



FUNDED RESEARCH 2015



MISSION

To fund research with the highest probability of preventing, slowing or reversing Alzheimer's disease.

2015 RESEARCH ANNUAL

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Message from the Chairman

Jeff Morby, Chairman and Co-Founder, Cure Alzheimer's Fund

Dear Friends,

At a recent meeting of our Research Consortium, made up of some of the world's most brilliant scientists in the field of Alzheimer's research, there was virtually unanimous agreement among the participants that CAF has been one of the principal leaders in the field and is unique among the research groups grappling with the disease.

The above described perception has motivated me to share with you what I believe are five important leadership roles CAF has carried out within the field:

First: Leadership in the Genetics of Alzheimer's Disease

Twelve years ago, when we first established the foundation, there were only 4 known Alzheimer's genes, representing about 30% of the causes of the problem. We knew that we had to find and understand the many other undiscovered genes in order to make headway against the disease. Consequently, in 2006 we performed the first genomic scan of the disease and made a truly breakthrough discovery at the time—we discovered 5 new genes, a discovery which was dubbed one of the “Top 10 medical breakthroughs in 2008” by *TIME Magazine*. Subsequently we were the first in 2011 to perform another genomic scan using the newest technology at the time. After that, in 2013, we were the first organization in the world to use new state-of-the-art technology “whole genome sequencing” to sequence the entire DNA of a database of more than 1,500 individuals from families afflicted by Alzheimer's disease.

Today we have one of the largest databases in the world of the genomics of Alzheimer's disease, having discovered more than 50 new Alzheimer's genes containing over 350 genetic variants associated with risk for the disease. As Rudy Tanzi will describe, this database has allowed us to undertake the **Genes to Therapies™ (G2T) program**, in which we have launched a major effort to understand 20 of the most significant Alzheimer's genes—how they interact individually and collectively to cause AD—and how we can stop them from doing so.

Second: Leadership in the Creation of Advanced Research Tools

Of course, the genomic database combined with the genomic analytics is our major research tool. But, also importantly, Rudy, Doo Yeon Kim, and their team have developed a breakthrough technology, called “**Alzheimer's in a Dish**” (ADD), which allows scientists for the first time to

grow a neuron in a 3-dimensional environment, a mini-brain, in order to see how the neuron develops its pathology and how it reacts to potential medications. The analytical tool is so powerful that it can be used for analysis of a variety of other neurological diseases. Its importance was underlined both by a full-page article in *The New York Times* as well as a special blog post by Francis Collins, director of the NIH.

ADD is also leading CAF to develop additional tools. One of these represents an extension of the mini-brain concept to include visualization of the formation and spreading of Tau, the actions of microglia, and the operation of the blood-brain barrier.

Another is the creation of rapid throughput analysis processes, using ADD, to ascertain whether or not existing drugs currently on the market can be repurposed to be used as preventatives for Alzheimer's disease. We have already analyzed approximately 1,200 drugs and have identified dozens of high-potential prospects for consideration in future trials.

Finally, an important analytical tool for most of biological science is what is called a “transgenic mouse,” which is a mouse grown with human DNA, which causes such mice to neurologically respond to stimuli as a human brain would do with the same set of genes. Such mice, with Alzheimer's DNA, can be used to test potential drugs for use against Alzheimer's disease. As mentioned earlier, in 2004, there were only 4 known AD genes. These same genes, incorporated into the brains of transgenic mice, have been for more than 10 years the “mice standard” against which potential drugs have been tested. However, we now know that there are far more AD genes than 4. Therefore, many more transgenic mice beyond those of the 4 genes are required if we are to have the proper vehicles for testing drugs. So, as part of the G2T project, we are now developing new transgenic mice with the information provided by our genomic database. Importantly, as we develop such mice, we make them available to both our researchers and the whole scientific community – a major contribution to science.

