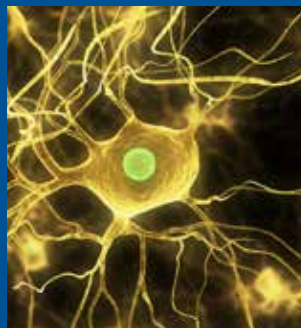


Amyotrophic Lateral Sclerosis Research Program



Congressionally Directed Medical Research Programs

CDMRP VISION

Transform healthcare for Service Members and the American public through innovative and impactful research

MISSION

Responsibly manage collaborative research that discovers, develops, and delivers health care solutions for Service Members, Veterans, and the American public

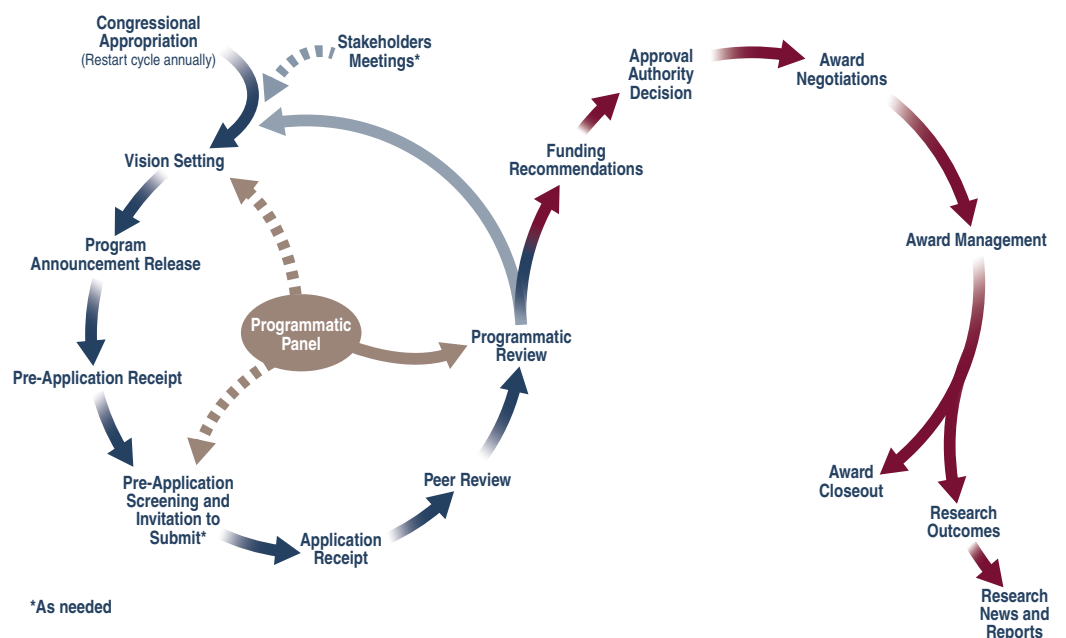
History

The Office of the Congressionally Directed Medical Research Programs (CDMRP) was born in 1992 from a powerful grassroots effort led by the breast cancer advocacy community that resulted in a congressional appropriation of funds for breast cancer research. This initiated a unique partnership among the public, Congress, and the military. Since then, the CDMRP has grown to encompass multiple targeted programs and has received over \$8.6 billion in appropriations from its inception through fiscal year 2015 (FY15). Funds for the CDMRP are added to the Department of Defense (DoD) budget, in which support for individual programs, such as the Amyotrophic Lateral Sclerosis Research Program (ALSRP), is allocated via specific guidance from Congress.

CDMRP Program Cycle

Two-Tiered Review Process

The CDMRP uses a two-tier review process for application evaluation, which is critical to ensuring that each of the research program portfolios reflects not only the most meritorious science, but also the research that best meets the program goals. The first tier of evaluation is a scientific peer review of applications measured against established criteria determining scientific merit. The second tier is a programmatic review, conducted by a Programmatic Panel composed of leading scientists, clinicians, and ALS consumers. In this tier of review, the Programmatic Panel compares the applications to each other and makes recommendations for funding based on scientific merit as determined in peer review, potential impact, relevance to program goals, and portfolio composition.



Amyotrophic Lateral Sclerosis Research Program

History

Amyotrophic Lateral Sclerosis (ALS), also known as “Lou Gehrig’s disease,” is an incurable, degenerative neurological disorder. The CDMRP ALSRP is guided by a vision to improve treatment and find a cure for ALS. The ALSRP was created in FY07 with a \$5 million (M) appropriation. Although the ALSRP was not funded in FY08, Congress subsequently appropriated funding in FY09 and has continuously provided funding since then, with a total appropriation of more than \$54M, including \$7.5M in FY15. Through its award mechanisms and funding recommendations, the ALSRP supports innovative preclinical research to develop new treatments for ALS.

