Target validation: the Parkinson disease perspective

David G. Standaert[1],* and Talene A. Yacoubian[1]

How much validation is enough?

Target validation is the process of determining the likelihood that modification of a certain molecule, mechanism or biological pathway may be useful for the development of pharmacological or molecular treatments for disease. Target validation is an essential part of the rational discovery of new therapies (Fig. 1). It may involve studies of cellular systems, animal models, human genetics, human biomarkers and even early-phase human clinical trials. Validation studies select the best candidates among a wide range of potential targets to focus resources and efforts on a small number of 'high-yield' targets for the development of target modifiers. Ultimately, targets are fully validated when they lead to the development of a clinically useful therapy.

Much of the current research related to Parkinson disease (PD) consists of efforts at target validation. PD is a common and disabling disorder, and the prevalence of the disease is expected to increase with aging of the population (Dorsey et al., 2007). Most current therapies are based on replacing dopaminergic function; although these improve quality of life for patients, they do not slow the progressive neurodegeneration observed with this disorder. Thus, the targets that are sought most eagerly by PD investigators are those that will slow or reverse disease progression.

Fig. 1. Rational discovery of new therapies.

Recent technological advances have greatly enhanced the capacity for the rapid discovery of potential targets for disease intervention. Among the most important of these new technologies are methods that enable genome-wide studies of heritable factors and alterations in gene expression in human disorders. Other high-capacity approaches to identify molecules to treat human disease include proteomics, analyses of small metabolites ('metabolomics') and studies of protein interaction (the 'interactome'). In PD, the use of these '-omics' methods has led to the identification of an expanding array of genes, including those encoding α-synuclein (α-syn), leucine-rich repeat kinase 2 (LRRK2), parkin, DJ-1 and PTEN-induced kinase 1 (PINK1), which may become targets for PD therapy. High-capacity methods are also applied to cellular and animal systems, and yield even more potential targets; in the case of PD, screening for inhibitors of α-syn toxicity in yeast and *C. elegans* identified more than a dozen additional targets (Hamamichi et al., 2008; Yeger-Lotem et al., 2009). The ever-increasing list of potential targets for just this one disorder illustrates the complexity and importance of target validation. This increasingly crucial part of the process allows drug development to focus on the targets with the most potential.

Target validation is a distinctly low-throughput, slow and painstaking process. It rarely involves a single method or assay; rather, it usually depends on convergent evidence collected from a variety of studies, including molecular analysis, model organisms and correlative studies in humans. In general, the more complex the underlying human disease state is, the more challenging the validation process will be. The evidence for

[1]Center for Neurodegeneration and Experimental Therapeutics, University of Alabama at Birmingham, Birmingham, AL 35294, USA
*Author for correspondence (dstandaert@uab.edu)

the validity of a target is usually acquired progressively and may be viewed as lying along a spectrum (Fig. 2) (Heemskerk et al., 2002; Colombo and Moll, 2008). At the bottom are in vitro biochemical and cellular studies, which are simple and may replicate some of the relevant disease processes, but lack the complexity of an intact animal system. In PD, such studies would include studies of the properties of disease-related proteins, such as the enzymatic activity of the kinase LRRK2, or evaluation of target function in a simple cellular model system. A more robust test is to establish that the target is effective in a multicellular model organism. Non-mammalian systems have important practical advantages, including speed and a moderately high throughput. For example, in PD, invertebrate models have been developed in yeast, Drosophila and *C. elegans* to study the effect of potential targets on α-syn aggregation or toxicity (Feany and Bender, 2000; Outeiro and Lindquist, 2003; Hamamichi et al., 2008). In most cases, validation in intact mammalian systems is sought; in PD, these would include rodent models based either on transgenic expression of PD-related genes or on neurotoxins. Human genetics can play an important role in the process, especially in disorders where the clinical phenomenology is well established but the nature of the underlying mechanism remains unclear.

