutant huntingtin to screen for drugs to treat HD. However, the use of primary cell-based assays is not easily adopted by industry's “target-directed” screening platforms due to the challenges of

preparing these cells reproducibly and in the quantity needed. Nevertheless, phenotypic screening returns to the drug discovery strategy that existed before the discovery of receptors and signaling pathways and may be appropriate in cases where the disease mechanism remains a black box and may have certain advantages (Fig. 2).

Two examples of phenotypic screening assays for neurodegenerative diseases are described below. The first objective was to identify small molecules that could be used as pharmacological tools both to understand the properties of the compound as well as explore mechanisms and activity in disease models. Where qualified, the ultimate goal is to continue their development to select a drug candidate and obtain proof of concept in human clinical trials.

#### EXAMPLE 1. TROPHIC FACTOR DEPRIVED MOTOR NEURONS AS A MODEL OF MOTOR NEURON DISEASE:

Motor neurons are highly dependent on target-derived trophic factors to maintain neuromuscular junction architecture, neuronal polarization, neurite outgrowth and retrograde survival signaling [8]. Since early breakdown of neuromuscular junctions precedes motor neuron cell death in ALS, it is reasonable to suppose that a reduced supply of muscle-derived neurotrophic factors may be an underlying mechanism in the disease [9-12]. It has been repeatedly demonstrated that trophic factor supplementation is beneficial in animal models of motor neuron disease [13-16]. Trophic factor deprived primary motor neuron cultures have been widely used to study neuronal cell death mechanisms and can identify all known protein neurotrophic factors [8]. Therefore, this system was established as a relevant system to base a phenotypic screening assay to discover small molecules that would promote motor neuron survival as effectively as trophic factors [17]. Hits coming from this screen were then used to design analogs which, along with pharmacologically active reference compounds, were studied in the primary assay and a number of secondary assays according to the strategy described below. This process led to the selection of a class of molecules, cholesterol-oximes, with a lead molecule, olesoxime (TRO19622) that was then tested in multiple *in vivo* models of nerve injury, while in parallel exploring possible targets that might account for its activity and provide information on its mechanism of action [18-21]. As a pharmacological tool, olesoxime has identified

## Drug discovery timeline

- 450 M years ago: plants & animals develop “chemical” survival strategies
- 2.5 – 0.2 M years ago: “humans” discover medicinal properties of natural products: By chance? By observing other animals? Bioassays?
- ~5,000 years ago: doctors, apothecaries, traditional medicine first recorded uses of natural products on Egyptian papyrus scrolls
- 1652 Nicholas Culpepper's herbal published
- 1800s: origin of synthetic organic chemistry – active principles identified; first attempts to synthesize: quinine, aspirin, heroin...
- 1900-1950: discovery of insulin, penicillin, streptomycin...
- 1960s: discovery of hormone receptors
- 1970s: recombinant DNA methods
- 1980s: high throughput screening & target-directed drug design
- 1990s: human genome project identifies the “druggable genome”
- 21st century: “systems biology” – a return to bioassays?

**Fig. (1).** A timeline of drug discovery illustrating the recent introduction of “target-directed drug design”. Bioassays, phenotypic screening, and chemical genetics are used when disease targets are unknown. This approach can discover products that modulate multiple targets that collectively provide a therapeutic benefit.

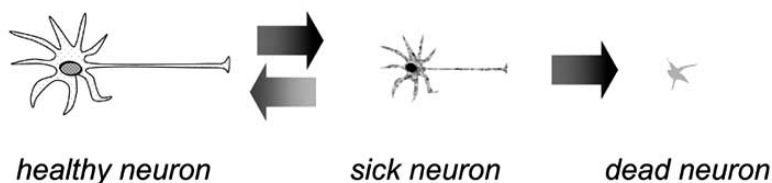
mitochondria as a possible drug target, which is potentially relevant to non-neuronal as well as neuronal cell degenerative diseases [22-25]. Its safety profile is satisfactory to allow long-term clinical trials and it is now being studied in patients with ALS, spinal muscular atrophy, and chemotherapy-induced neuropathy.

