

Translational Research in Central Nervous System Drug Discovery

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Summary: Of all the therapeutic areas, diseases of the CNS provide the biggest challenges to translational research in this era of increased productivity and novel targets. Risk reduction by translational research incorporates the “learn” phase of the “learn and confirm” paradigm proposed over a decade ago. Like traditional drug discovery *in vitro* and in laboratory animals, it precedes the traditional phase 1–3 studies of drug development. The focus is on ameliorating

the current failure rate in phase 2 and the delays resulting from suboptimal choices in four key areas: initial test subjects, dosing, sensitive and early detection of therapeutic effect, and recognition of differences between animal models and human disease. Implementation of new technologies is the key to success in this emerging endeavor. **Key Words:** CNS, drug discovery, translational research, biomarkers, proteomics, imaging.

INTRODUCTION

In the pharmaceutical industry, translational research refers specifically to those activities conducted to bridge the gap between drug discovery in animals and drug development in human patients. The need for such activities has been driven by two major changes in drug discovery: increased productivity and the focus on novel drug targets not yet pharmacologically proven in man. Availability of the complete genome sequence of humans and the partial ability to deduce probable gene function informatically and experimentally has increased the number of potential drug targets from the 500 or so that underlie our current pharmacopeia¹ to potentially tens of thousands. Only a small minority of these potential targets have been pharmacologically proven in human disease. Combinatorial chemistry and high-throughput screening ensure that small molecules interacting with a substantial majority of these potential drug targets can be devised in a matter of few years and optimized by increasingly standardized methods. Humanized monoclonal antibodies provide still another source of readily developed therapeutic agents against virtually any cloned protein target, whether or not its function in human disease is understood. As a result, the sheer volume of

potential drug candidates is providing a challenge to development organizations that must really on labor-intensive, increasingly expensive clinical trials to identify which of these candidates can become drugs, and ultimately products.

Drug development in humans has always provided special challenges because of inescapable practical, ethical, and regulatory constraints. This was true even when discovery organizations focused on incremental improvements –“me-too” drugs directed against targets whose relationship to human disease had already been established. Whereas preclinical validation is conducted in isogenic animals reared under identical conditions, development is conducted on outbred populations with equally heterogeneous lifestyles. Treatment in animal models commences at a precise interval after induction of an identical provocative lesion, whereas developmental testing occurs in naturally occurring disease of variable severity and duration. Treated animals can be sacrificed for examination of pathology and effects of treatment, using endpoints directly related to the induced pathology.

In contrast, ethical constraints require noninvasive measurements in humans. Drugs can only be registered if they demonstrate improvement in the less direct measures of clinical improvement and quality of life. The resulting variability requires sample sizes of hundreds or thousands of human subjects, rather than the dozens required for studies in animals. Costs are further in-

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creased by the regulatory requirements for tamper-proof locked databases. Instead of the multiple small iterative experiments typical in discovery laboratories, traditional development relies on relatively few meticulously pre-planned large trials, with few opportunities for mid-course corrections informed by emerging data.

Nevertheless, the traditional paradigm of phase 1–4 studies was adequate to the task of developing the relative trickle of candidates that used to emerge from discovery laboratories, especially because the majority of the drug targets were well understood and development and regulatory strategies were well established. Although translational research may have been useful in this by-gone era, it was hardly necessary. Even then, proposals were made to reshape the drug development paradigm into a “learn and confirm” paradigm, that encouraged more exploratory studies in early development,² a harbinger of what we now refer to as translational research. Such a paradigm was not accepted then because of concerns that it would have resulted in unnecessary expense and delay to market. In the era of “me-too drugs” the likelihood of efficacy was relatively high. The paramount concern was speed to market. This is no longer the case given the risks inherent in not being certain that the target is relevant to human disease. In recent years, only 9% of compounds that entered phase 1 survived to launch. Over 50% of this attrition resulted from failure to demonstrate efficacy in phase 2 studies, a 15% increase in phase 2 failures over the last decade. Compounds that worked beautifully on cloned human proteins and in animal models more often than not proved ineffective in human disease.

Experience demonstrated that behavior of a compound in an patient cannot be predicted perfectly from interactions with isolated human molecules or cells *in vitro*, nor from animal models.

