

NOVEL TRENDS IN ORPHAN MARKET DRUG DISCOVERY: AMYOTROPHIC LATERAL SCLEROSIS AS A CASE STUDY

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1. ABSTRACT

As new lead discovery technologies of high throughput screening and rational drug design have been incorporated into pharmaceutical and biotechnology drug discovery programs, researchers have focused on the applying these new technologies in diseases traditionally neglected by for-profit drug discovery efforts. This article reviews general trends in orphan disease lead discovery, identifies best practices of orphan market drug discovery and provides an overview of recent ALS lead discovery programs and drug development according to these metrics. Best practices in orphan market drug discovery embodied by programs like the NIH Anticonvulsant Screening Program include the (1) management of timelines and priorities, (2) engagement of for-profit partners, (3) creative application of technology, (4) collaboration, and (5) flexibility. Recent trends in ALS lead discovery have been shaped not only by the predominance of animal models of disease over *in vitro* models, but also by the successes and

best practices of these earlier orphan market drug discovery programs. The ALS Treatment Initiative, the Johns Hopkins Center for ALS Research, the ALS Association, and the ALS Therapy Development Foundation have all initiated lead discovery programs in the past several years which seek to utilize existing experimental models of the disease and challenge assumptions about the linear nature of the lead discovery and development process. The compounds currently in clinical evaluation for ALS were identified as leads from a variety of sources, further reinforcing the transforming effect these new lead discovery programs have had on drug discovery and development in ALS. We conclude our review with an overview of the challenges and opportunities lead discovery in ALS currently faces, ultimately concluding that ALS lead discovery, and indeed orphan market drug discovery in general, would most benefit from more centralized lead discovery management, expanded national

access to core facilities for lead discovery, and matrixed simultaneous screening of multiple compounds for multiple neglected diseases.

2. OVERVIEW

Lead discovery programs seek to identify which candidate compounds have the potential to become treatments for specific diseases. These programs ask the question: do a compound's physical, biological and chemical properties qualify it as a potentially active, safe and effective treatment for use in one or more human clinical applications? To answer this question, lead discovery programs must identify the mechanisms and experimental models of the disease, characterize the biological, physical and chemical properties of compounds, recruit compounds with the appropriate properties, and ultimately determine the likelihood that a compound will safely and effectively enter a system to alter disease progression. Traditionally, these programs are applied largely to proprietary chemical libraries or patented novel compounds.

The different disciplines of lead discovery and drug development (disease modeling, ADME, toxicology, formulation and pharmacokinetics) are often conceived as a linear and segmented "assembly line," guiding uncharacterized chemical entities from creation through pre-clinical and clinical trials toward FDA approval and marketing. Indeed, most entirely for-profit lead discovery programs proceed in a systematic path from the initial creation of intellectual property through to approval (1).

However, for a number of diseases this traditional lead discovery process has proven relatively fruitless (2). Though recent trends in lead discovery overall have sought to accelerate the process of lead discovery or more accurately target compounds to diseases, researchers in a number of orphan market diseases have challenged this traditional conception of lead discovery, crossing disciplinary boundaries and challenging the linear timeline. Amyotrophic lateral sclerosis (ALS) is one of these diseases. A number of new programs in ALS lead discovery initiated within the past several years build on the strengths of recent novel lead discovery programs for other orphan market diseases ranging from epilepsy to cystic fibrosis. These programs operate on the principle of integrated research, recruitment, and development procedures.

Several features of amyotrophic lateral sclerosis make novel approaches to lead discovery particularly salient. ALS is a neurodegenerative disease in which motor neurons progressively degenerate, causing paralysis and death within two to five years. Though ALS qualifies as an orphan disease under the Orphan Drug Act of 1983, the risk of failure in accelerating compounds for ALS through early stage pre-clinical and clinical development are generally too great to justify the potential rewards (in the form of tax benefits) later in the disease. Thus traditional lead discovery efforts proceed slowly in relation to those for other diseases. In addition, serious gaps in

basic knowledge on ALS hinder the lead discovery process further: the mechanisms and biological pathways implication in the causation and perpetuation of ALS-related motor neuron degeneration are not clearly established. Because of this, basic research and clinical intervention efforts must work side-by-side, often blurring the boundaries among stages of clinical development and constantly reworking the timeline of lead discovery.