### EXAMPLE 2. STRIATAL NEURONS TRANSDUCED WITH MUTANT HUNTINGTIN AS A MODEL OF HUNTINGTON'S DISEASE

Although the functional role(s) of huntingtin, the protein product of the gene associated with HD, is still unknown, it is hypothesized that an N-terminal fragment of mutant huntingtin containing 35 or more glutamine-repeats has selective toxicity for striatal neurons [26]. An *in vitro* model of mutant huntingtin-

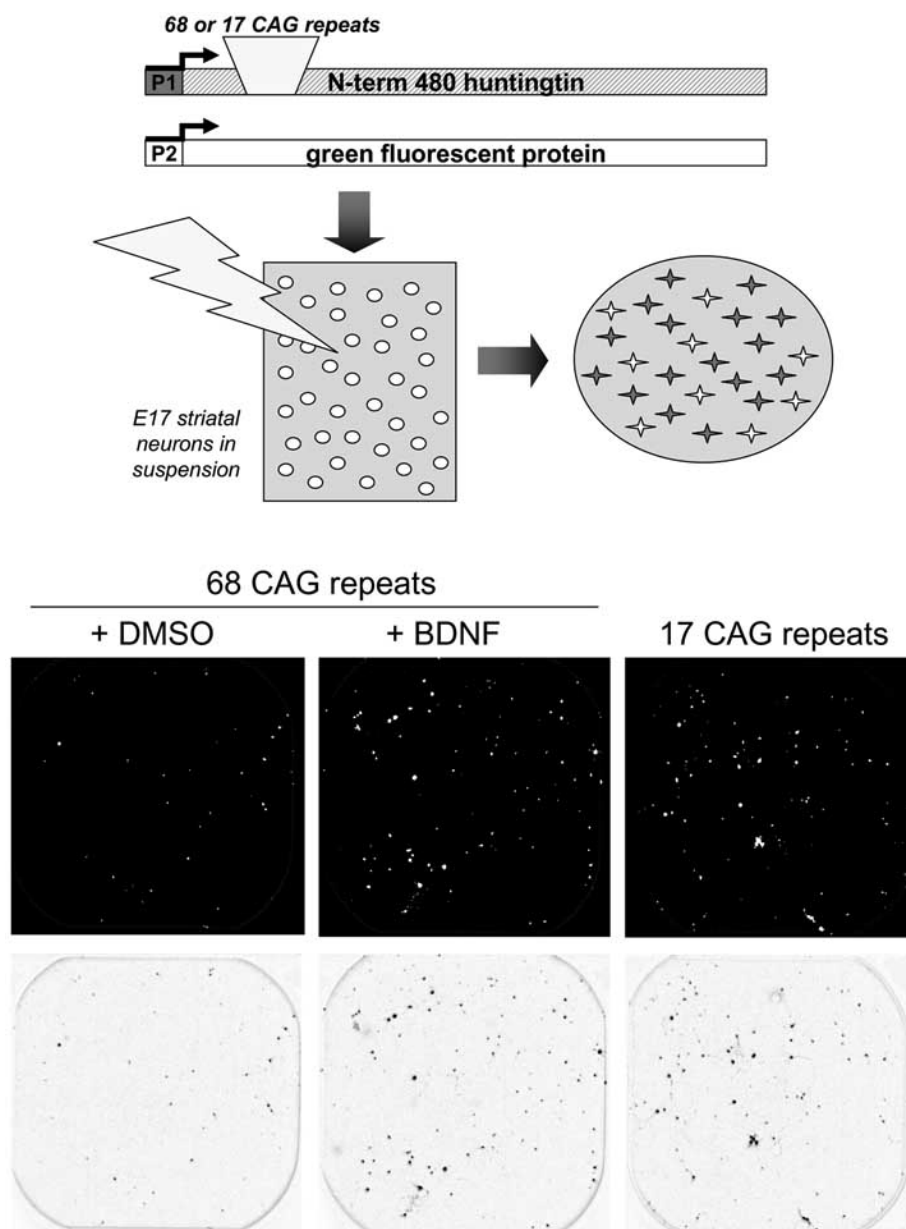
induced toxicity in primary striatal medium spiny neurons has been used extensively to study how mutant huntingtin causes striatal toxicity [27-32]. This cell-based model can be adapted to high throughput screening by the use of electroporation to rapidly and efficiently transfer DNA coding for the N-terminal 480 amino acid fragment of mutant huntingtin (containing 68 CAG repeats) along with green fluorescent protein into freshly prepared rat embryonic day 17 striatal neurons. Using green fluorescent protein-positive cells as a reporter for surviving mutant huntingtin-expressing striatal neurons, it was possible to measure expression efficacy (~30% of all cells in the well), mortality after 6 days in culture (~50% compared to cultures electroporated with DNA coding for the same fragment of huntingtin with only 17 CAG repeats) and show that brain-derived neurotrophic factor (BDNF) could rescue mutant huntingtin-induced mortality (Fig. 3). Limited only by the