Significant metabolic pathways in laboratory animals may be minor or redundant in humans. Animals models are not identical to human disease. In no other therapeutic area is that more true than for diseases of the CNS, demonstrably the organ system most uniquely distinguishing humans from laboratory animals. Indeed, only 3–5% of CNS candidates ever become marketed therapeutics. Waiting for demonstration of efficacy in a traditional phase 2 study is becoming an increasingly costly proposition. Further risk reduction is necessary before embarking on lengthy and expensive phase 2 trials using registrable endpoints on samples of the broadly defined populations that the target product profile aims for. Developing the tools for as well as execution of this risk reduction is the role of translational research.

In the largest sense, translational research could be taken to encompass the entire breadth of medicine: diagnosis, prognosis, and management. Such a definition is not helpful. For industrial translational research to be a

practicable, nonduplicative addition to the current activities of integrated pharmaceutical companies, it is critical that the mission is focused sharply and its deliverables be defined clearly. There is no better way of defining the focus than by examining the root causes of failure in drug development in this era of novel, unproven targets. Drug candidates fail for one of four major reasons:

- 1) The compound is given to the wrong subjects.
- 2) The compound is given at the wrong dose or schedule.
- 3) The favorable effects of the compound are not detected.
- 4) The compound has a significant effect in laboratory species, but not in humans.

It is the mission of translational research to minimize the risk associated with the first three causes of failure and to permit efficient identification of the fourth, to ensure that the resources required for development and registration are allocated optimally.

WHAT PATIENTS WILL RESPOND TO THE DRUG? PATIENT IDENTIFICATION/STRATIFICATION BIOMARKERS

Animal models are imperfect predictors of human efficacy

Before a compound is judged suitable for testing in humans, it must first demonstrate safety and efficacy in animal models. The increasingly high failure rates of CNS compounds in human trials has demonstrated that this success in animals is no guarantee. No animal model is a perfect mimic of human disease. Animals can serve as models of disease mechanisms, but not of the disease itself. Arguably, nowhere is the disparity greater than for neuropsychiatric diseases. Failure rates in clinical development attest to the disparity.

Given our imperfect understanding of the pathophysiology of most neuropsychiatric diseases, the best guide is past experience with predecessor compounds that impact the same target or metabolic pathway. In psychiatric diseases, pharmacology has driven the science. The serendipitous observation that putative antimalarials calmed inmates of a psychiatric asylum led to the dopamine theory of schizophrenia and the serotonin theory of depression and anxiety. These theories remain the mainstay of the animal models used for preclinical validation. Although predictive for certain classes of compounds, such an approach is inherently circular and limiting. It is likely that there is more to the major psychoses than either direct or indirect failure of these two transmitter systems.

Even in diseases where there is a greater mechanistic understanding, there are still significant disparities between the animal models used in discovery validation and the human diseases being targeted for treatment.

Transgenic models of Alzheimer disease show relatively little neurodegeneration, neuroinflammation, cognitive, or behavioral impairment. Multiple classes of compounds have proven effective in middle cerebral artery occlusion models of ischemic stroke, but almost all have failed in the clinic. Even in simple pain models, the discovery investigator can only observe the speed of withdrawal from painful stimuli, rather than pain itself.

The challenge of finding responsive human subjects is further compounded by the possibilities of disease heterogeneity. In all the aforementioned categories, we know that there are subtypes. Some of these may be pharmacologically relevant.

In the absence of previous pharmacological experience, how can translational research guide the choice of patients that may benefit from a novel putative therapeutic?

GENETIC MUTATIONS IN HUMANS CAN HELP GUIDE CHOICE OF HUMAN SUBJECTS FOR DRUG DISCOVERY AND TRANSLATIONAL RESEARCH

A potential substitute for a pharmacologic challenge to a putative drug target is a mutation that modifies its activity.³ Thus far, the greatest contribution of genetics to neurological drug discovery has been the support for the amyloid hypothesis of Alzheimer disease afforded by mutations of amyloid precursor protein or its metabolizing enzymes, presenilin 1 or 2. These mutations are found in affected individuals in rare families segregating an early-onset disorder that is clinically and histologically similar to the sporadic old age disease that is the intended target of these therapies. Transgenic animal models are used widely for preclinical evaluation of putative disease-modifying therapies for treatment of sporadic Alzheimer disease. Certain features of the human disease are mimicked more closely than others.^{4,5} The strong association of apolipoprotein E with propensity to sporadic Alzheimer disease has not influenced drug discovery as strongly despite the availability of animal models.^{6,7} However, interest of the pharmaceutical industry in these models has increased belatedly with the independent epidemiological demonstration of the relationship of cholesterol metabolites and statin treatment on the incidence of sporadic Alzheimer disease.^{8,9}