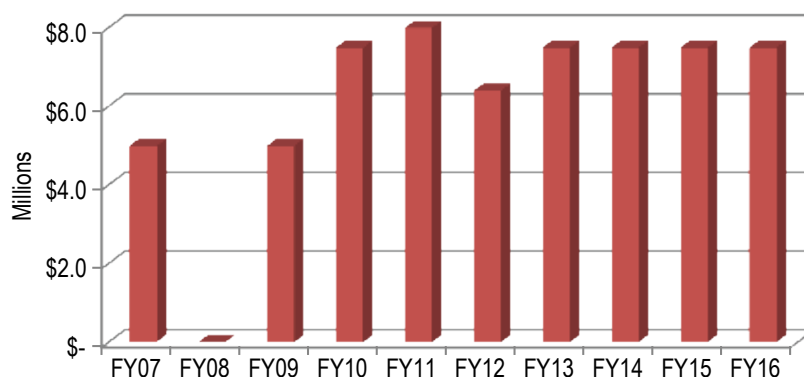
The ALSRP has focused on awards that support preclinical development of therapeutics for ALS. Areas of emphasis include development and/or validation of high-throughput screens to define targets with therapeutic potential identification of lead agents for ALS treatment, or the development of pharmacologic agents through the adsorption, distribution, metabolism, excretion, and toxicity (ADMET) stage or Investigational New Drug application submission.

ALSRP VISION

Improve treatment and find a cure for ALS

MISSION

Fund innovative preclinical research to develop new treatments for ALS



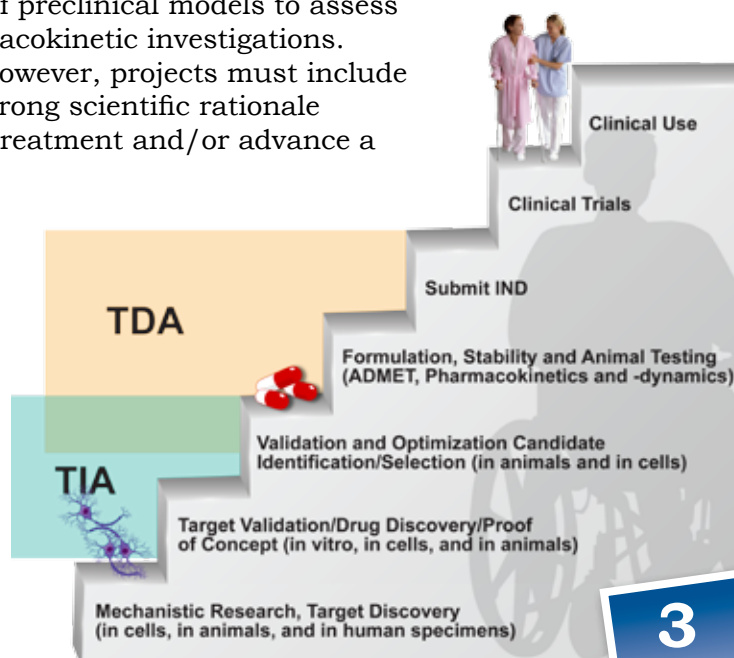
ALSRP Research Mechanisms

Therapeutic Idea Award (TIA)

First offered in FY10, the TIA is supports new ideas aimed at early stage drug/target discovery and development beyond the stage of investigations of ALS pathophysiology. These awards can include target and drug identification efforts including development, validation and use of high-throughput screens, development and validation of preclinical models to assess lead compounds, and pharmacological and pharmacokinetic investigations. Presentation of preliminary data is not required; however, projects must include a well-formulated, testable hypothesis based on strong scientific rationale that holds translational potential to improve ALS treatment and/or advance a novel treatment.

Therapeutic Development Award (TDA)

First offered in FY07, the TDA is intended to support more advanced preclinical research aimed at translating therapeutic compounds and other treatments closer to clinical trials. The TDA is narrowly focused on moving a specific previously identified candidate or narrow class of candidates along the pipeline toward the clinic. Research supported by this mechanism includes validation of leads, demonstration of target selectivity and mechanism of action, and efforts to optimize the therapeutic or pharmacologic properties of candidates.



Unique Partnership for ALS Research

FY16 Programmatic Panel:

Bryan Traynor, M.D., Ph.D., M.M.Sc., M.R.C.P.I. (Chair)
National Institute on Aging, National Institutes of Health

Lucie Bruijn, Ph.D.
(Chair Emeritus)
ALS Association

Pierre Drapeau, Ph.D.
University of Montreal

Amelie Gubitza, Ph.D.
National Institute of Neurological Diseases and Stroke, National Institutes of Health

Jim Humay
Les Turner ALS Foundation

Carlos Maldonado, Ph.D.
U.S. Department of Veterans Affairs

Larry Mink, Ph.D.
ALS Association, Michigan

Lyle Ostrow, M.D., Ph.D.
Johns Hopkins University

John Ravits, M.D.
University of California at San Diego School of Medicine

The impact of the DoD ALSRP can be attributed to the collective wisdom and synergistic efforts of many talented and dedicated ALS patients and/or their family members (i.e., consumers), clinicians, scientists, and the military. This partnership brings together stakeholders that typically might not collaborate, to help shape the ALSRP and accelerate research to accomplish a vision of improving treatment and finding a cure for ALS.

The Programmatic Panel is composed of prominent members of the ALS research community, including scientists representing the National Institutes of Health as well as the non-profit advocacy community (ALS Association) and academia. All aspects of the ALSRP, including setting program priorities, designing funding opportunities, evaluating and recommending applications for funding, and conducting high-impact research, integrate the diverse expertise of these scientists with the perspectives of consumers. During the annual vision setting meeting, the Programmatic Panel advises the ALSRP on programmatic focus and areas of research interest that can be addressed through certain award mechanisms. Later in the program year, the Programmatic Panel meets to recommend to the ALSRP which applications best fulfill the program's vision and mission while also demonstrating cutting-edge research to benefit ill ALS patients. This innovative approach is a proven and effective way to support and advance research that reflects the needs of survivors and their families, as well as the clinicians who treat them.