This paper reviews recent trends in ALS lead discovery, focusing on novel discovery programs implemented by researchers and clinicians in light of limited for-profit attention to the disease. To that end, although experimental models of ALS used in lead discovery are touched upon in this review, more attention is paid to the structure of these novel discovery programs than on the technologies utilized in them. These technologies are not unique to ALS; a technical review of them here would not do them justice and they have been covered extensively in other publications. The first section of this paper reviews general lead discovery trends in the past twelve years, including an overview of the aforementioned new technologies as well as the scientific "best practices" exhibited by novel lead discovery programs for orphan market diseases like epilepsy and cystic fibrosis. Next, we provide a general overview and resource guide to ALS pathology, clinical drug development parameters, animal models, and *in vitro* assays used in the process of lead discovery and development. After this, we provide a brief tour of four recent novel lead discovery programs and their structures, arguing that two of the most salient trends in ALS lead discovery are the creative application of high throughput technologies and values, and the management and alteration of the linear timeline of lead discovery. A sampling of compounds currently in clinical (or late preclinical) development for ALS reinforces this latter point, providing an overview of the multiple non-traditional sources of drugs developed for ALS. Finally, after distilling these programs' efforts into distinct trends, we conclude with an analysis of the challenges facing these lead discovery programs and reasoned speculation on future developments.

3. BACKGROUND: GENERAL LEAD DISCOVERY TRENDS, 1990-PRESENT

3.1. Overview

In recent years, a number of novel lead discovery programs have been initiated for orphan market diseases. The successes of these programs in identifying lead compounds and developing drugs, as well as the "best practices" they represent, are the foundation on which recent trends in ALS lead discovery build. Understanding these model programs, particularly in the context of novel trends in non-orphan drug discovery, allows us to better contextualize and evaluate recent efforts at ALS lead discovery.

Lead discovery, in very general terms, is the process of connecting information on chemical substances with knowledge on disease pathways to yield a candidate for future development into a drug. In recent years, this

essential step of drug development has been the subject of considerable attention and effort by researchers, clinicians, and other scientists seeking to increase the clinical productivity of the academic and pharmaceutical research industry. In the early 1990's, as it became increasingly clear that traditional strategies of drug discovery were not the most efficient process for discovering new drugs, two new scientific trends radically altered the traditional lead discovery paradigm. One strategy, high-throughput screening and its corresponding microarrays, chemical libraries, and robotics systems, sought to increase quantity of leads produced by increasing the sheer number of lead discovery experiments run (3-6). The other strategy, rational drug design, sought to increase the quality of the lead (7-9) – technologies like gene therapy, though their clinical potential has tarnished in recent years, promised to produce substances specifically designed around existing knowledge on a disease.

The success of these strategies, however, is limited to only a few diseases. Despite the promise of recent high-throughput-screening and rational drug design trends in lead discovery, there are still hundreds of diseases for which the necessary basic etiological consensus does not exist or in which the small size of the disease prohibits the investment of pharmaceutical and biotechnology discovery resources. A number of novel programs have served as alternative models for lead discovery, incorporating new technologies of lead discovery in novel ways appropriate to their specific disease. These programs cross disciplinary and functional boundaries within the research community and challenge assumptions about the predetermined linear progression of drug discovery and development.

3.2. Drug Discovery for Huntington's Disease

The Cure Huntington's Disease Initiative (CHDI), a program initiated by the Hereditary Disease Foundation in 1997, identifies lead compounds for Huntington's Disease through the strategic engagement of researchers and for-profit partners. Headed by two full-time Directors from the biopharmaceutical sector, the program recruits lead discovery proposals, largely for screening in the mouse model of Huntington's disease. The CHDI also engages scientists in comprehensive methods to identify potential therapies for Huntington's Disease. By acknowledging the different approaches to basic research used in different disciplines, and by prioritizing the screening of FDA-approved substances, CHDI pursues a policy of lead discovery that will both produce drugs for patients as quickly as possible and also contribute to knowledge of disease mechanisms. The work of this program has led to a number of approved drugs being identified as potential treatments for Huntington's Disease (10,11). By prioritizing approved drugs and linking pre-clinical efficacy observations to basic research on the disease, the CHDI not only takes charge of the timeline of Huntington's Disease lead discovery, but also challenges the assumption that lead discovery must be predicated on comprehensive knowledge on disease pathways and mechanisms.

3.3. NIH Anticonvulsant Screening Program

Formed in 1975, the NIH Anticonvulsant Drug Development (ADD) Program directly engages the pharmaceutical industry in developing novel therapies for epileptic seizures (12). Under this program, the first new epilepsy drug approved in two decades, Felbamate, was finally approved in 1993 through collaboration between ADD and a pharmaceutical sponsor (13,14). Within this broad collaboration, the NIH Anticonvulsant Screening Program (ASP) of the ADD program provides government contracts for lead discovery and early toxicology experiments in assorted assays as well as induced *in vivo* mouse and rat models of epilepsy. The specific contractors of the ASP serve as a national resource for epilepsy lead discovery, screening nearly 1000 compounds per year from a network of 300 suppliers worldwide. Most compounds have had basic characterization performed but require additional work before they can be considered a 'lead' for epilepsy. Simultaneous to efficacy and biological activity evaluation of potential leads, toxicology work evaluates safety and optimal dosing for each compound *in vivo* and *in vitro*. The anticonvulsant screening program places a high emphasis on animal models of disease as well as on simultaneous lead discovery and early pre-clinical development, blurring the boundaries between the traditional realms of discovery (bioassays) and pre-clinical development (animal models) in ways that are most appropriate to the disease at hand (15,16).