These genetic insights into Alzheimer disease have already been accepted widely by drug discovery organizations. There remains an opportunity for translational research to bring the many other findings of neurogenetics to the pharmaceutical industry. This impact that has been relatively modest to date, especially given the prominent contribution that neuropsychiatric diseases have played in human genetics.¹⁰

Monogenic disorders

There are two main reasons for this disparity. First is the rarity of the disorders for which simple Mendelian inheritance has allowed the ready identification of the causative mutation. A disproportionate number of diseases—about 200 neurologic diseases of the 500 in all categories for which the causative mutation has been identified by positional cloning—are neurological.¹¹ However, most of these diseases are individually rare, and thus not attractive markets for the pharmaceutical industry. Nevertheless, just as in the case of the rare Mendelian cases of early-onset Alzheimer disease, both discovery and translational research of these rare but well characterized disorders will provide insight to a broader category of human diseases. For example, single-gene mutations causing cognitive impairment in multiple mental retardation disorders can provide a molecularly proven insight into the molecular mechanisms of human cognition.¹²

Complex disorders

The second reason for this disparity between the potential contribution from human genetics and its uptake by the pharmaceutical industry is that the psychiatric disorders that have provided most of the market for CNS drugs are heritable not as simple Mendelian traits, but in complex inheritance patterns. In the previous decade, the pharmaceutical industry spent millions of research dollars sponsoring association and linkage studies of common disorders, perhaps expecting a pay-off as rapid as that in genetic studies of single-gene Mendelian disorders. It was not that simple. The pay-offs are only now beginning to come in, and slowly at that. A combination of multiple genes and environmental influences contribute to susceptibility to schizophrenia, the depressive disorders, attention disorders, autism as well as some neurological disorders like multiple sclerosis. Just as was the case with apolipoprotein E, the lack of a clear cause and effect relationship hindered the development of animal models. More importantly, even though genetic associations have been demonstrated in these complex disorders, the genetic loci identified in genetic studies of complex disorders are too large to permit definitive identification of a specific gene, without corroborating biological evidence.^{13–15}

Schizophrenia

Nevertheless, there have been some recent successes, several of them achieved by adding biological information from transcriptional profiling to standard genetic analyses. These combined approaches offer a more promising approach for translational research in pharmaceutical organizations than the more open-ended gene-hunting genetic studies of the previous decade.^{16,17} Comparison of transcriptional profiles of prefrontal cortices harvested from schizophrenics and matched controls

demonstrated a selective decrease of transcripts encoding proteins involved in presynaptic functioning, albeit variable among subjects. These findings were corroborated by *in situ* hybridization. The most consistently diminished transcripts were those for synapsin 2 and N-ethylmaleimide-sensitive factor,¹⁸ a protein involved in the recycling of vesicles.¹⁹ Further support for the role of synapsin 2 in schizophrenia came from both case control and transmission disequilibrium studies demonstrating association of synapsin 2 haplotypes with schizophrenia in population and family studies.²⁰ Another transcriptional profiling analysis with a limited number of candidate schizophrenia genes demonstrated a 2.6-fold increase in expression of apolipoprotein L1,²¹ a high-density lipoprotein known to be encoded on chromosome 12q12, previously demonstrated to be a high-susceptibility locus for schizophrenia.

Promising leads have come from other approaches as well. Mutations of proline dehydrogenase contribute to susceptibility to schizophrenia in some individuals.^{22,23} Transmission disequilibrium analysis has demonstrated association of schizophrenia with the PPPeCC gene and the calcineurin γ catalytic subunit.²⁴ These findings are intriguing given the observation of Miakawa²⁵ that mice with forebrain-specific conditional knockout of calcineurin had behavioral difficulties similar to those of schizophrenia. Variation in the epsin-4 gene, which encodes the clathrin-associated protein entrophin²⁶ (with a role in the stability of synaptic vesicles) and is in linkage disequilibrium with a locus conferring susceptibility to schizophrenia. The finding of a translocation in a family with mental and behavioral disorder DISC1 (disrupted in schizophrenia 1)²⁷ were followed by linkage with chromosome in schizophrenics.^{28,29} Associations with schizophrenia have also been found with variants in nicotinic acetylcholine receptor 7 and diminution of prepulse inhibition³⁰ as well as with variants in neuregulin 1.^{31,32}