Third: Leadership in the Development of Effective Forms of Scientific Collaboration

It is hard to imagine a more talented group of scientists than the list of our Research Consortium and SAB collaborators. This last year we have added another

group of exceptional scientists, as Rudy will describe below. But apart from capabilities, what truly distinguishes these groupings of scientists is their willingness, in fact eagerness, to collaborate. Over time, all of them have developed with us an atmosphere of trust and cooperation and are willing to share their unpublished insights and participate in quarterly brainstorming sessions focused on attacking the disease in new and creative ways. Additionally, they are guided in their research decisions by **CAF's Roadmap**, a jointly shared strategy, which is changed as new scientific insights are attained.

Fourth: Conceptual Leadership in the Attainment of New Scientific Insights

I believe that it is safe to say that we have been a conceptual leader in the field of Alzheimer's research. Our emphasis on genomics, the development of new tools, and our collaborative approach to scientific exploration, as described above, are some examples. But there are others. One of these is the conceptualization of a comprehensive model of Alzheimer's disease. This model has allowed us to identify what we call "intervention points" to use in potentially combatting the disease as it spreads. Each one of these intervention points represents a stage of development of the disease, which stage, if stopped, will arrest the progression of the disease. We have organized our research around those intervention points and the G2T project, and this year have committed to fund **44 different research projects totaling \$10 million.**

Of significant importance to the understanding of Alzheimer's disease is our new conceptualization of what is called the **"Anti-Microbial Protection Hypothesis."** Rob Moir and Rudy Tanzi of Mass General have been working for 5 years to validate this concept, and their paper providing strong evidence for the hypothesis in various experimental models was published in *Science Translational Medicine* in May 2016. This revolutionary hypothesis has the potential to fundamentally alter the current paradigm regarding how Alzheimer's pathology is triggered in the brain. They have shown that Abeta is a component of the brain's innate immune system, which is protective of the brain. Abeta traps pathogens in plaques when they enter the brain and kills them, which is a good thing and essential for the protection of the brain. However, too much of this activity, or genetic defects, may cause the brain to overproduce Abeta or fail to clear Abeta, thereby leaving too much Abeta in the brain. This results in "tangles" which kill neuronal cells from within, ultimately leading to Alzheimer's disease. This radical, new view has major implications for drug discovery.

As new discoveries are made in the field, we adjust our priorities. One example of that is the human "microbiome" and what is called "epigenetics." Based on solid scientific evidence, we now know that our human microbiome (made up of bacteria and other microbes in our gut and elsewhere within our body) has the capacity to influence how our genes code for proteins, with major implications

for the disease. The gut microbiome also regulates brain inflammation, a key pathological feature of Alzheimer's disease. We have already undertaken two new research projects with leaders in that field.

Fifth: Leadership in Foundation Management (Venture Philanthropy)

Many of the founders of this organization are former venture capitalists. The founders and directors have, over time, personally contributed \$23.8 million to the foundation. These monies are used to pay the operating expenses of the foundation so that any contributions received from third parties go 100% into research. Given our backgrounds and financial contributions to the organization, we don't want to waste money or waste time. We want a cure or preventative as soon as possible, and we manage the institution with this aim. We call the way we manage "Venture Philanthropy." And the management processes we have set up have distinguished us as risk takers, fast decision makers, and strategists. These processes represent one of the reasons for our success (and one of the reasons we have been given a four star rating by Charity Navigator for the fifth consecutive time).

Cures in the Pipeline

Potential cures/preventatives have been long in coming, but we now have two in the pipeline. One of these, a gamma secretase modulator, is the equivalent of a statin for AD. It is, in essence, a preventative for Abeta accumulation in the brain in much the same way a statin is a preventative for the over-accumulation of cholesterol in the arteries. The second, Amylyx, is a medicine developed to protect neurons from cell damage which might occur as a result of over-concentration of Amyloid in the brain and brain inflammation. We expect Amylyx to go to human trials in 2016 and the gamma secretase modulator in early 2017. We also have preliminarily identified numerous existing medications which could possibly be repurposed for Alzheimer's disease and, of course, have underway a great number of scientific studies, any one of which could identify a promising new drug at any time.