### phenotypic screening using primary neurons



- **Primary neurons:** physiologically relevant
- **Target independent:** no validated targets for neurodegeneration
- **Assay endpoint:** neuronal survival
- **Advantages:** non-toxic, stable, tool to identify novel targets

**Fig. (2).** Phenotypic screening using primary neurons. A simple screen can be developed using the appropriate class of neurons, those affected in a specific neurodegenerative disease, exposed to a relevant pathophysiological stress, then screening for cell survival up to a week later. Fluorescent dyes or protein reporters can be used as sensitive indicators of cell survival. Trophos developed a new type of fluorescence plate imager, the PlateRunner HD<sup>®</sup> to capture the area of an entire well of a 96 well plate in a single image in under 200 milliseconds. This allows counting a plate of live neurons in less than 2 minutes. Image analysis software can then count the cell bodies or other objects on the bottom of the well. Details of the instrument can be downloaded at [http://www.trophos.com/download/pr\\_datasheet.pdf](http://www.trophos.com/download/pr_datasheet.pdf). Hits coming from the screen may maintain cell survival by stimulating survival pathways, mimicking trophic factors, or inhibiting death signalling. Higher content screening and profiling in target-directed secondary assays can then be used to identify targets and mechanisms of action of promising hits.

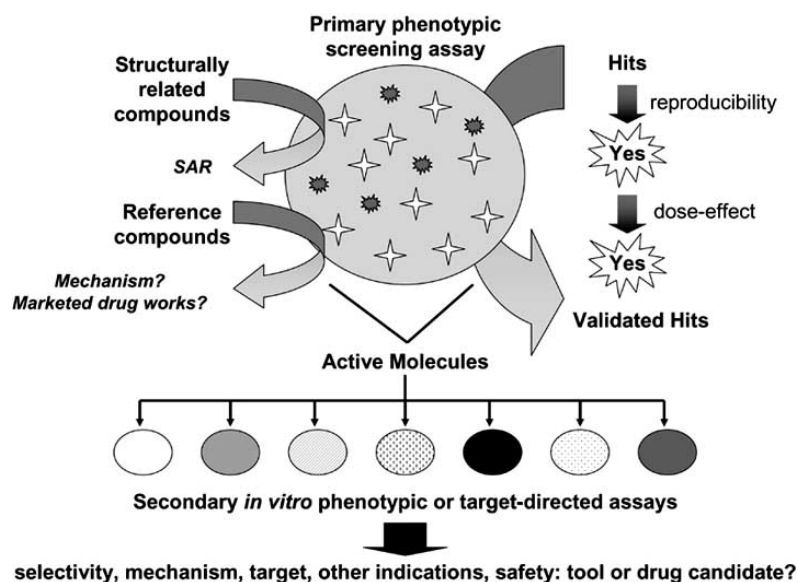


**Fig. (3).** An illustration of the screening assay used to detect compounds that block mutant huntingtin toxicity. Upper drawing: DNA coding for huntingtin and a fluorescent protein reporter are mixed and introduced by electroporation into primary striatal neurons freshly prepared from embryonic day 17 rat brain. To avoid competition for transcription factors, different promoters (P1 or P2) are used to drive expression of huntingtin and the fluorescent reporter. The cell suspension is seeded into 96 well dishes and the number of fluorescent cells (e.g., white versus dark in the drawing) are counted 6 days later using the PlateRunner HD<sup>®</sup> (formerly called the “Flash Cytometer”) as described in Fig. (2). Lower images: Fluorescent images (white objects on a black background above, reversed images below) show a typical experiment in which huntingtin containing either 68 (mutant) or 17 CAG repeats are introduced (left and right images, respectively) showing the relative decrease in fluorescent cells expressing mutant huntingtin compared to wild type huntingtin. Treatment with BDNF (1-10 ng/ml) at the time of plating fully rescues striatal neurons from mutant huntingtin-induced toxicity (middle images).

volume of the electroporation cuvette, it was possible to produce enough cells to screen 40,000 compounds in about 4 months. Compounds were considered active if they had at least 40% of the efficacy of BDNF relative to dimethyl sulfoxide-treated cultures. Several compound families were identified and, through the process of chemical analoging and pharmacological profiling as described below, one interesting family is now satisfactory for long-term studies in animal models of HD and can be considered an interesting pharmacological tool. Should activity in animal models be compelling, this lead may be useful for identifying new drug targets or even considered as a possible drug candidate.