Other psychiatric disorders

Genetic studies of depression have identified multiple susceptibility loci, but many of these have proven difficult to reproduce. Of these, the most promising leads are polymorphisms in the FK binding protein 5 gene that plays a role in the stress hormone-regulating hypothalamic-pituitary-adrenal axis, associated with a faster response to drug treatment and increased recurrence of depressive episodes.³³ Recently, a mutation in the tryptophan hydroxylase gene has been found in individuals with unipolar major depression.³⁴ Similar promising leads have been found in attention deficit disorder.³⁵

In summary, although the many pharmaceutical companies have been discouraged by the poor return on investment in their initial genetic studies of neuropsychiatric disorders, a small proportion of these studies have

begun to yield specific information that may be directly applicable to drug discovery and to the identification of biomarkers identifying susceptible patients. Identification of mutations allows the production of transgenic mouse models and small interfering RNA knockdowns for preclinical validation by discovery biologists. Clinical and biological investigation of patients with these causative or predisposing mutations is likely to be a fertile area for translational research.

Choice of patients/subjects: presymptomatic disease

Development of disease-modifying therapy for neurodegenerative disorders is hampered by late diagnosis. Many neurodegenerative diseases cannot be diagnosed clinically until after substantial tissue loss has occurred.^{36–38} This provides a severe, possibly insurmountable, hurdle for novel disease modifying therapeutics. A useful endeavor for translational research is the development of reliable presymptomatic diagnosis that would allow institution of disease-modifying therapy prophylactically, before much tissue has been irretrievably lost.

Neuroimaging provides an opportunity to identify early signs of neuronal dysfunction in a characteristic pattern, before the development of significant irreversible tissue atrophy. This was first demonstrated using resting glucose positron emission tomography (PET) technology in asymptomatic individuals carrying pathogenic mutations in families segregating autosomal dominant early-onset Alzheimer disease.³⁹ This finding was later extended to individuals at high risk for the development of typical late-onset sporadic Alzheimer disease by virtue of their apolipoprotein E status and an affected first-degree relative.⁴⁰ These clinically healthy individuals demonstrated the same characteristic anatomical pattern of decreased glucose uptake as is seen in individuals with symptomatic Alzheimer disease, albeit with decreased severity, even though their age was up to two decades lower than the mean age of onset of dementia. Corroboration of this finding and searches for other methods of presymptomatic diagnosis by translational research would likely improve the chances of achieving efficacy with neuroprotective drugs.

Choice of patients/subjects: challenge models

Another approach to the translational goal of reducing patient heterogeneity in early clinical development of novel therapeutics is based not on the etiologic homogeneity discussed above, but on standardization of the timing and/or severity of the pathological insult that the drug candidate is intended to treat. Ethical considerations clearly preclude the induction of most pathologies in human beings, as is standard practice for animal validation experiments. However, for certain situations either naturally occurring or induced clinical models of disease do permit initial evaluation of novel therapeutics.

Challenge models for pain

A mainstay in the evaluation of analgesics for the initial evaluation of analgesics for inflammatory pain is extraction of third molars.⁴¹ Volunteers are recruited from those individuals scheduled for such a procedure for therapeutic reasons. This model allows intervention at a precisely timed interval with respect to the lesion, typically after induction, but the model is also amenable to evaluation of prophylactic treatments. Other models create no lasting tissue damage and are sufficiently mild as to permit their induction in normal volunteers. Intradermal injection of capsaicin,⁴² with or without the additional application of noxious heat, provides a standardized pain stimulus for the initial evaluation of analgesics. Such a lesion provides the additional advantage of producing easily identified and measurable areas of primary and secondary hyperalgesia, each of which is subserved by different neurological mechanisms, pharmacologically distinct. This model can be extended further by the use of occlusion to selectively eliminate input from large myelinated fibers. It is in these mechanistic studies that the capsaicin volunteer model offers the greatest advantage for the evaluation of compounds targeting as yet unproven pain targets. A recently introduced variant of such testing involves oral administration of capsaicin.⁴³ Muscular pain can be modeled by injection of hypertonic saline.⁴⁴ Volunteer studies such as these can be extended by application of painful electrical stimuli.⁴⁵ The delivery of these stimuli can be graded not only in severity but also in frequency, providing quantifiable measurements of temporal summation and wind-up, important indicators of central sensitization, an important component of chronic neuropathic pain.⁶⁴