Many, Many Thanks

All of the above would not have been possible without your support and the support of the more than 23,000 contributors to our organization. In 2015 we raised \$11.7 million, our 11th record fundraising year. Thanks to all of you very much for your significant generosity. A thank you, also, to our wonderful researchers and staff, all of whom continue to be inspired by our quest to rid this planet of the dread disease and as a result produce inspirational results, which continue to amaze.

Very Best Wishes,

Jeffrey L. Morby
Chairman and Co-Founder



Message from the Research Consortium Chairman

Rudolph E. Tanzi, Ph.D., Chairman, Research Consortium, Cure Alzheimer's Fund

Dear Cure Alzheimer's Fund Friends,

2015 was another great year for the research funded by Cure Alzheimer's Fund (CAF). I am very pleased to report that we capitalized on our previous momentum and made immense progress.

During the year, we added many new esteemed colleagues to our Research Consortium and our Scientific Advisory Board, as well as collaborators and grantees. These additions to our already significant group of accomplished scientists has the impact of substantially expanding our research efforts while maintaining our standards for only funding research of the highest quality and with the greatest potential impact on understanding and treating Alzheimer's disease (AD).

For 2015, we increased our funded research to over \$10 million, double the amount from 2014 of just over \$5 million. I am confident in stating that Cure Alzheimer's Fund is operating at an entirely new level of research excellence, which is unparalleled in the field of Alzheimer's disease research.

Most of our projects remain focused on the Genes to Therapies™ (G2T) initiative, which is now in high gear. G2T involves taking the top Alzheimer's disease genes, including the previously established (4) as well as a dozen of our new ones, and using them to create new disease models to study AD. The primary purpose of G2T is to translate our unprecedented database of novel genetic results into a deeper understanding of the causes of Alzheimer's disease as well as novel drug discovery and development for treating and preventing the disease.

In addition to studying the above Alzheimer's disease genes in various mouse and cell models, we are incorporating the new disease gene-derived data into our 3D stem cell-derived neuronal cell cultures (Alzheimer's in a Dish). We have also used Alzheimer's in a Dish to initiate the 3D Drug Screening (3DDS) project with several collaborators. Our approach is to use 3DDS to screen all existing approved drugs (~1200) and many clinically safe, but not yet approved, investigational drugs. The objective is to evaluate which drugs can be repurposed to stop beta-amyloid deposition, tangle formation and neuronal cell death in 3D cultures. The studies have already resulted in the

identification of several drugs that appear to block tangle formation. In other studies, we are screening for drugs that will stop neuroinflammation using the AD genes involved in innate immunity in the brain, such as CD33. And, we are developing novel 3D systems to address innate immunity and the blood-brain barrier (BBB) in Alzheimer's disease.

In addition to screening for new drugs, there are other projects in development that will take drugs funded by Cure Alzheimer's Fund into clinical trials in patients with Alzheimer's disease. Most notably, these include the gamma secretase modulators (GSM), aimed at lowering Abeta levels, and the Amylyx compounds, aimed at protecting neurons from neuroinflammation. This research effort is currently being conducted and will continue through 2016.

The Cure Alzheimer's Fund portfolio of projects aimed at diagnosis and detection of Alzheimer's disease has been steadily increasing, especially pre-symptomatically for the purposes of early prediction, early detection and early intervention.

An area of great growth is in the study of the role of neuroinflammation and innate immunity in the pathological pathways of Alzheimer's disease. In addition to the G2T projects focusing on several new AD genes involved in these pathways, such as CD33 and TREM2, we are also investigating how microglial cells and astrocytes contribute to the death of nerve cells in AD.

Cure Alzheimer's Fund was one of the first foundations to appreciate the key role played by innate immunity in Alzheimer's disease, when we discovered in 2008 that CD33 is an AD gene. Today, entire AD research programs around the world are focused on CD33 and its counterpart gene, TREM2, first discovered by the recipient of a grant from Cure Alzheimer's Fund.