"The ALSRP is an important program supporting cutting edge research. Over the years, this program has become a stalwart of the ALS research community. This has been achieved through rigorous and fair review process, together with careful governance of how funds are spent. The overarching aim of the program is to enable research that will ultimately lead to effective treatments."

— **Bryan Traynor, M.D., Ph.D., M.M.Sc., M.R.C.P.I. (Chair ALSRP)**
National Institute on Aging, National Institutes of Health



"Through my service on the ALSRP integration panel for the last years, I have been struck by the consistent thoughtfulness of the reviewers, and the overall quality and fairness of the review process. Equally impressive is the genuine effort to constantly improve the manner in which the CDMRP for ALS serves the mission of funding preclinical research to support the development of therapeutics for patients with ALS."

— **Michael Benatar, MBChB, DPhil**
Professor of Neurology and Walter Bradley Chair in ALS Research at the University of Miami

Consumers Serve Critical Roles in the ALSRP

A unique aspect of the CDMRP is the active participation of consumer advocates throughout the program. This innovative approach, recommended by the National Academy of Sciences' Institute of Medicine and adopted by other funding organizations, has proven to be a highly effective way to evaluate research applications for their potential to meet the program's goals for those we seek to serve.

Consumers are a vital part of all CDMRP programs as they represent the collective views of survivors, patients, family members, and those affected by and at risk for a disease. Consumers for the ALSRP are ALS patients and supportive family members. They sit side by side with research professionals on both peer and programmatic review panels, and their voices play a pivotal role in maintaining an appropriate focus within the program.

"I believe that as a Consumer Reviewer I can bring into the process the perspective of an actual ALS patient who is living with this progressive disease - the viewpoint of how someone with the disease feels about how the proposals, if successful, would affect him and others in the ALS community. I feel that my participation could help make a difference for those diagnosed with this disease now, and more so for those yet to be diagnosed. The scientific reviewers enlightened me on their efforts to find a cure. There are so many talented researchers/scientists diligently working on new ideas, which is so encouraging to those diagnosed with the disease."



— **Fred Carlson**

**Robert Packard Center for ALS Research
Consumer Peer Reviewer**

"My wife and I joined the battle against ALS after losing both our mothers to the disease, only three years apart. Since that time, a great deal has been learned about the causes of ALS, but there is much work to be done to get to an effective treatment. It has been my privilege to be part of the ALSRP Programmatic Panel as a consumer reviewer. The quality of research projects that ALSRP funds gives me hope that effective treatments for this horrific disease will be found in the not too distant future."



— **Larry Mink, Ph.D.**

**ALS Association, Michigan
Programmatic Panel Member**



Consumer Reviewer Spotlight



Matt Bellina: “ALS is a worthy adversary, but each day I am hopeful.”

Matt Bellina had been a naval aviator flying the EA-6B Prowler out of Naval Air Station Whidbey Island on Washington’s Puget Sound when the twitching and loss of coordination began. By the time he was given a preliminary diagnosis of amyotrophic lateral sclerosis (ALS) in January 2013, Matt had already been grounded due to his worsening symptoms, and he had relocated to Minnesota. The diagnosis was confirmed in April 2014 when Matt was only 30 years old. His life as an active father of two boys, ages 1 and 3, was completely turned around as he had been forced into administrative duty and faced upcoming medical retirement from the Navy.

While living with the preliminary diagnosis, Matt connected with Dr. Carol Hamilton from the ALS Therapy Development Institute (ALS-TDI). After learning more about ALS-TDI, Matt was inspired to drive to Cambridge, Massachusetts, to meet Carol and see the ALS-TDI facility. This led to helping with fundraisers for that facility, as well as the ALS Cellucci Fund at the University of Massachusetts and Johns Hopkins University’s Packard Center, along with events involving Major League Baseball’s New York Yankees (Lou Gehrig Memorial Game) and Philadelphia Phillies (Phillies Phestival). Matt was also selected to help lead an ALS-TDI awareness campaign called Young Faces of ALS (YFALS). Serving as an YFALS Ambassador has given Matt the opportunity to network with many ALS patients and their families, and to be a catalyst for accelerating research at ALS-TDI. Matt has also participated in a gene mapping program within the ALS clinic at the University of Pennsylvania, and ALS-TDI’s Precision Medicine Program.

By participating in these opportunities and raising his own awareness, Matt is now committed to being fully present and fighting this devastating disease. Through the ALS-TDI, Matt found the opportunity to serve as a peer reviewer for the CDMRP’s ALSRP, and he welcomed the prospect of immersing himself further in therapeutic development

efforts. Matt relishes the opportunity to discuss medical research with some of the brightest minds in the industry, and he says he wishes every ALS patient could do the same. Participating in peer review has been an exciting and humbling learning experience for Matt, and the scientists’ extensive knowledge and the content of the research proposals has given him hope for the future. As a consumer reviewer, Matt tries to emphasize the urgency of pushing meaningful research forward, and he was happy to see that scientists share his forward-looking perspective.