Stroke model in humans

Another potential model system is available for the study of stroke in humans. This is provided by the use of heart-lung bypass procedures necessary to support patients during certain types of cardiac surgery, such as coronary artery bypass. Depending on the center and the type of procedure, there is a 2–5% incidence of ischemic stroke in the first few hours of the procedure and recovery. An even higher risk group can be identified preoperatively by simple clinical criteria.⁴⁶ In this group, magnetic resonance imaging (MRI) scanning demonstrates a higher incidence of stroke than observed clinically.⁴⁷ Translational studies of neuroprotective compounds with this high risk group are likely to provide a useful method of risk reduction for putative neuroprotective drugs. Although very many compounds have proven effective in animal models of stroke, almost none have been successful clinically. In large part, the disparity between animal and human results can be attributed to an exquisite time dependence of such therapy. In traditional human stroke trials, patients are binned in large intervals—typically 3

or 6 h after the onset of stroke symptoms—to achieve enrollment goals. This introduces a great deal of variance in the results, a variance that is difficult to calculate precisely. Animal studies have uniformly shown that the earlier therapy begins, the more effective the treatment. Even a few minutes have a significant effect. Ideally, the drug is on board before the vessel is occluded.

The predictable high incidence of stroke in a narrowly defined temporal window in heart-lung bypass patients permits testing of neuroprotective agents either prophylactically or at a carefully predetermined interval after the injury. Translational studies using this model would test novel neuroprotectants in an ideal setting to judge the efficacy of neuroprotective compound in humans. Only those compounds that prove effective in this model would be advanced to standard phase 2 trials.

Models of anxiety and panic

Other provocative models have been adopted for initial testing of CNS drugs. Anxiolytics can be tested in normal volunteers asked to perform public speaking^{48,49}; or in those individuals anxious in anticipation of a scheduled dental extraction.^{50,51} Other provocative tests for the induction of anxiety in normal volunteers include inhalation of carbon dioxide,^{52,53} infusion of lactic acid,⁵⁴ or cholecystokinin.^{55–57} Of these various pharmacologically inducing anxiety models, cholecystokinin infusion is thought to most closely mimic the physiologic changes associated with generalized anxiety disorder, its attendant changes in the stress-hypothalamic pituitary axis, and consequently for its predictive value of therapeutic effect of a wide variety of anxiolytics in the less easily studied spontaneous anxiety disorders.^{58,59}

Model of cognitive impairment in Alzheimer disease

Less widely used challenge models include scopolamine-induced cognitive impairment, which mimics some of the cognitive impairments associated with Alzheimer disease.^{60,61} Scopolamine challenge has been applied both in animal models⁶² as well as in normal human volunteers. However, the cognitive deficits produced by scopolamine differ in substantial respects from that seen in Alzheimer disease.⁶³ Although the scopolamine model has been used with some success in the human validation of anticholinesterase drugs, its predictive value for putative cognitive enhancers working by other mechanisms has yet to be established.

Model of positive symptoms of schizophrenia

Challenge with several different pharmacologic compounds that induce psychosis or delirium in normal human volunteers have been used to model the positive symptoms of schizophrenia, with varying degrees of success.⁶⁴ Of these the most widely used is ketamine.^{65,66} Ketamine has also been used to model schizophrenia in rats, permitting similar evaluations of putative antipsy-

chotic drugs preclinically and clinically.⁶⁷ However, the predictive value of either human or rodent challenge models for antipsychotic drugs working through nondopaminergic mechanisms have yet to be established.

2) IS THE DRUG INTERACTING APPROPRIATELY WITH THE TARGET? PHARMACODYNAMIC BIOMARKERS

Central to the success of any drug development project is ensuring that the drug reaches its target with the appropriate degree of saturation and for the appropriate duration. Because of fixed dosing as well as pharmacokinetic variation between human subjects, direct measurements of receptor occupancy are desirable. This is true in all therapeutic areas. However, development of CNS drugs poses the additional twin challenges of the blood brain barrier (impenetrable to the majority of drugs⁶⁸) and the inaccessibility of brain targets to direct measurements *in vivo*.⁶⁹ A useful undertaking for translational research for the development of CNS drugs is the development and execution of estimates of drug-receptor occupancy.