As an extension of studies of innate immunity in the brain and its role in AD, our research has been advancing our ongoing studies of the role of microbial organisms, such as bacteria, viruses and fungus (yeast) in Alzheimer's disease pathology. The ongoing work has resulted in a very exciting new paper, now in press. This paper is poised to rock the

very foundation of our current models of the etiology and pathogenesis of Alzheimer's disease. We have now shown in several different animal models and cell culture models (mice, fruit flies, dirt worms and neurons) that beta-amyloid is clearly an anti-microbial substance produced in the brain to protect against infection, including yeast (candida), herpes simplex virus 1 and various bacteria.

Our research has demonstrated that by infecting the brain of a very young (one-month-old) AD mouse model, in which no plaque would normally be present until 6-8 months of age, abundant amyloid deposition could be seeded in the brain, virtually overnight. Moreover, each amyloid plaque that formed overnight was observed to contain at its center a single bacterium. **The resulting theory is that, in the brain, just a single bacterium that gains entry across the blood-brain barrier can lead to a senile plaque.** As revealed with Alzheimer's in a Dish, amyloid deposition can then trigger tangles to form in neighboring nerve cells, leading to cell death. In view of these results, we are now actively investigating our hypothesis that as we age, low-grade and clinically non-symptomatic infections of the brain from viruses, bacteria and yeast, trigger beta-amyloid deposition in the brain as a protective mechanism. Beta-amyloid then leads to tangle formation, followed by cell death, inflammation and, ultimately, dementia. If this hypothesis is borne out, we can envision potentially stopping the pathological process of Alzheimer's disease at its earliest pre-symptomatic stage, by therapeutically targeting specific microbial infections in the brain.

The other role of microbes in Alzheimer's disease involves beneficial microbes that make up our "microbiome." The microbiome is the total collection of the several thousand species of bacteria that live in our gut, on our skin and in body cavities. In particular, the gut microbiome is directly connected to the brain and has been shown to affect mood and neuroinflammation in the brain. Two projects have now been funded by Cure Alzheimer's Fund to explore the role of the gut microbiome in Alzheimer's disease. The goal of these studies is to determine how we might treat and prevent AD, and otherwise enhance brain health, by managing the gut microbiome.

As 2015 came to a close, we had a new paper accepted describing (3) new Alzheimer's disease genes, all of which offer new targets for drug discovery, including one involved in cholesterol metabolism and two others that appear to be involved in tangle formation. We have also **finalized our whole genome sequencing data to arrive at roughly 350 different gene mutations and variants in about 50 genes that directly affect risk and/or age-at-onset for Alzheimer's disease.** Our growing database of detailed genomic data on AD continues to be the most comprehensive and highest quality, worldwide.

We are currently preparing the publication of our unprecedented whole genome sequencing data and plan to make all of it available to the entire research community.

In summary, this has been a landmark year for Cure Alzheimer's Fund. Not only are we supporting some of the most exciting and state-of-the-art research in the field of Alzheimer's disease, we have also dramatically expanded our team of investigators. We now have the breadth, depth and focus in our research portfolio to move forward with true momentum.

2015 was a very exciting year, and 2016 is even more so. Our work is only possible because of the generosity of all of those who have donated to and supported Cure Alzheimer's Fund. Thank you to my colleagues, to the Board and staff of Cure Alzheimer's Fund. We are all enthusiastic about our progress and know that our work continues. We will not stop until we have discovered a way to end Alzheimer's disease.

Thank you,

Rudy

Rudolph E. Tanzi, Ph.D.
Joseph P. And Rose F. Kennedy Professor of Neurology
Harvard Medical School

Vice Chair, Neurology
Director, Genetics and Aging Research Unit
Massachusetts General Hospital