While not initially as optimistic, Matt has learned to embrace the life challenge of living with ALS. He now says, “With ALS, every day could be seen as a new defeat as muscles and function fall away. I have learned that every day is actually a blessing because of the things I can still do. I can’t fly anymore or lead sailors, but if I dwell on the losses, then I think I will have lost. It is such a victory that I can wake up in the morning and see my kids’ smiling faces. It is a victory that my wife gives me a kiss when I head out the door, and it is a staggering victory that today is one day closer to us finding a cure for ALS.”

Therapeutic Targets Investigated by the ALSRP

Translocator Protein (TSPO) ligand

Expressed in motor neurons and glia, may be neuroprotective.

Investigating response to astrocyte mediated toxicity.

Apoptosis Signal regulating Kinase 1 (ASK1)

Involved in the endoplasmic reticulum (ER) stress response and associated with motor neuron death.

Lead ASK1 inhibitors shown to inhibit ER stress-induced cell death in cellular models of ALS.

NADPH oxidases (Nox) (neurotoxic)

Drug development to block Nox-2 production of ROS implicated in neuro-inflammation and degeneration.

SOD1 mutations (neurotoxic)

Associated with familial ALS. Developing compounds to inhibit aggregation of SOD-1.

TAR DNA Binding Protein-43 (TDP-43, neurotoxic)

Associated with neurodegeneration.

Developing chemical screens for agents that suppress or augment TDP-43 mutations.

Pimozide identified and put into human trials.

c9RAN Proteins

Generated from C9ORF72 repeat expansion-associated translation, contribute to neuropathology and clinical phenotypes, including cognitive impairments.

Investigating this mechanism of ALS pathology.

IGF-II (neuroprotective)

May protect motor neurons from glutamate-induced toxicity and promote axon regeneration.

Vardenafil HCl shown to promote IGF-II and should protect motor neurons.

TAR DNA Binding Protein-43 (TDP-43, neurotoxic)

Associated with neurodegeneration.

Developing chemical screens for agents that suppress or augment TDP-43 mutations.

Pimozide identified and put into human trials.

P-glycoprotein (multi-drug resistant protein)

Therapeutic target in spinal cord for increasing ALS drug efficacy.

P-glycoprotein inhibitor Elacridar increases Riluzole bioavailability.

c-Jun N-terminal kinases (JNKs)

Contribute to cell death.

Developing brain-penetrant JNK inhibitors.

C9orf72

While some GGGGCC (G₄C₂) repeats are normal, the mutated form produces thousands, leading to the neurotoxicity of ALS.

Investigating ways to reduce C9ORF72 toxicity.

Net Charge of Protein

Achilles heel of misfolded protein.

Increasing the net charge of a protein to reduce aggregation.

Heat Shock Factor protein 1 (HSF-1)

Reduction in HSF-1 activity accelerates neuronal toxicity.

Peripherin

Overexpression of protein aggregates of the neuronal intermediate filament peripherin has been linked to neuronal injury and may actually play a therapeutic role in reversing neuronal degeneration.

Studying Per-28, peripherin isoform, to see if it may serve as a mechanism for attenuating motor neuron toxicity.

Establishing Research Resources



In Vitro Models of TDP-43 Aggregation and Toxicity

Dr. Leonard Petrucelli, Mayo Clinic, Jacksonville, FL

Tar DNA binding protein 43 (TDP-43), which aggregates into toxic inclusions in brain and spinal cord neurons of ALS patients, has been recognized as a major player in ALS pathophysiology. TDP-43 exhibits a disease-specific biochemical signature, though the mechanisms through which it mediates neurodegeneration remain unclear.

Dr. Leonard Petrucelli and his team at the Mayo Clinic in Jacksonville, FL, used an FY09 TDA to refine and validate in vitro models of TDP-43-driven ALS and screen a qualified small molecule library of compounds looking for candidate therapeutic agents that prevent or reverse TDP-43 aggregation.

The team had previously generated a model of human neuroblastoma cells that recapitulates the intracellular aggregation of TDP-43. These cells, termed M17D3 cells, inducibly express a pathological TDP-43 C-terminal fragment, TDP220-414, fused to enhanced green fluorescent protein (GFP) for easy monitoring of TDP220-414 expression and aggregation (using the tetracycline-off technique). With the ALSRP award, the in vitro screen was successfully miniaturized from 24-well plates to a 384-well assay, and further efforts markedly improved the assay through optimizing conditions for the 384-well format. Not only was the dynamic range enhanced, leading to a Z-factor over 0.5 (a benchmark for robustness), but this format also afforded a much more efficient approach for screening the small molecule library, as the number of compounds that could be screened was more than triple the number possible using a 96-well plate. In addition, GFP is measured by microplate reader, a faster, more cost-effective technique compared to other alternatives.

In validating the assay, Dr. Petrucelli confirmed that GFP fluorescence levels correlated with the actual amount of inclusions present within the cells and, just as importantly, that treatment-induced reductions in GFP fluorescence resulted from decreased TDP220-414 aggregation and not from the degradation of the GFP molecule itself. The team also screened hits for their ability to prevent aggregation of GFP-TDP208-414, another C-terminal fragment linked to TDP-43 proteinopathies and phosphorylated at pathologically relevant sites.