3.4. Aurora Biosciences and the Cystic Fibrosis Foundation

In 2000, Aurora Biosciences and the Cystic Fibrosis Foundation entered into a 5-year partnership to develop high-throughput assays and screen compounds for effectiveness in cystic fibrosis. Aurora Biosciences normally develops and runs high-throughput assays for specific for-profit pharmaceutical customers with proprietary chemical libraries; through the partnership with the Cystic Fibrosis Foundation, Aurora Biosciences will screen compounds from a variety of researchers. The collaboration agreement is the largest contract ever awarded by a non-profit health research organization, expected to total more than \$45 million over its duration, and is partially funded through nominal technology access fees charged of CF researchers who wish to screen a compound in the assay. Although such contracts between biotechnology companies and non-profit research organizations are common, particularly between Aurora Biosciences and other orphan disease drug discovery programs like the Cure Huntington's Disease Initiative, the Cystic Fibrosis agreement is unique in that a single entity provides coordination and project management for the screening process and any resulting clinical development. The CFF and Aurora partnership harnesses the efficiency and expertise of the for-profit industry in high throughput screening, but manages the project according to timelines and priorities that make most sense to patients (17).

3.5. Best Practices

These models emphasize the novel ways recent trends in lead discovery have been applied to neglected

diseases. There are several best practices that can be distilled from these model lead discovery programs.

3.5.1. Management of Timelines and Priorities

The organizations above, dissatisfied with the progression of lead discovery efforts in their diseases, have taken charge of lead discovery and subsequent development timelines. As a further step, several have prioritized already approved drugs for lead discovery efforts in an attempt to drastically reduce the time from lead identification to clinical use.

3.5.2. Engagement of for-profit partners

Although in many orphan market diseases for-profit pharmaceutical and biotechnology companies have difficulty justifying investment of time and resources, the programs above have all found ways to utilize the high throughput and other technological expertise of this industry toward their non-profit patient treatment and research goals.

3.5.3. Creative application of technology

Where the research knowledge does not exist to run high throughput assays in a disease, these lead discovery programs have either engaged partners in simultaneous screening and assay development programs, or applied high throughput concepts and values to other experimental models, particularly animal models of disease.

3.5.4. Collaboration

The organizations above recognize that cross disciplinary collaboration is essential to facilitating the spread of ideas and knowledge in a relatively small disease research and development community.

3.5.5. Flexibility

The preceding lead discovery programs all rely on extensive flexibility in conceptions of the lead discovery process and on the sources of potential lead compounds.

These practices outline a policy of lead discovery that is quickly becoming the standard for orphan market disease lead discovery. As we shall later see, these practices directly inform novel lead discovery programs for ALS.

4. ALS: CLINICAL CHARACTERISTICS AND EXPERIMENTAL RESOURCES

4.1. Overview

Amyotrophic Lateral Sclerosis (ALS), also called Lou Gehrig's disease, is a fatal neurodegenerative disease affecting motor neurons of the cortex, brain stem and spinal cord (18). Onset of ALS occurs anywhere from the late teens to the late eighties, although median onset is age 57, and is generally fatal within two to five years after diagnosis (19). Depending on the course of the disease, the symptoms can appear as general muscle weakness, loss of fine and/or gross muscle control, and eventually loss of visceral muscle activity creating difficulties in life-sustaining conditions such as swallowing and breathing

(20-22). ALS affects approximately 30,000 Americans with anywhere from 3,600 to 8,000 deaths reported in the US each year (23-25).

4.2. Trends in ALS Basic Research

The cardinal feature of ALS is the loss of spinal motor neurons, which causes the muscles under their control to weaken and waste away leading to paralysis. Generally, this paralysis is initially localized and progresses asymmetrically. It may present in the upper or lower motor neurons, known colloquially as "bulbar" or "limb" onset. ALS has both familial (5-10%) and sporadic forms and the familial forms have now been linked to several distinct genetic loci (26, 27). About 15-20% of familial cases are due to mutations in the gene encoding Cu/Zn superoxide dismutase 1 (SOD1) (28).

Although a great deal is known about the pathology of ALS little is known about the pathogenesis of the sporadic form and about the causative properties of mutant SOD protein in familial ALS (20). Many models have been speculated, including glutamate toxicity (29), mitochondrial dysfunction (29), trace element and environmental toxicity (30), and oxidative stress (31). However, though most models appear to have direct relevance to observed biochemical phenotypes of the disease, no single model has comprehensively described the disease pathogenesis or causation.