As examples of the progressive nature of target validation, our laboratories have focused on two sets of genes, VPS-41 and 14-3-3s, which were identified through high-throughput studies to identify potential new targets. VPS-41 was identified in a high-throughput RNA interference (RNAi) screen in *C. elegans* to evaluate modifiers of α-syn aggregation and α-syn toxicity (Hamamichi et al., 2008). 14-3-3s were identified as potential candidates through a gene microarray study evaluating gene alterations in the substantia nigra of transgenic α-syn mice (Yacoubian et al., 2008). As the first step in target validation, both VPS-41 and 14-3-3s have been demonstrated to
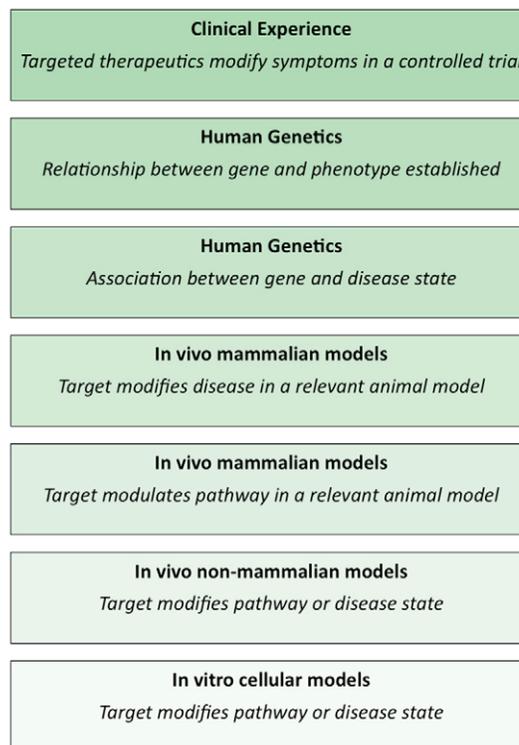
Clinical Experience
Targeted therapeutics modify symptoms in a controlled trial

Human Genetics
Relationship between gene and phenotype established

Human Genetics
Association between gene and disease state

In vivo mammalian models
Target modifies disease in a relevant animal model

In vivo mammalian models
Target modulates pathway in a relevant animal model

In vivo non-mammalian models
Target modifies pathway or disease state

In vitro cellular models
Target modifies pathway or disease state

Fig. 2. Spectrum of studies employed to evaluate the validity of a target.

reduce toxicity against neurotoxins in dopaminergic cell lines (Ruan et al., 2010; Yacoubian et al., 2010). As the next step in validation, both VPS-41 and 14-3-3s have shown neuroprotection in the transgenic α-syn *C. elegans* model (Ruan et al., 2010; Yacoubian et al., 2010). The next phase for both of these targets is the evaluation of neuroprotection in mammalian models, which are now in progress. If these targets do show efficacy in mammalian systems, then one can imagine that high-throughput screens to identify modifiers of these targets will be the next step towards the development of potential therapeutic agents.

How much validation is enough? This is not a simple issue, and involves the interactions between the demand for new treatments, the high cost of therapy development, and the social and business structures that support drug discovery. The highest degree of validity is associated with targets with established efficacy in human trials, but restricting therapy development to these targets is likely to lead to duplication of existing therapies and little in the way of progress. In the case of PD, the most valid target at present is the dopamine system, but the development of additional drugs based on this target is not likely to make fundamental progress towards the goal of curing the disorder. However, investing large amounts of resources into targets with limited evidence for validity is also unlikely to succeed, and diverts resources that would be better used elsewhere.

Linked to the question of how much validation is required, is the predictive validity of models used to study human disease. For many human disorders, the existing cellular and animal models do not adequately replicate the complexity of the human disease. For example, none of the existing mouse models of PD replicates the selective and progressive degeneration of dopamine neurons, which is considered to be an essential component of the human disorder. Indeed, the validity of a disease model for progressive PD cannot be fully established in the absence of any effective therapy for the progressive aspect of the human disorder.

It is essential to appreciate that the goal of target validation is usually not to establish 'proof' of validity; this is usually impractical in all but the simplest of disorders. Rather, target validation is a probabilistic exercise, where the goals are to accumulate evidence supporting the likelihood of eventual success in treating disease, and to provide justification for continued investment in the target as a therapeutic candidate. The ultimate 'validation' of a target would be the success of a therapy directed against that target in patients.