Because the M17D3 neuroblastoma cells did not exhibit neurotoxicity resulting from conditional expression of TDP-43 fragments, the team tested seven lead compounds that reduced GFP-TDP220-414 inclusions in the initial screen in mouse primary cortical neurons transduced to express GFP-TDP220-414 through recombinant adeno-associated virus vectors. A novel approach developed by the team using the BD Pathway 855 system was employed to measure the formation of GFP-TDP220-414 inclusions and associated toxicity, the latter characterized by significantly shorter neurite outgrowth. It was observed that GFP fluorescence in the model was increased in a dose-dependent manner

with treatment of GFP-TDP220-414 virus. Moreover, GFP-TDP220-414 inclusions dose-dependently inhibited neurite outgrowth compared to GFP-expressing control neurons. Of the seven library compounds screened at this second level, three significantly decreased GFP fluorescence in a dose-dependent manner. However, these compounds failed to rescue neurite outgrowth deficits or inhibit cytotoxicity as assessed by LDH release. Based on their impact on TDP-43 inclusions, the three top compounds will be further evaluated in a transgenic mouse model of ALS and for structure-activity relationship studies, and may have therapeutic potential.

The two in vitro models refined and developed by Dr. Petrucelli with ALSRP funding have been published and offer a valuable resource to the ALS research community.

Establishment of a Human-Based in Vitro Functional NMJ System for ALS Drug Screening

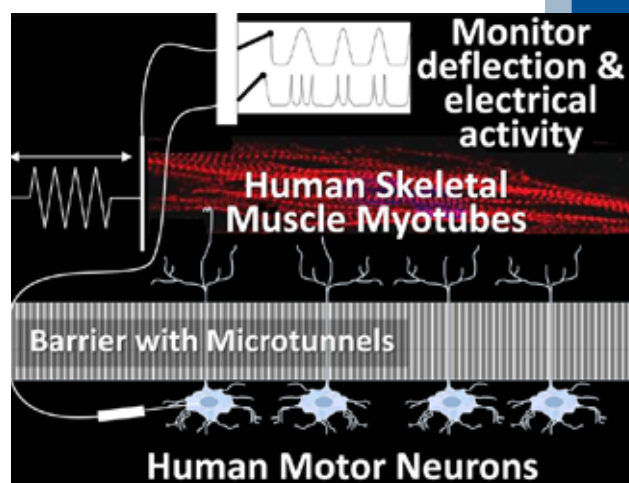
Dr. James Hickman, University of Central Florida

ALS is a motor neuron disease, and accumulating evidence suggests that pathology at the neuromuscular junction (NMJ) (i.e. deterioration and/or muscle denervation) is a more sensitive landmark for monitoring the progression of ALS than previously thought. To date, detecting NMJ deterioration has been notoriously difficult because it happens before detectable symptoms develop, and there is currently no suitable model to investigate the early pathology expressed within these synapses. Dr. James Hickman, with support from an FY13 ALSRP TIA, is undertaking the creation of an in vitro model of the NMJ environment in order to characterize ALS-related events, identify factors that may act as initiators of disease onset, and conduct drug screening.

In Dr. Hickman's first-ever human-based functional NMJ system for ALS, human pluripotent stem cells from ALS patients or normal controls (hMNs) are induced to form neurons and grow through microtunnels to interface and innervate human skeletal muscle (hSKM) myotubes. Separation of hMNs and hSKMs as well as axonal growth along the microtunnels can be clearly monitored utilizing phase microscopy and confirmed by immunocytochemistry. Electrical stimulation of hMNs can induce correspondent myotube contractions, and electrical properties of the nerve-myotube junction and myotube contraction after neuron depolarization can be monitored under various conditions. A recording system has been developed to record and quantify myotube contractions in the chamber.

Dr. Hickman conducted preliminary studies characterizing the NMJ model using drugs known to interact with the NMJ. Increasing dosage of curare, for example, gradually inhibited NMJ function as quantified by monitoring corresponding myotube contractions. These types of measurements have not been possible previously except in in vivo models or using animal tissue explants.

Dr. Hickman's chambered NMJ system provides the first human stem cell-derived in vitro NMJ model in which NMJ function can be analyzed quantitatively. This model provides a good system for understanding the basic physiology of the NMJ system, such as the identification of essential factors for their formation, function, and regeneration. Currently, Dr. Hickman is optimizing, refining, and updating his NMJ system in order to conduct drug screening for ALS therapeutics.



Developing Drug Products



Chemical Genetic Screens for TDP-43 Modifiers and ALS Drug Discovery

Dr. Pierre Drapeau, University of Montreal

Recent findings have shown that the TARDBP gene, which encodes for the DNA/RNA binding protein TDP-43, is mutated in familial ALS cases and is a major contributing factor to ALS progression. Development of novel therapies targeting TDP-43 activity may be beneficial for at least a subset of individuals with ALS.