#### FROM HITS TO LEADS TO DRUG CANDIDATES OR PHARMACOLOGICAL TOOLS

The phenotypic screening approach described here can be a strategy for drug discovery but is also referred to as chemical genetics: a means to identify pharmacologically active molecules for the purpose of identifying disease mechanisms and new potential drug targets [33]. One of the initiatives of the National Institutes of Health Roadmap is providing screening platforms and chemical libraries for just this purpose. The pharmaceutical industry has provided many such pharmacological tools that scientists use routinely to explore how various pathways mediated by receptors, ion channels or enzymes are used in their model systems even though many of these compounds have been abandoned as drug



**Fig. (4).** Hit profiling strategy. Hits are validated using the phenotypic screening assay on the basis of repeatability at the initial screening concentration (usually between 1 and 10  $\mu\text{M}$ ) then dose response over a 3-log concentration range. Compounds with similar structures as the validated hits (commercially available or newly synthesized analogues) can be tested in the same primary screen to explore SAR. Reference pharmacologically active compounds and previously marketed drugs can also be benchmarked in the assay to identify potential mechanisms of the validated hits or pathways that can provide a positive outcome in the screen. It could also identify a drug that has already been used in humans.

candidates for various reasons. While a pharmacological tool does not need to pass all the criteria necessary for a drug candidate, a hit from a primary screening assay still needs to have passed sufficient hurdles to be considered a “lead” for more extensive pharmacological evaluation. A structure-activity relationship (SAR) should be established including identification of similar but inactive compounds to use as controls. Some degree of specificity and selectivity in relevant secondary *in vitro* assays also needs to be established before using the compound to fish for its target. Before testing a compound in animal models, even as a pharmacological tool, it is also important to study its absorption, tissue distribution, metabolism, elimination and toxicity (ADMET properties), which requires analytical chemistry expertise.

Hit-to-lead optimization based on potency and selectivity is relatively straightforward for target-based assays: a lead molecule is a compound that could be suitable for testing in a relevant animal model. When hits come out of a phenotypic screen, chemical optimization relies on a black-box assay that can be a conceptual challenge for chemists who rely on target-based drug design to interpret SAR. Activity is based on the original phenotypic screen and additional pharmacological assays are introduced to assess selectivity. At the same time, confronting the primary screening assay with reference compounds may also help to identify or eliminate possible targets or mechanisms of action. The process of deciphering the activity of hits coming from cell-based phenotypic screens can be broken down into a series of secondary assays (Fig. 4 and Table 2).