Recent new data has suggested that greater attention be focused on the role of the immune system in propagating the disease. Though ALS is not considered a classical autoimmune disease there is growing evidence supporting the involvement of microglia and the innate immune system in the progression of the disease. For quite some time, changes in immune function such as complement deposits in neurons, anti-neuronal antibodies, Ca++ channel antibodies, anti-Fas antibodies and increased incidence of lymphoproliferative disorders have been observed in sporadic ALS patients (29-44). In addition, immune reactivity in transgenic mouse models of ALS correlates with disease progression (45). Gene chip analysis of both human post mortem ALS and murine ALS spinal cord demonstrated significant increases in macrophage/microglial activation markers as compared to normal (46-49). Neutrophilia associated with increased metabolic rate, increased oxidative stress in the plasma, and increased levels of IL-6 in skin and serum are all detected in ALS patients (50-54). Supporting these findings, immuno-modulatory drugs such as Celebrex, a JAK3 kinase inhibitor and cyclosporin that affect a variety of targets in the immune system show beneficial effects in the mSOD mouse model (55-57). The role of the immune system appears to be a promising topic of current and future research and drug development.

4.3. Clinical Development

Riluzole, manufactured and marketed by Aventis since 1995, is the sole drug approved to treat ALS. Current data suggests the drug has only a moderate effect, slowing disease progression and prolonging life by anywhere from 2.3 months to 1 year (60-62). Clinical development of

drugs in ALS has proven difficult in the past; most trials have been powered such that only drastic changes in disease progression can achieve significance; their time frame has often been quite short – 3 months in many cases to about 1 year for Phase III trials – and the number of patients enrolled is too low to generate statistically significant moderate effects. In addition, further complicating clinical evaluations of ALS drugs, most ALS patients self-medicate with a cocktail of approved drugs, off-label drugs, and nutraceuticals that affect multiple observed biological contributors to disease progression, including glutamate inhibitors, anti-oxidants, and anti-apoptotic drugs (58,59). In many cases, patients do not report alternative therapies or nutraceutical medication to their physicians, which can complicated the design and implementation of controlled clinical trials in ALS.

Though clinical development has been complicated by difficulty in measuring clinical outcomes and the lack of standard biomarkers of disease progression, in recent years several novel endpoints have been proposed to improve the clinical evaluation of drugs for ALS. Although measures of survival were used to demonstrate efficacy for Riluzole, such measures have been criticized as inefficient for measuring outcomes of trials under 18 months duration (63). Alternatives that have been proposed include natural history trials based on the Functional Rating Scale, a widely accepted measurement of ALS progression based on patients' answers to a questionnaire on their ability to perform activities of daily living (64,65). In addition, a variety of alternative tests of muscle strength and motor neuron loss have been proposed, including maximal voluntary isometric contraction (66), hand-held dynamometry (67), sniff nasal pressure (68), and motor unit number estimates (69-71). Though many of these tests appear to promising diagnostic tools, few have been implemented in a controlled clinical trial setting.

4.4. Animal Models

One of the strongest areas of ALS research and drug development has traditionally been the animal models used to research the disease and its potential treatments. These models are predominantly murine and produce a variety of ALS-like diseases with similar observed pathology and progression similar to the human clinical course of the disease. Additionally, researchers have created transgenic *drosophila* and *c. elegans in vivo* models but preliminary studies indicate that the disease translates poorly to non-mammalian species: the *drosophila* model has no visible phenotype and the *c. elegans* model shows only a biochemical phenotype (72-76).

4.4.1. SOD G93A Transgenic Mouse

In 1993, Rosen *et al.* published their definitive paper linking mutations in superoxide dismutase to nearly 20% of all familial ALS cases (77). The first transgenic mouse model for ALS, based on this mutation, was developed the following year (78-82). These animals show many similarities in pathology and disease progression to the human familial and also the sporadic form of ALS (83). Most interestingly this model was used in testing the efficacy of Riluzole (post FDA approval); murine results

mirrored observed clinical efficacy (84). Currently the SOD1 G93A model is one of the most widely used models in ALS research, used in over 110 published papers over the past 9 years. Over 25 papers have been published on various drugs' efficacy in the SOD mouse alone (84-108). A number of other SOD mutations, including G37A and G85R, have also been used to create transgenic mice, all of which developed ALS-like symptoms. Although it appears that a low copy version of the mouse may have the phenotype that most closely resembles the human disease, high copy transgenic mice are most frequently used for these efficacy studies: the low copy live for more than 400 days; high-copy mice succumb to ALS by day 120 – 150 (109).

4.4.2. Wobbler Mouse

The Wobbler mouse is bred to develop murine disease that bears some similarity to human ALS. However, the disease appears quite early in the lifespan of the mouse, at around 3 weeks of age (110). In addition, symptoms appear mainly in the forelimbs and diaphragm (111). The Wobbler mouse, however, has been partially accepted as a suitable model for exploring the efficacy of novel compounds against sporadic ALS. A number of papers have been published on drug efficacy in the Wobbler model; however, recent research suggests that certain compounds may have a beneficial effect in the Wobbler model that is only minimally relevant to clinical practice (112-119). Because the genetic cause of the disease is not known, use of this model in efficacy screening or lead discovery is problematic.