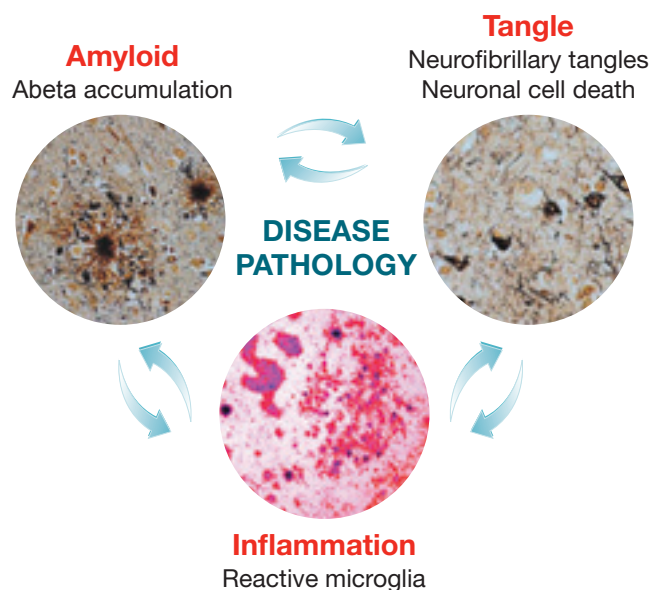
OUR RESEARCH IS

Cure Alzheimer's Fund has a focused plan to end Alzheimer's disease, and we make significant strides towards that goal each year. Our research has contributed to a more thorough understanding of how Alzheimer's pathology progresses from the earliest to latest stages of the disease, including the identification of key genes and the functions of these genes.

Alzheimer's Disease Model

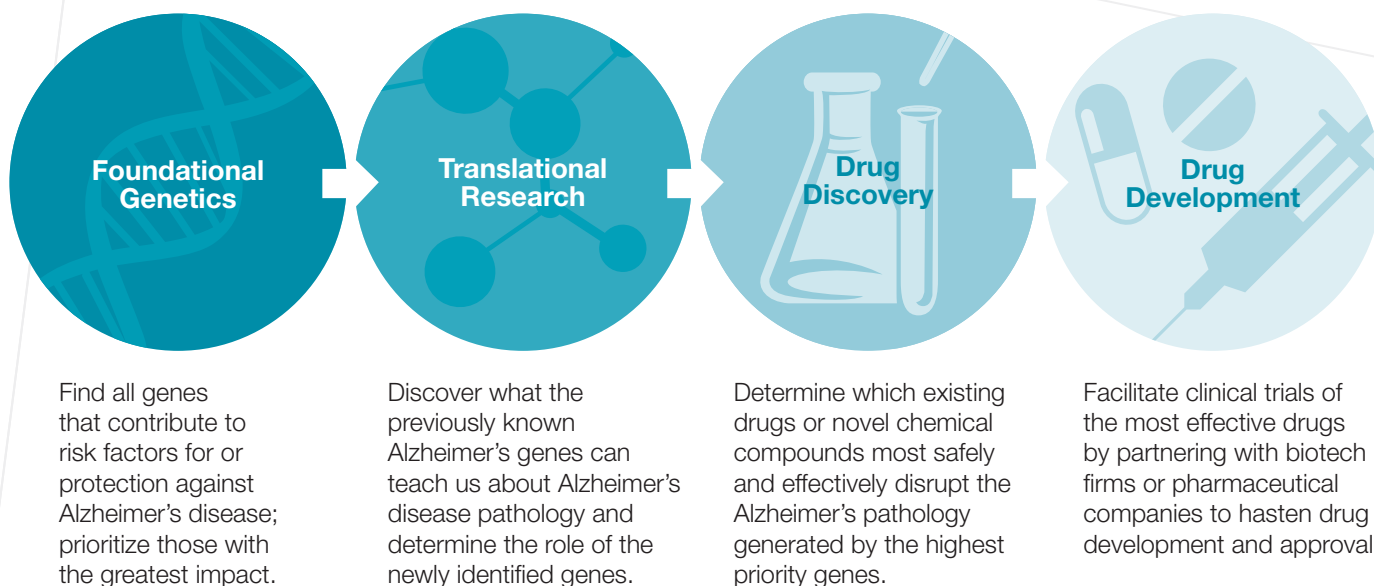
This model of Alzheimer's disease allows us to identify three basic strategies for intervention in the process:

1. An early-stage intervention inhibiting the production of the Abeta protein (the primary component of plaques characteristic of Alzheimer's disease) and/or clearing it from the brain after it forms.
2. An early- to mid-stage intervention that would inhibit the formation of tau tangles (proteins abundant in the central nervous system that have become defective and twisted into microscopic strands) and protect neurons from undue stress.
3. A late-stage intervention that would fight inflammation and thus slow down or even stop the disease process.