#### SECONDARY ASSAYS OF SELECTIVE ACTIVITY

Hits that suppress toxicity or promote survival in the primary neurodegeneration assay can be tested on other neurodegeneration models. This will provide a first indication if the neuronal environment is important and whether the compound may have disease selectivity or have more general applications. This can be followed up in appropriate cellular or animal models.

#### SECONDARY ASSAYS TO EXPLORE POTENTIAL TARGETS AND MECHANISMS

Reference pharmacologically active compounds or existing drugs can be tested in the primary neurodegeneration assay as a way of exploring the effects of known signalling pathways and identifying possible targets engaged by the active hits. It can even be a shortcut to identify a drug that has already been tested in humans. While this appears to be a fast track for clinical development, the known pharmacological and ADMET properties of the drug need to be compatible with chronic treatment and CNS penetration is necessary for a drug to treat a neurodegenerative disease.

Comparing pathways activated by hits with those activated by trophic factors or other pharmacologically active reference compounds can be done using the same or other primary assay or even cell lines, to see if they are trophic factor mimetics or activate other known signalling pathways (for example, second messenger assays or protein phosphorylation patterns). Conventional target-based binding or functional assays with characterized receptors, ion channels, transporters, enzymes, etc. can also be used as a way to identify or eliminate potential targets. Knowledge of interactions with physiologically important targets is important not only for target identification purposes but also as a way of anticipating secondary effects before the molecule is tested in animals. In addition to such established drug or “druggable” targets, other unconventional targets may be identified using a hit’s chemical scaffold as a clue and searching patents, scientific literature or chemical databases to identify possible targets or activities. This strategy led to the discovery that olesoxime binds to two outer mitochondrial membrane proteins, and the hypothesis that its neuroprotective activity is *via* a mechanism of action involving an interaction with the mitochondrial permeability transition pore (mPTP) [18]. This demonstrates that phenotypic screening can identify novel, disease-relevant drug targets such as mitochondrial dysfunction, thereby implicating the mPTP in both lesion and transgenic animal models of ALS [34-39]. That a general target like

**Table 2. Evaluating Phenotypic Screening Hits as Possible Drug Candidates or Pharmacological Tools.**

The following is a series of profiling activities that can be used to confirm the interest of a new chemical entity as a lead for development as a drug candidate or pharmacological tool. Drug candidates require further assessment of bioavailability, chemical and metabolic stability, safety, toxicity and manufacturing feasibility. sm for identifying disease relevant targets or mechanisms. usefulness as a pharmacological tool is not strictly dependent on these latter issues

Assay	Utility	Conclusion
Activity in primary assay	Repeatability at the screening concentration	Hit confirmed
	Dose-effect in an acceptable concentration range (10 <sup>-9</sup> -10 <sup>-6</sup> M)	Hit validated
	Effect of known pharmacologically active compounds or existing drugs	Possible mechanism(s) identified; short cut to a drug candidate
	Effect of structurally similar compounds	Structure-activity relationship; possible mechanism
Activity in secondary <i>in vitro</i> assays	Other cell-based neurodegeneration models	Potential disease specificity
	Binding or functional assays for specific targets or pathways activated by trophic factors or by reference compounds or drugs active in the primary screen	Confirm or eliminate potential targets or mechanisms
	Binding or functional assays for CNS or other physiologically important targets	Eliminate possible safety issues prior to <i>in vivo</i> testing; identify/eliminate possible targets
	Binding or functional assays related to putative target/mechanism	Exploration/validation of target/mechanism hypothesis
Activity in preclinical animal models	Disease related: non-transgenic (if available)	Dose ranging; pharmacokinetic/pharmacodynamic relationship
	Disease related: transgenic (if available)	Pre-symptomatic or post-symptomatic activity
	Other models in which putative targets/mechanism is implicated	Exploration/validation of target/mechanism hypothesis
	Safety (CNS, cardiovascular, acute and repeated maximum tolerated dose)	Potential for side effects in long term treatment studies

the mPTP might be responsible for olesoxime's cytoprotective properties was validated using non-neuronal cell secondary *in vitro* assays [21].