Receptor occupancy by ligand PET

Of these, the most direct is the use of PET⁷⁰ scanning using site-specific ligands radiolabeled to high specific activity with positron emitting isotopes—usually carbon 11 or fluorine 18. The high specific activity of such labels has the advantage that even miniscule, subpharmacologic doses of such ligands can register a signal detectable by a PET camera, providing a quantifiable three-dimensional image of receptor occupancy in the brain. The receptor occupancy required of the radioligand is so low that there is no physiological perturbation of the system. Radioligand PET can be used to estimate receptor occupancy of an unlabeled drug of interest, administered at pharmacologic doses, by measuring displacement of the radiolabeled tracer. Although expensive and laborious, when a suitable PET tracer is available, this is the procedure of choice for the estimation of drug receptor occupancy in the otherwise inaccessible CNS. Currently, there exist suitable PET radioligands for only a few dozen receptors,⁷¹ limiting the generalizability of this procedure. Of the PET ligands currently available for displacement studies, the most widely used include: raclopride, for assessment of D2 dopamine receptor occupancy⁷²; WAY 100635, for detecting occupancy at the serotonin 1A receptor⁷¹; and risperidone for 5HT2A receptors.⁷³

The utility of direct measurements of receptor occupancy by PET radioligand displacement is demonstrated by the example of F18 SPA-RQ, which labels the neurokinin 1 receptor, in the development of the centrally active antiemetic compound aprepitant.^{74–76} Demonstra-

tion of complete occupancy of the central NK1 receptor with an intermediate dosing regimen provided a ceiling above which no further dose escalations were warranted. This accomplished two purposes. It set the maximal dose for use of this compound as an antiemetic for which partial efficacy was demonstrated at the intermediate dose. Furthermore, it blocked further dose escalation in search of a hypothesized antidepressant effect, antidepressant efficacy being absent even when the NK1 receptors were fully occupied by the drug.

For other compounds, other values of drug occupancy may be informative, although clearly not as definitive as in the example given above. As a general rule, for receptor antagonists, which represent the largest proportion of drugs targeting seven transmembrane G protein-coupled receptors, efficacy is achieved only when the majority of receptor sites are occupied by drug. In contrast, the less frequent agonist drugs need only achieve a few percent occupancy to be effective. Further guidance to receptor occupancy may be provided by determining receptor occupancy in the effective dose range of an appropriate animal model.

When no suitable PET ligand is developed, this provides an opportunity for translational research. The development and validation of a novel PET ligand requires several years of effort by a team of specialized chemists, biologists, and additional personnel.

CSF sampling

Some investigators propose a less exact approximation based on sampling of CSF.^{77,78} Although this method is safe, tolerable, and less expensive than radioligand PET displacement, it does not offer a direct estimate of receptor occupancy, the critical variable for drug action.

Other pharmacodynamic biomarkers

A third approach to the estimation of drug-target interactions in the CNS is through the use of other pharmacodynamic biomarkers: measurable downstream consequences of drug-target interactions that may have nothing to do with the intended consequences of the drug but may represent activation of a parallel pathway. Well recognized examples are uses of pupillometry to measure activation of central autonomic pathways⁷⁹; measurement of anterior and posterior pituitary peptides to assess perturbation of the hypothalamic pituitary axis⁸⁰; and assessment of other autonomic responses.⁸¹ An electrophysiological measure known as the bispectral index and related electrophysiological measures have been used widely in clinical settings to judge the depth of anesthesia.^{82,83} The utility of these measures as more general pharmacodynamic measures have yet to be explored fully.

3) IS THE DRUG WORKING? EFFICACY BIOMARKERS

In this era of novel drug targets, it may happen that the first two translational questions were satisfied—the optimal human subjects were selected for dosing and the dosing regimen provided optimal receptor occupancy by the drug—and yet the drug has no positive therapeutic effect. What may have been a significant metabolic or physiological pathway in an animal model may not be so in a human disease. Indeed, phase 2 proof-of-concept studies are currently the major source of failure for CNS drugs, as well as others. Phase 2 registration studies demonstrate efficacy using clinical endpoints, which are often indirect and noisy. This in turn requires large sample sizes and prolonged observation. In contrast, translational studies using efficacy biomarkers and more highly selected test populations could provide a test of efficacy with smaller test groups and shorter observation periods. Only those compounds that demonstrate efficacy in such idealized translational studies, would progress to phase 2 registration studies. The potential utility of efficacy biomarkers is greatest for chronic disorders for which there is a long delay before clinical improvement can be observed reliably even though the initial beneficial effects on the disease pathophysiology may have commenced early in the course of drug treatment.