4.4.4. Other models

Other murine diseases that bear some similarity to human ALS include murine progressive motor neuropathy (120), murine motor neuron disease (121), and wasted mouse syndrome (122). Both wasted mouse and progressive motor neuropathy (*pnn*) are characterized by early onset and a rapid clinical course: the average lifespan of each is, respectively, 4 weeks and 7 weeks. The Motor neuron disease mouse lives much longer, developing the disease at around six months of age and dying five months later (110). Alternative transgenic models of ALS include a mouse bred to over express human genes encoding mutant neurofilament subunits (123) and a mouse bred to over express Interleukin 3, which develops an auto-immune related ALS-like disease (124). In addition, a novel gene has recently been identified that causes a juvenile form of familial ALS. Animal models based on this mutation are currently in development (125-127).

4.5. Assays

Tissue culture methods allow detection of injury at the cellular level and also allow detection of cellular interrelationships in the protective or deleterious effects of a particular compound. Mixed cultures including astrocytes, neurons and sometimes microglia allow better dissection of the biological process on a molecular and cellular level as compared to *in vivo* experimentation. Most screening assays in ALS have focused on the general vulnerability of normal neuronal cell lines to injury such as glutamate, growth factor withdrawal, ALS sera or free

radical damage (128-134). More specialized assays to determine the selective vulnerability of primary culture motor neurons have also been developed. Understanding the basis of selective motor neuron susceptibility is a key issue in ALS. However, the absence of supporting cells significantly hinders these cultures' suitability to detecting clinically relevant mechanisms and pathways. Organotypic cultures such as dissociated fetal spinal cord or spinal cord slice cultures have allowed detection of the complex interrelationship between astrocytes and neurons (135). The discovery of the neuroprotective role of EAAT2 by Rothstein *et al.* used this approach (136, 137). More recently the role of the intrinsic CNS immune system in the form of microglia has been investigated through complex organotypic cultures (138). Other novel approaches in ALS assays have focused on the formation of intracellular protein aggregates in ALS (139, 140). The tissue culture exploration of the formation of ubiquitin reactive inclusion bodies and high molecular weight SOD aggregates, for example, is expected to expand the breadth of knowledge on ALS pathology and accelerate the drug discovery process.

5. NOVEL LEAD DISCOVERY PROGRAMS FOR ALS

5.1. Overview

As discussed earlier, several complications to basic knowledge on and clinical characteristics of ALS have hampered traditional and late 20th century methods of lead discovery. In forty years of research on the disease, the pharmaceutical industry has produced a single approved drug for ALS that only moderately extends survival. As researchers and clinicians became increasingly concerned about the lack of effective treatments for ALS, attention focused on lead discovery and development programs that were isolated from patient and physician input. In 1997, Dr. Theodore L. Munsat presented a paper to the World Federation of Neurology Research Group on Motor Neuron Disease entitled "The Therapeutics of ALS" (141, 142). In his presentation, he noted the importance of interrelationships among concerned ALS communities in advancing lead discovery and drug evaluation efforts. More importantly, Dr. Munsat encouraged clinical investigation via poly-pharmaceutical trials to test the potential for drug cocktails to affect disease progression, a move which would force collaboration among pharmaceuticals and between the industry and the academic researchers interested in already-approved compounds. Munsat's official publications through leading scientific journals echoed the themes of his 1997 paper (143-146). This and other early statements, though they mainly expressed dissatisfaction with then-contemporary lead discovery programs and offered only general suggestions for remedies, fed a growing sense of frustration in the clinical and research community. In the years immediately before the new millennium, several novel lead discovery programs were initiated to address these frustrations.

5.2. ALS Treatment Initiative

The ALS Treatment Initiative (ALSTI), founded in August 1998, united ALS interest groups, institutions, and scientists to advance applied research on the disease

into tangible treatments for ALS patients (147). The collaboration of leading ALS researchers marked the first active step in advancing effective ALS lead discovery programs. The scientific board included Drs. Steve Gullans and Robert Brown of Harvard, Drs. Serge Przdborski and Lewis Rowland of Columbia, Dr. Jeffrey Rothstein of Johns Hopkins, and Dr. Flint Beal of Cornell. The ALSTI agenda was defined by four goals: increase research in the field of Functional Genomics, search for potential markers of ALS onset and progression, develop a bioassay for high-throughput screening, and construct a program based on drug libraries and high-throughput screening technologies for rapid lead discovery. The outlined scope of the ALSTI program concurrently fills gaps in applied research abilities in ALS and creates high-throughput leads discovery programs to immediately apply new knowledge to the identification of potential treatments for ALS. But these two arms of ALSTI are not officially separated; leading researchers and clinicians expected high-throughput screening data to inform the search for biological markers of ALS onset and progression and *vice versa* – a step that challenged the linear architecture of disease research, leads discovery, and drug development. In addition, ALSTI supports the sharing of important scientific and technological advancements with other ALS research efforts.