Dr. Pierre Drapeau and a team at the University of Montreal, with support from an ALSRP FY10 TDA, screened libraries of thousands of bioactive molecules to identify chemical modifiers of TDP-43 as therapeutic approaches to ALS treatment. The project was carried out in three previously developed *in vivo* models of ALS (expressing a mutant TARDBP gene) in worms, zebrafish, and mice. Screening “hits” were able to suppress the *in vivo* phenotypes observed in worm and fish models, and were then further validated in a mouse model. It was found that almost all of the confirmed hits were neuroleptics (antipsychotics), including the FDA-approved compound pimozone. Given this, Dr. Drapeau turned to screening pimozone derivatives, but found none were more potent than the base compound. Closer analysis of the mutant zebrafish phenotype pointed to a decrement in neuromuscular transmission through disrupted calcium channels that was restored by pimozone. Thus, this decremental response to repetitive nerve muscle stimulation may be a novel biomarker for ALS. Dr. Drapeau’s work has identified the neuroleptic drug pimozone as a potential ALS therapeutic which is now incorporated in an early-stage clinical trial (see inset).

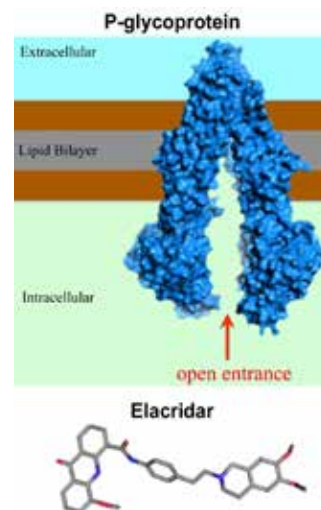
Dr. Drapeau teamed with Dr. Lawrence Korngut at the University of Calgary and Dr. Lorne Zinman at Sunnybrook Health Sciences Centre to obtain follow-on funding for a double-blinded Phase 2 clinical trial of pimozone in ALS patients. The new decremental response marker is also being used as a part of this trial. The one-year trial of 50 subjects is ongoing and will be expanded for an additional 2 years if promising results are obtained.



Rethinking Drug Treatment Approaches in ALS by Targeting ABC Efflux Transporters

Dr. Piera Pasinelli, Jefferson Medical College

Despite researchers’ best efforts, there is only one FDA-approved drug available for treatment of ALS, and this drug, riluzole, exhibits only modest efficacy. Dr. Piera Pasinelli hypothesized that the reason that the efficacy of riluzole is so modest might be due to the action of proteins called ABC efflux transporters that can remove riluzole from the interiors of cells. These transporter proteins normally protect the brain by actively pumping “foreign” substances out of the central nervous system (CNS) and were previously shown by her group to be upregulated in the spinal cords of both an ALS mouse model (SOD1-G93A) and ALS patients. Dr. Pasinelli received an FY10 ALSRP TIA to



investigate efflux as a possible mechanism of resistance to riluzole and develop methods to improve the efficacy of the drug.

Dr. Pasinelli's group was able to show that riluzole became more effective in ALS mice when administered in combination with the ABC efflux inhibitor elacridar, which has already undergone Phase I clinical trials for combination therapy. Specifically, they observed an increase in CNS penetration, muscle function, slowing down of disease progression, and a significant extension in survival. Mass spectrometry methods demonstrated riluzole bioavailability in the blood and CNS of test animals in the study. This new approach may enhance the effectiveness of riluzole in a clinical setting, as well as provide a mechanism to improve the action of other potential pharmacotherapies. Dr. Pasinelli is optimistic that if proven effective, this treatment should quickly move to help those suffering with ALS.

Developing ER Stress Inhibitors for Treating ALS

Dr. Nicholas Cosford, Sanford Burnham Prebys Medical Discovery Institute

While the exact cause of ALS is unknown, a prominent pathological feature of the disease implicates upregulation of endoplasmic reticulum (ER) stress signaling in motor neurons of affected individuals. Apoptosis signal-regulating kinase 1 (ASK1) is an important signaling molecule involved in the ER stress response, and its activation has been associated with motor neuron death in ALS models. Dr. Nicholas Cosford of the Sanford-Burnham Institute used an ALSRP TDA to design and evaluate benzodiazepinone analogue compounds as small molecule modulators of the ASK1 pathway.

Benzodiazepinone analogues were synthesized with an established protocol and then analyzed for their ability to protect SH-SY5Y neuroblastoma cells from thapsigargin-induced cell death as an initial benchmark of efficacy. The lead compound from this screen was then tested to characterize its potential for therapeutic efficacy in ALS. This began with a motor neuron cell line expressing an ALS-specific mutation, neuroblastoma spinal cord 34 cells (NSC-34), which express mutant superoxide dismutase 1 (SOD1), the gene most commonly mutated in familial ALS. The lead compound was able to protect the cells from ER stress-induced cell death, generally supporting the hypothesis that benzodiazepinone derivatives could be promising leads for therapeutic development.

Subsequent analyses examined the impact on calcium dysregulation, a hallmark of ER stress. The lead compound prevented elevated CHOP transcription associated with ER stress in H4 glioma cells. Intracellular calcium concentration was monitored in parental NSC-34 cells and cells expressing mutant or wild type SOD1 with stimulation from ATP. The lead compound was found to facilitate calcium mobilization through modulation of store-operated channel-mediated calcium entry. However, this effect was not present following stimulation with the calcium enhancer spiperone (similar to thapsigargin). The lead compound was also not cytoprotective against insult with either calcium regulators ionomycin or 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid. Finally, the lead compound was tested for its ability to decrease the activity of phospholipase A2 (PLA2) signaling, whose upregulation has been demonstrated to be critical in triggering inflammatory and neurodegenerative processes. A decrease in PLA2 activity was seen in benzodiazepinone-treated cells.