Most efficacy biomarkers are not surrogates

Efficacy biomarkers may, at the discretion of the company, be used for internal decision making even if they have not received the full degree of validation that would be required for the status of surrogates.^{84–86} A surrogate biomarker is one that tracks so closely with the clinical outcome that it may substitute for that clinical outcome as a primary endpoint in a registration study.^{88,89} To be acceptable as a surrogate, the biomarker must demonstrate that the behavior of the marker with respect to clinical outcome is not an accidental association⁹⁰ as had been the case in agents that increased radiographic bone density but did not protect against osteoporotic fractures.⁹¹ Part of that requirement is validation by more than one drug. Current surrogates are few: hypertension, cholesterol, and HIV viral titer, among them. Less validated efficacy biomarkers cannot be used as primary endpoints in registration studies. They can, however be used for risk reduction in translational studies, saving development resources for those compounds that have demonstrated some potentially useful activity in man.

Imaging efficacy biomarker for multiple sclerosis

Several widely accepted efficacy biomarkers that have not yet achieved the validation required for surrogate status involve neuroimaging. The seminal example is the use of contrast MRI to demonstrate the periodic appearance of new inflammatory demyelinating lesions during

the course of relapsing-remitting multiple sclerosis, many of them clinically silent.^{92–94} A number of putative treatments, initially the interferons, were able to demonstrate convincingly a reduction in the appearance of such new enhancing MRI lesions during controlled clinical trials. This was only a secondary endpoint in registration studies, but it gave a strong signal, bolstering the claim of efficacy supported by a much weaker signal from the primary endpoint of clinical disability score.^{95,96} This success lifted multiple sclerosis from the ranks of those diseases judged intractable by the pharmaceutical industry, to an active area of both commercial as well as academic activity.

Volumetric imaging biomarkers for stroke and Alzheimer disease

Indeed, volumetric MRI measurements are likely to be more sensitive than clinical outcome measures in other multifocal brain disorders, because of the unique juxtaposition of eloquent and clinically silent regions in the human brain. Reduction in stroke volume is acceptable to many investigators as a measure of efficacy of neuroprotectants or thrombolytics in treatment trials for ischemic stroke, even though there is as yet insufficient validation for its use as a surrogate. The recently initiated Alzheimer Disease Neuroimaging Initiative (ADNI) is systematically investigating the relationship of brain atrophy to clinical outcome in Alzheimer disease. If successful, this study may lead to the adoption of assessments of global or regional brain volume by MRI as an efficacy biomarkers for disease modifying treatment of Alzheimer disease.^{97–100}

Imaging amyloid

Other imaging methods merit investigation as potential efficacy biomarkers for disease modification in Alzheimer disease. For those therapies that aim to ameliorate brain amyloid levels, quantification of brain amyloid burden by PET imaging may prove an early efficacy biomarker.^{101–104} It seems likely that compounds working through this mechanism may favorably alter amyloid burden before cognitive improvement occurs or atrophy is halted. If so, translational studies using reduction of amyloid burden as an endpoint may be useful addition to an early development program. Other potentially useful efficacy biomarkers may come from resting^{105,106} or functional glucose uptake PET studies.¹⁰⁷

CSF biomarkers for Alzheimer disease

The use of soluble biomarkers as efficacy measures of disease-modifying treatment of Alzheimer disease and other neurodegenerative disease has lagged behind the development of neuroimaging biomarkers.¹⁰⁸ Much of the work on soluble biomarkers in Alzheimer disease has been focused on the CSF, which is not only more proximal to the CNS than blood, but also has a simpler

composition, facilitating analysis.^{109,110} The relationship between CSF amyloid and clinical stage is not straightforward, following an inverted U-shaped distribution. The relationship of tau appears monotonic: steadily rising,¹¹¹ as does the relationship for byproducts of oxidative stress.¹¹² The search for other biochemical parameters that may track with disease progression have extended to proteomic analyses of cerebrospinal fluid^{113,114} and brain parenchyma^{115,116} of patients with Alzheimer disease.