#### PROOF OF CONCEPT IN PRECLINICAL ANIMAL MODELS

Once a compound appears to have an interesting activity-side effect liability profile it may then be considered useful as a pharmacological tool to explore activity in animal models. It is normally at this stage that the drug discovery process, even if target-directed, is confronted with black-box, bioassay screening in order to assess ADMET properties. While various informatic tools have been developed to screen compounds for these properties "in silico", they are not infallible: there is a real danger that unfavourable properties predicted by a computer can kill a useful compound (e.g. drugs derived from natural products) while positive scores do not guarantee appropriate behavior in animals or humans. Simple assessment of bioavailability and CNS penetration should be performed with doses in the mg/kg range. If exposure is satisfactory (plasma and tissue concentrations in a pharmacologically active range), assessment of acute toxicity and a safety margin with repeated administration in the appropriate species should be performed. These preliminary steps will also be helpful to design the dosing strategy (frequency and route of administration) and avoid confounding side effects or toxicities that appear only after chronic treatment. In the absence of biomarkers or other short-term measures of disease modification (symptomatic treatments are not considered here), long-term treatment (more than one month) is necessary to evaluate effects on survival, behavior or histological endpoints as measures of disease progression or regeneration. Besides transgenic animal models of neurodegenerative diseases in mice or rats (or even model organisms like *C. elegans*, *drosophila*, or zebra fish) lesion or intoxication models may also be

informative. Olesoxime was indeed found to be well-tolerated, CNS penetrant, and to have general neuroprotective properties in lesion and transgenic models of peripheral or central neurodegeneration [18-20].

#### CONCLUSIONS: PHENOTYPIC SCREENING CAN FIND PHARMACOLOGICAL TOOLS THAT CAN LEAD TO DRUG CANDIDATES

Two examples of phenotypic screening were described. One, based on motor neuron survival identified a novel cholesterol-oxime family that served as a pharmacological tool to identify targets and mechanism of action. Because the compound targets mitochondrial proteins, it is possible that compounds in this family may be useful to treat other indications where mitochondrial dysfunction is implicated. Olesoxime advanced rather rapidly to drug candidate status based on activity in a number of animal models relevant to motor neuron disease and has satisfactory ADMET properties allowing clinical testing in patients with ALS. Developing new chemical entities from hit to lead to drug candidate for treatment of HD is more challenging. First, although there are many transgenic animal models of HD, whether they mimic the human disease or can provide sufficient evidence of efficacy to treat HD in humans remains to be determined [40-42]. Second, there are no specific underlying mechanisms that have been identified that could serve as pharmacological endpoints or biomarkers of potential efficacy. Third, HD progresses slowly and long-term treatment will be required to see modification of the disease process in contrast to studies focused on symptoms like chorea. Therefore, a potential neuroprotective drug must pass additional pre-requisites before starting long-term clinical trials. Although currently not considered a drug candidate, one of the hits coming from the screen based on the rescue of primary striatal neurons from mutant huntingtin toxicity may be a useful

pharmacological tool. This compound is currently being tested in a rat model of HD. Promising results may inspire further use of this compound to identify potential targets and better understand HD pathology, regardless of whether it has all the properties needed in a future drug.

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#### ABBREVIATIONS

AD	= Alzheimer's disease
ADMET	= Absorption, distribution, metabolism, elimination and toxicity
ALS	= Amyotrophic lateral sclerosis
BDNF	= Brain derived neurotrophic factor
CNS	= Central nervous system
HD	= Huntington's disease
mPTP	= Mitochondrial permeability transition pore
PD	= Parkinson's disease
SAR	= Structure-activity relationship

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