ALSTI remains an active collaborative forum for ALS researchers and is spearheading the movement toward efficient strategies in drug screening and treatment development performed in academic settings. ALSTI has not publicly released a list of the cooperative findings, and is not funded as a research facility, but many of these findings have been published as separate papers by the individual researchers involved. The laboratory of Dr. Steve Gullans remains active in Gene Expression Research and the FDA 2000 project, a high-throughput screening program to identify neuroprotective properties of FDA-approved compounds. Currently, Dr. Robert Brown is developing a program through ALSTI to promote the evaluation of compounds currently in clinical development for other neurological conditions as potential treatment options for ALS. The ALS Treatment Initiative has operated, however, on only a very limited scope in recent years due to funding concerns; most official programs of the initiative are on hold.

5.3. John Hopkins Center for ALS Research

The Center for ALS Research at Johns Hopkins University (148), directed by leading ALS researcher Dr. Jeffrey Rothstein, provides grants for ALS related research and encourages collaboration among these researchers. Last year, the Center awarded \$2.5 million in grants to 22 researchers. Ten of these projects are conducted off-site; the remaining 12 research projects are conducted at the ALS Center. The ALS Center contract requires both internal and external investigators to attend monthly meetings and yearly symposia therefore providing a forum for research collaboration. More importantly, the contract clearly specifies the types of data disclosure and information sharing that constitutes successful attendance. By removing the barriers between individual research

laboratories, Rothstein's program ensures a free and open flow of ideas and cross-disciplinary collaboration.

Research projects at the Center for ALS Research are divided between those that target basic molecular and cellular mechanisms of ALS and those that investigate therapies that might affect these mechanisms. Similar to ALSTI, the strategic research plan of the Center for ALS Research simultaneously targets biological mechanisms and therapeutic interventions. Within the Center for ALS Research, the specific agendas of investigators and staff are defined through assignment a team(s) with clear goals and responsibilities. These teams include: Basic Mechanisms of the Disease, Models of Human Disease, Experimental Therapy, Clinical Investigations, and Clinical Trials. In addition to funding and organizing ALS research, the ALS Center provides valuable resources such as cell models, animal models and facilities to ALS researchers.

5.4. ALSA/Hereditary Disease Foundation Collaborative

The ALS Association (ALSA), the Hereditary Disease Foundation (HDF) and the Huntington's Disease Society of America (HDSA) recently joined in a Collaborative Initiative to encourage screening of FDA-approved compounds for a variety of late-onset neurodegenerative diseases (149-151). Funded by the National Institute of Neurological Disease and Stroke (NINDS), the Collaborative Initiative sponsors efforts to bridge screening procedures and ultimately, to increase treatment options in the areas of Huntington's disease (HD), Alzheimer's disease (AD), and ALS. The researchers involved in this project compare the effects of over 1000 FDA-approved compounds in twenty-seven assays in five separate laboratories, developed for HD, ALS, or AD. Findings from preliminary screenings of these common compounds are to be shared publicly in database format. The original intent was that online publication of data through alternate routes would reduce the time it takes research results to move from the laboratory into the knowledge base on the disease; however, this issue (and the potential lack of peer review controls) has been contested among researchers. The data from these studies is, at the time of publication, still embargoed, and there have been few firm decisions made on the nature of its eventual release. Similar to other lead discovery programs in ALS, researchers intend to extract basic research information on the disease from indication of compounds' efficacy and biological activity. These outcomes will provide material for understanding the overlap in degenerative disease research through highlighting the (possible) pathogenetic commonalities among HD, AD, and ALS. Moreover, this collaborative effort will establish a more comprehensive list of assays suitable for investigating leads in ALS.

5.5. ALS Therapy Development Foundation

The ALS Therapy Development Foundation (ALS-TDF), a not-for-profit biotechnology company, was founded in 1999 (152). In mid 2001, the Foundation launched a core facility for ALS lead discovery and development that would rapidly become the largest *in vivo* drug screening facility for ALS. Operating on the premise

that the SOD G93A transgenic mouse model of ALS was the most likely to provide clinically relevant results, the newly formed Research & Development team at the foundation developed a process for "high-throughput *in vivo* screening." While high-throughput screening assays may test thousands of compounds simultaneously, "high throughput" in an *in vivo* context translates into initiating four to eight animal efficacy studies per month. Each study is performed according to standard protocols based on drug delivery; only the specific drug administered changes from study to study. A comprehensive formulations team and laboratory staff recruited from the pharmaceutical industry rather than academia ensure for-profit level efficiency, process improvement, and quality control. In the first twelve months of operation, ALS-TDF initiated fifty drug studies of FDA-approved drugs, nutraceuticals, and compounds in late-stage clinical development for other relevant indications. ALS-TDF is engaged in partnerships with twelve pharmaceutical and biotechnology companies to obtain these late-stage clinical compounds for screening and to plan future drug development collaboration when appropriate.