Proteomic biomarkers for Parkinson disease

Similar proteomic searches for potential efficacy biomarkers have been undertaken for another progressive neurodegenerative disorder, Parkinson disease.^{117–119} These initial searches for potential efficacy biomarkers are at an earlier stage than are those for Alzheimer disease. Analysis thus far has been limited to the CNS parenchyma in autopsy specimens, inaccessible in life as a clinical biomarker, but a potential source of protein that could be released into the CSF.

Proteomic biomarkers for schizophrenia

Proteomic analytic searches for potential efficacy biomarkers have been extended even to nonprogressive encephalopathies such as schizophrenia. Analysis of CSF in schizophrenics has demonstrated significant increases in levels of apolipoprotein A-IV, as well as other proteins.¹²⁰ It is not yet known how these alterations track with disease activity and, thus, whether they would be useful as suitable efficacy biomarkers. Proteomic analyses of brain tissue from schizophrenic brains may identify products that are released into the CSF.^{121,122} Of the studies completed to date, the most promising appears to be a combined proteomic and metabolomic analysis that has provided evidence of oxidative damage,¹²³ a finding interesting not only as source of efficacy biomarkers, but in the potential understanding of a basic disease mechanism. Corroboration or refutation of such findings is a high priority for translational research.

Proteomic and transcriptional biomarkers for pain

For certain CNS therapeutic areas, notably analgesics for either neuropathic or inflammatory pain, there is little need for molecular efficacy biomarkers to aid drug development. Efficacy can be most conveniently monitored by standard clinical endpoints such as patient report of symptoms. Nevertheless, translational studies may prove useful in elucidation of mechanism. Proteomic and transcriptional analyses of pain models have thus far been limited to preclinical species.^{124–130}

Physiological biomarkers for schizophrenia

Long known electrophysiologic abnormalities offer potential efficacy biomarkers for schizophrenia, contrary to prior expectations. Characteristic abnormalities of pre-

pulse inhibition,¹³¹ auditory gating,¹³² delay and/or diminution of amplitude of other evoked potentials have long been recognized characteristics of schizophrenia, as have been abnormalities of saccadic eye movements.¹³³ However, these abnormalities have also been observed in nonpsychotic first-degree relatives and were not altered with antipsychotic treatment of symptomatic schizophrenics.¹³⁴ For these reasons, they have been considered endophenotypic, trait biomarkers rather than markers of disease severity. As such, they not been used as efficacy biomarkers in the development of antischizophrenic drugs, most of which target positive symptoms only. However, recent findings indicate they may be worth revisiting as potential biomarkers for the development of drugs designed to ameliorate the equally problematic negative symptoms and cognitive impairment in schizophrenia, present in first-degree relatives and not significantly ameliorated, if at all, by antipsychotic medications.^{135,136}

CONCLUSION

In this challenging time for the pharmaceutical industry, translational research offers an opportunity to bridge the gap between discovery in animals and development in humans. Nowhere are the challenges or potential rewards greater than in the discovery and development of drugs for the CNS. Although formal translational research efforts in fully integrated pharmaceutical companies are rather new, enough of technical and scientific background in the understanding of human disease has already been established that many of the tools necessary for implementation are already in place. Much of this work has already been accomplished in academic, government-sponsored as well as industrial laboratories. Continued collaboration between these institutions will facilitate translation of advances made at the bench to therapies for neurological and psychiatric diseases, many of them previously thought intractable.

What is needed from translational scientists is careful selection and prioritization from these myriad of data and technical advances, only those refinements that best serve the early developmental needs of the discovery portfolio in a timely manner. Whatever the risks, the industry will not tolerate delay. A few clear successes will far surpass the impact of a more diffuse effort. This is best accomplished by close collaboration of preclinical counterparts as well as the developmental clinicians and scientists who will ultimately perform the registration studies that will bring drugs to registration and to market.

The application and interpretation of the output of the new technologies of proteomics, transcriptional profiling, metabolomics, and neuroimaging falls directly into the purview of translational research. As was predicted nearly 10 years ago,² the “learn” phase of development is

becoming more and more critical for the success rate of phase 2 studies and the eventual registration of new drugs. Translational research has become a key factor in the production of new innovative therapies.

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