Our view is that, at present, the most promising road to success is to distribute the components of the target validation process, and the associated risks, among the organizations and institutions that are best equipped to manage them. Early-stage discovery and validation efforts are probably best accomplished by academic investigators who are better able to absorb the risk that a molecule or pathway will prove interesting but not an effective target. At the other end of the spectrum, the costs of clinical studies are exorbitant and necessitate industrial and commercial partners with capital resources. The 'weak link' in this formulation is the hand-off between academic investigators and industry partners. The collaboration between academic researchers and the pharmaceutical industry requires ongoing consultation and interaction, which is often lacking in current practice. Indeed, this gap has been referred to as the 'valley of death' for drug development (Moran, 2007) because of the number of potentially promising targets that are not pursued.

In the long term, better metrics for the validity of targets are needed. These should emerge from analyses of preclinical data in the light of later clinical efficacy. Most target validation efforts are currently ad-hoc, and not part of a broader effort to produce

correlated data. Indeed, studies of this kind are often hindered by the lack of transparency associated with industry-based investigators, particularly when the eventual outcome is not successful. Important steps have been taken in this direction, but continued vigilance from patients, doctors, scientists, funding agencies and industrial partners is required to move this area forward.

REFERENCES

Colombo, R. and Moll, J. (2008). Target validation and biomarker identification in oncology: the example of aurora kinases. *Mol. Diagn. Ther.* **12**, 71-76.

Dorsey, E. R., Constantinescu, R., Thompson, J. P., Biglan, K. M., Holloway, R. G., Kieburtz, K., Marshall, F. J., Ravina, B. M., Schifitto, G., Siderowf, A. et al. (2007). Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. *Neurology* **68**, 384-386.

Feany, M. B. and Bender, W. W. (2000). A Drosophila model of Parkinson's disease. *Nature* **404**, 394-398.

Hamamichi, S., Rivas, R. N., Knight, A. L., Cao, S., Caldwell, K. A. and Caldwell, G. A. (2008). Hypothesis-based RNAi screening identifies neuroprotective genes in a Parkinson's disease model. *Proc. Natl. Acad. Sci. USA* **105**, 728-733.

Heemskerk, J., Tobin, A. J. and Ravina, B. (2002). From chemical to drug: neurodegeneration drug screening and the ethics of clinical trials. *Nat. Neurosci.* **5 Suppl.**, 1027-1029.

Moran, N. (2007). Public sector seeks to bridge 'valley of death'. *Nat. Biotechnol.* **25**, 266.

Outeiro, T. F. and Lindquist, S. (2003). Yeast cells provide insight into alpha-synuclein biology and pathobiology. *Science* **302**, 1772-1775.

Ruan, Q., Harrington, A. J., Caldwell, K. A., Caldwell, G. A. and Standaert, D. G. (2010). VPS41, a protein involved in lysosomal trafficking, is protective in Caenorhabditis elegans and mammalian cellular models of Parkinson's disease. *Neurobiol. Dis.* **37**, 330-338.

Yacoubian, T. A., Cantuti-Castelvetri, I., Bouzou, B., Asteris, G., McLean, P. J., Hyman, B. T. and Standaert, D. G. (2008). Transcriptional dysregulation in a transgenic model of Parkinson disease. *Neurobiol. Dis.* **29**, 515-528.

Yacoubian, T. A., Slone, S. R., Hamamichi, S., Harrington, A. J., Schieltz, J. M., Caldwell, K. A., Caldwell, G. A. and Standaert, D. G. (2010). Differential neuroprotective effects of 14-3-3 proteins in models of Parkinson's disease. *Cell Death Dis.* **1**, e2 [doi: 10.1038/cddis.2009.4].

Yeger-Lotem, E., Riva, L., Su, L. J., Gitler, A. D., Cashikar, A. G., King, O. D., Auluck, P. K., Geddie, M. L., Valastyan, J. S., Karger, D. R. et al. (2009). Bridging high-throughput genetic and transcriptional data reveals cellular responses to alpha-synuclein toxicity. *Nat. Genet.* **41**, 316-323.