More importantly, ALS-TDF makes explicit the connection between non-profit-initiated drug discovery and patients. Drug screening decisions and scientific projects are prioritized according to their ability to deliver the highest theoretical efficacy to patients on the shortest timeline. ALS-TDF also prioritizes clinical actions based on their ability to provide patients with access to promising approved treatments, rather than exclusively develop new commercial drug entities. Clinical researchers at the Foundation have prepared documents enabling physicians to obtain compassionate access for their patients to certain novel and promising treatments for ALS. In addition, the Foundation recently managed the first intrathecal transplantation of stem cells (153).

Entirely funded through private donations, ALS-TDF's drug discovery projects are based on the observed best practices of existing programs in ALS lead discovery. In exploring the novel "not-for-profit biotech" business model, the Foundation seeks to further leverage the value of academic/clinical research and the non-profit mission with the efficiency and management expertise of for-profit drug discovery and development organizations (154).

6. SOURCES OF RECENT DRUGS IN CLINICAL DEVELOPMENT

6.1. Overview

The profile and categories of drugs currently in pre-clinical and clinical phases of investigation give a more complete picture of the novel alterations to the traditional timelines of lead discovery programs. A large percentage of the lead compounds that are later developed clinically come from "non-traditional" sources – i.e. drugs in simultaneous development for other indications, nutritional supplements, leads identified through clinical practice and disease interactions, and potential leads identified through patient advocacy and self-medication. Although the presence of these types of novel lead sources is fairly

common in other diseases, in ALS these sources of leads are unusually predominant. Below, we have summarized several compounds each in a variety of categories of lead sources in recent years. This is not intended to be a comprehensive list of drugs in development; other reviews and resources provide much more detailed analysis and information on current drug development in ALS (155, 156). The intent is to show the extent to which the diverse discovery and research agendas of academic institutions and non-profits are matched by extensive diversity in the types of treatments that enter clinical and late pre-clinical testing.

6.2. Traditional Lead Discovery

6.2.1. AVP-923

Avanir Pharmaceutical's novel compound AVP-923, a mixture of dextromethorphan hydrobromide and quinidine, is currently in clinical evaluation for pathological laughing and crying observed as a common but secondary symptom of ALS and other neurodegenerative disorders. Pathological laughing and crying, also referred to as emotional lability or pseudobulbar symptoms is a puzzling complication of ALS (157). However, recent studies have indicated that its prevalence is overestimated (158). The Phase II/III trial is open for enrollment at the publication of this review.

6.2.2. Myotrophin

Cephalon's biologic Myotrophin, also known as insulin-like growth factor, is currently under clinical investigation in a two-year phase III trial with the unusual primary endpoint of manual muscle testing. Insulin-like growth factor appears to be implicated in disease progression (159), and has shown efficacy in induced *in vivo* models of neurodegeneration (160). Previous clinical trials have been inconclusive on the efficacy of similar compounds (161).

6.3. Theoretical Secondary Indications

6.3.1. Celebrex

Celebrex, manufactured by Pharmacia, is currently approved for use in treating arthritis. A powerful COX-2 inhibitor, the drug was introduced as a potential treatment for ALS through *in vivo* studies performed by Dr. Jeffrey Rothstein and the Center for ALS Research (162). Previous research demonstrated increased expression of COX-2 in the neurons and glial cells of the anterior horn of SOD mouse spinal cords, and in post-mortem spinal cord samples from patients (163). In addition to the *in vivo* studies, Rothstein had earlier demonstrated efficacy of COX-2 inhibition in *in vitro* models of ALS (164).

6.3.2. NeotrofinT

NeotrofinT, leteprenim potassium, is currently being developed by Neotherapeutics for use in Alzheimer's Disease. Based on preliminary results in Alzheimer's (165), it is now in late-stage pre-clinical evaluation for ALS. A purine derivative, NeotrofinT appears to promote transcription of genes encoding neurotrophic factors, though it remains unclear whether this trophism is limited to specific populations of neurons. Development is still in the pre-clinical phase for ALS and other neurodegenerative diseases.

6.4. Vitamins, Minerals, and Dietary Supplements

6.4.1. Coenzyme Q10

Coenzyme Q10 is a nutritional supplement that plays a key role in electron transport and free radical scavenging. Its metabolic and anti-oxidant properties have long been connected to observed pathology in ALS (166). *In vitro* and *in vivo* data suggest a beneficial effect of administration (167,168). In addition, an earlier high-dose pilot study by the Eleanor and Lou Gehrig Center at Columbia appeared to produce promising results meriting further study (169). Trials are currently proceeding on a pilot basis to assess the potential for future controlled trials of coenzyme Q10 in ALS.