In addition to these mechanism-of-action studies, various benzodiazepinone compounds have been tested for microsomal stability, plasma stability, and parallel artificial membrane permeability (an indicator of blood-brain barrier permeability). These pharmacokinetic assays have supported further in vivo evaluation of several candidates. Further characterization in zebrafish and mouse ALS models is ongoing, as these benzodiazepinone compounds hold promise as potent ER stress inhibitors for continued clinical development.



Other Therapeutic Approaches



Overcoming the Practical Barriers to Spinal Cord Cell Transplantation for ALS

Dr. Nicholas Boulis, Emory University

The promise of stem cell regeneration has been the hope of many ALS patients, as well as others suffering from neurological disorders. Although multiple clinical trials are currently testing different stem cell therapies as treatment alternatives for neurodegenerative diseases and spinal cord injury, optimal injection parameters have not yet been defined nor are there sufficient data to effectively minimize post-surgery complications such as graft rejection. With support from an FY10 ALSRP TDA, Dr. Nicholas Boulis from Emory University is pursuing two aims in order to generate needed data to help guide clinical practice. The first part of his project entails determining the tolerance and toxicity of cell dosing, and numbers of permissible spinal cord injections in order to establish basic safety and injection parameters. The second part deals with optimizing immunosuppression following surgical spinal cord stem cell transplantation in order to minimize graft rejection. Taken together, the data generated from this project will significantly help translate stem cell therapy to patients with ALS.

For the first aim, Dr. Boulis injected minipigs with human neural progenitor cells and then assessed the pig's gait and motor function, as well as general morbidity. All animals, despite increases in the volume and number of injections, returned to their preoperative baseline within 14 days, showing complete motor function recovery. However, swelling of the spinal cord with escalating volume doses prevented complete healing of the spinal cord in some cases. Dr. Boulis also was able to safely increase the number of injections to 40 in the swine model, as long as the volume of each injection was kept low. Ultimately, Dr. Boulis concluded that 25 micro liters is likely the ideal injection volume in order to maximize stem cell delivery and minimize tissue damage. These experiments support the functional safety of various injection volumes and numbers in the spinal cord, and provide critical insight to consider when developing safety thresholds for other studies.

The second part of Dr. Boulis' project, focusing on the immune response to intraspinal stem cell therapy, is still ongoing. Graft rejection remains a significant risk for intraspinal stem cell therapies, and an assay to non-invasively monitor the immune response to transplanted intraspinal cell grafts is essential in order to move the field forward. Dr. Boulis hypothesized that graft-specific host antibodies generated after stem cell transplantation may be detected in the peripheral blood and can be used as a diagnostic marker of cellular graft rejection. To test this hypothesis, levels of graft-specific antibodies were measured in the peripheral blood of ALS patients and minipigs following either no immunosuppression or treatment with tacrolimus. Preliminary results provide evidence for a decreased immune response to transplanted intraspinal stem cell grafts with tacrolimus immunosuppression. In future studies, Dr. Boulis plans to correlate the peripheral blood findings to immunohistological analysis of transplanted grafts. Taken together, Dr. Boulis' quest to overcome the barriers to spinal cord cell transplantation could significantly advance the treatment of ALS.

Preclinical Studies of Induced Pluripotent Stem Cell-Derived Astrocyte Transplantation in ALS

Dr. Nicholas Maragakis, Johns Hopkins University



Induced pluripotent stem cell (iPSC) methodology has made it feasible to generate stem cells directly from adult cells. Adult cells are genetically reprogrammed to express genes and factors important for maintaining the defining properties of embryonic stem cells. This technique allows for the creation of cell lines from patients with a variety of ALS presentations and helps answer questions regarding differences in the disease. With support from an FY09 ALSRP TDA, Dr. Nicholas J. Maragakis from Johns Hopkins University focused on making cells called iPSC-glia restricted precursor (iPSC-GRP) cells. These cells can develop into astrocytes, which are key players in the ALS disease process and are important for neuroprotection in general. Previous ALS stem cell transplantation studies have focused primarily on motor neuron replacement. However, astrocytes are thought to play a key role in protecting neurons, and astrocyte replacement using these iPSC-GRP cells represents a new way of protecting host motor neurons.

At the start of the award, it was not known whether iPSC-GRP cells from ALS patients would harbor ALS-specific abnormalities that could exacerbate disease. Dr. Maragakis' first objective was to determine whether human iPSC-GRP cells derived from either sporadic ALS, familial (SOD1-mediated) ALS, or control subjects have the same capacity for engraftment, survival, and neuroprotective qualities following transplantation. Dr. Maragakis was able to generate an extensive library of iPSCs from control, familial ALS, and sporadic ALS patients. He demonstrated that the cells resemble normal cells after numerous passages, and that they can be differentiated into astrocyte precursors regardless of the presence of SOD1 mutations. To date, Dr. Maragakis has shared cells from this collection with collaborators at numerous institutions.