6.4.2. Creatine

Creatine is another widely available nutritional supplement involved in muscle metabolism in ALS. Creatine appears to be neuroprotective in the SOD transgenic mouse model of ALS (170). Early clinical data indicates creatine increases voluntary isometric muscular contraction. Gains in function were observed after 7 days of high dose creatine supplementation, but decline at expected rates of disease progression over the next six months. The current trial will test the effects of chronic creatine administration over nine months, with muscle strength, muscle size, and breathing capacity as the major endpoints.

6.5. Clinical Observations

6.5.1. Tamoxifen

A dose-ranging clinical trial of Tamoxifen in ALS was initiated after an ALS patient suffering from breast cancer experienced improvement in her ALS symptoms while treated with Tamoxifen. Tamoxifen appears to operate through a number of pathways implicated in ALS, including estrogen elevation (171) and subsequent inhibition of protein kinase C (172). The relation of motor neuron disorders to cancer has long been the subject of some debate; it is unclear whether drugs that act on paraneoplastic syndrome-related motor neuron phenotypes will also affect the sporadic disease (173); the tamoxifen trial will hopefully shed some light on this area of research.

6.5.2. Indinavir

In a similar clinical situation, trials of indinavir, a protease inhibitor approved to treat HIV, were initiated after two case reports in Neurology indicated an HIV-related motor neuron phenotype appeared to be affected by standard HIV treatments (174,175). However, the clinical relevance of the HIV-related syndrome to ALS are currently unclear. In addition, given the increasing data on potential aberrant immune activation in the pathogenesis of ALS, it remains to be seen whether indinavir, a CD4 lymphocyte anti-apoptotic, would have any beneficial effect at all.

7. CONCLUSION

Novel lead discovery programs in ALS have faced, and will face in the coming years a number of serious obstacles to success. One of the most pressing obstacles is funding. As we have seen, the ALS Treatment Initiative has been all but dismantled by funding shortages. Other institutions,

though currently at adequate levels of funding, could potentially double their lead discovery efforts (and, one assumes, increase the number of lead compounds identified per year) with sufficient funding. These organizations encounter the same problem that dissuades pharmaceuticals and biotechnology companies from investing in the disease: a lack of money. The most successful orphan market drug discovery programs have been funded by visionary individuals or through large government grants, providing them the flexibility to execute quickly and effectively on lead discovery plans and bring promising compounds to clinical evaluation.

Additionally, these organizations face the challenge of maintaining legitimacy while presenting data in unorthodox and potentially controversial mediums, such as online databases. Current research, at least in the academic and medical *milieu* in which these institutions operate, is legitimized by publication in major medical and scientific journals – peer review, though it may delay the official release of results, exerts a quality control process on the act of publication that judges results according to the oversight of an objective third-party panel. Projects like the ALSA/HDF/HDSA collaboration and our own foundation (ALS-TDF), because they work at the intersection of the academic research community and the for-profit pharmaceutical industry, must carefully contextualize the ways they present their research, data, and operations to both sides of this spectrum. These organizations must be doubly cautious due to their close relationship to patient populations and the constant potential for data and research to be misconstrued by desperate populations as medical advice. These foundations face the challenge of working out the aforementioned issues while operating on timelines that value the earliest possible release of information.

In addition, if we consider lead discovery for *all* orphan market diseases, current policies of disconnected and independently operating screening programs – all operating on the same principles, using similar technologies, and working with the same suppliers and for-profit partners – point to inefficiencies in the disease-by-disease strategy of orphan market drug discovery. All four novel lead discovery programs, particularly ALSA/HDF/HDSA and the ALS Therapy Development Foundation, have found their efforts significantly enhanced through collaboration with researchers and lead discovery programs in *other* orphan market diseases, particularly the neurodegenerative diseases. This, together with the similar form that other orphan market drug discovery programs have taken, seems to suggest that the next big trend in lead discovery for these neglected diseases will involve coordinated lead discovery efforts for multiple diseases. This might take the form of a lead discovery program based on a network of patented and generic drug manufacturer suppliers and a national core facility capable of simultaneously screening multiple compounds for *in vitro* and *in vivo* efficacy in multiple neglected diseases. Or, it might simply take the form of an institute or consortium coordinating separate efforts, negotiating shared resources, collaborating buying groups to reduce the price of for-profit

collaboration, and serving as a national think-tank and consulting resource for affiliate lead discovery programs.

Lead discovery technologies for all diseases have the potential to experience dramatic advances in the next decade. But at their heart, lead discovery programs will always focus on a single task: connecting a compound to a disease in which it might have efficacy. Lead discovery programs in diseases traditionally served poorly by the for-profit lead discovery community will need to incorporate existing and future technologies into uniquely efficient orphan market drug discovery programs that utilize the strengths of the research and patient communities they serve. It remains to be seen how soon these programs will develop or from which discipline the leaders will emerge.

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