Dr. Maragakis next demonstrated *in vivo* transplantation of these control human iPSC-derived astrocyte progenitor cells into rat spinal cords. His results show that human embryonic stem cell- and hiPSC-derived astrocyte progenitors survive long-term after spinal cord engraftment and differentiate to astrocytes *in vivo* with few cells from other lineages present. Gene profiling of the transplanted cells demonstrates that the astrocyte progenitors continue to mature *in vivo* and upregulate a variety of astrocyte-specific genes. These observations are critical in demonstrating that cells grown in a dish can actually mature into adult cells when transplanted. This work has important implications as we think about the translational potential of these cells in ALS (Haidet-Phillips et al. 2014).

His lab also wanted to know how human glial progenitor cells would behave when placed in an ALS environment. Using human fetally-derived glial restricted progenitor cells, he found that engraftment and gene expression was independent of the neurodegenerative ALS spinal cord environment, and that these cells maintained their autonomy.

Dr. Maragakis further demonstrated that human glial restricted progenitor cells continued to mature into astrocytes following transplantation. Efforts are under way to design a clinical trial using human glial restricted progenitors (Haidet-Phillips et al. 2015).

Dr. Maragakis believes that the ultimate success of iPSC-derived astrocyte progenitor cells may be highly variable and dependent upon the individual clone and donor, but that the transplanted cells may be able to function as normal cells even in an ALS environment.

Research in the Pipeline

Principal Investigator	Title	Project Description
Therapeutic Development Awards		
Dr. Steven Finkbeiner, J. David Gladstone Institutes	Development of Novel Neuronal Autophagy Inducers to Block Neurodegeneration and Treat ALS	The PI plans to develop effective ALS therapeutics by focusing on the development of drugs that activate autophagy, a natural cellular protective mechanism that degrades and recycles unnecessary or dysfunctional cellular components. Buildup of mutant proteins such as SOD1, TDP43, FUS, and C9orf72 translation products are thought to be involved in ALS pathology and progression. Therefore, identifying and developing drugs that activate autophagy may help block neurodegeneration in ALS by increasing the clearance of mutant proteins.
Dr. Joseph Beckman, Oregon State University	Development of Copper ATSM as a Therapeutic for SOD- Familial and Sporadic ALS	In previous work, the PI found the copper-delivery molecule CuATSM protects certain ALS prone mice bearing the G93A SOD1 mutation by facilitating maturation of the mutant SOD1 molecule. The objective of this follow-up study is to optimize the pharmacological properties and formulation of the CuATSM lead compound and conduct preclinical studies in order to submit an IND application for clinical trials.
Dr. Justin Ichida, University of Southern California	A High-Throughput Phenotypic Screen for C9ORF72 ALS Therapeutics Using Patient- Specific Motor Neurons	The purpose of this project is to develop a high-throughput screening capability based on motor neurons induced from stem cells bearing the C9orf72 repeat. The screen will then be used to assay a large library of (~40,000) compounds to identify leads for drug development.
Therapeutic Idea Awards		
Dr. Jiou Wang, Johns Hopkins University	Developing Therapeutic Agents for Nucleotide Repeat Expansion-Mediated ALS	This project addresses the hypothesis that secondary and tertiary structures of C9orf72 hexanucleotide repeat DNA (and RNA) possess toxic properties. The PI will identify molecular partners that interact with C9orf72 hexanucleotide repeat DNA structures and to identify compounds that can interfere with formation of these structures as potential ALS therapeutics.
Dr. David Borchelt, University of Florida	Inhibitors of SOD1 Interaction as an Approach to Slow the Progressive Spread of ALS Symptoms	The PI proposes to develop an assay and screen for drugs that stabilize the SOD1 dimer to prevent aggregation. This is based on the hypothesis that compounds that stabilize native SOD1 structure will provide therapeutic benefit to patients with SOD1-linked familial ALS and could possibly benefit a subset of sporadic ALS cases.

Research on the Horizon

Dr. Antonius Bunt, Izumi Biosciences Inc.

Therapeutic Development Award

Small-Molecule Efflux Inhibitor for Enhanced Treatment of ALS

Dr. Evan Snyder, Sanford-Burnham Medical Research Institute, La Jolla

Therapeutic Idea Award

Enabling Widespread, Minimally Invasive Distribution of Multimodal Therapeutic hNSCs throughout the Neuroaxis of ALS Mice

Dr. Keith Gagnon, Southern Illinois University

Therapeutic Idea Award

Chemical Library Screening for Potential Therapeutics Using Novel Cell-Based Models of ALS

Dr. Brent Stockwell, Columbia University

Therapeutic Idea Award

Motor Neuron-Protecting Agents as Therapeutics for Treating ALS

Dr. Joseph Puglisi, Stanford University

Therapeutic Idea Award

RAN Translation as a Therapeutic in ALS

Dr. Gong Chen, Pennsylvania State University

Therapeutic Idea Award

Reprogramming Reactive Astrocytes Directly into Functional Motor Neurons in the Spinal Cord of ALS Model

Dr. Jacob Robinson, Rice University

Therapeutic Idea Award

High-Throughput, High-Dimensional (HT-HD) Phenotyping of *C. elegans* for ALS Drug Discovery

Dr. Claudio Hetz, Biomedical Neuroscience Institute

Therapeutic Idea Award

Targeting the ER Stress Sensor IRE1 to Treat ALS

Dr. Justin Yerbury, University of Wollongong, New South Wales, Australia

Therapeutic Idea Award

Delivery of Ubiquitin to Motor Neurons Using a Targeted, Sterically Stabilized Liposome Delivery System



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