Research Roadmap

By addressing Alzheimer's at its origins and finding the major causes of the disease, we are accelerating developments that may lead to effective therapies. The projects we fund are based on this research roadmap, which we believe to be the quickest way to a cure.



MAKING A DIFFERENCE

9 Areas of Research Focus


The four roadmap categories focus our funding and have helped us make significant progress in our understanding of the disease. This growing understanding has allowed us to further delineate our roadmap into the following areas of focus:




Genes to Therapies™/ Drug Screening
Finding mechanisms of actions by which Alzheimer's genes affect risk and identifying targets for potential drug intervention



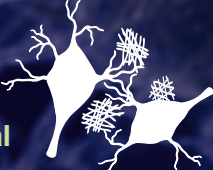
Identification and Early Detection
Improving our methods of detecting Alzheimer's in its early stages, opening the doors for treatments that stop the disease before symptoms begin or progress




Innate Immunity
Understanding the role of the brain's immune response—including inflammation—in the development and progression of Alzheimer's disease



Microbiome
Understanding the ways in which microbes living in the body may affect brain health




Pathological Pathways and Systems
Researching the underlying causes and mechanisms of Alzheimer's, such as Abeta accumulation, tau tangle propagation and inflammation




Stem Cell Models
Using stem cells to create highly accurate models of the Alzheimer's brain in the lab, in order to observe disease pathology and test possible therapies



Therapeutic Strategies
Identifying, testing and perfecting small molecules that could function as therapies for Alzheimer's disease



Whole Genome Sequencing and Epigenetics
Assessing the entire human genome, including intergenic DNA, for variants that influence risk for Alzheimer's



Individual Projects (Other)
Studying Alzheimer's disease using compelling methods or perspectives not covered by our other Areas of Focus

By funding the most promising research in each of these categories, we will build an in-depth and multifaceted picture of Alzheimer's disease. Approaching the disease from these perspectives will allow convergence on the ultimate goal—a **cure**.

RESEARCH PROJECTS

Cure Alzheimer's Fund distributed **\$10 million to support 44 research projects** across our focus areas, an all-time high that allowed us to fund even more of the most innovative research in 2015.

Visit curealz.org/research/funded-research to read about all of our current research projects.

Project/Researcher	Distribution Amount
Genes to Therapies/Drug Screening	
ABCA7 in Brain Homeostasis and Alzheimer's Disease Guojun Bu, Ph.D., and Takahisa Kanekiyo, M.D., Ph.D., Mayo Clinic Jacksonville	\$200,000
3DDS: A 3-D Human Neural Cell Culture System for Studying Neuron-Microglia Interaction in Alzheimer's Disease Hansang Cho, Ph.D., University of North Carolina at Charlotte	\$150,000
Role of Blood-Brain Barrier Function in Alzheimer's Disease Pathogenesis Investigated Using a 3-D Microfluidic Platform Se Hoon Choi, Ph.D., Massachusetts General Hospital, and Roger D. Kamm, Ph.D., Massachusetts Institute of Technology	\$291,056
Discovery of CK1 Activators for Inducing the Autophagic Degradation of APP Beta-CTF Paul Greengard, Ph.D., The Rockefeller University	\$450,000
Investigation of sTREM2 in CSF as a Potential Biomarker for Neuronal Cell Death Christian Haass, Ph.D., DZNE, Ludwig Maximilians University of Munich	\$144,182
The Biological Impact of TREM Locus Mutations in Alzheimer's Disease David Holtzman, M.D., and Marco Colonna, M.D., Washington University, St. Louis	\$250,000
The Role of the KIBRA Gene in Abeta Regulation of AMPAR Trafficking Richard L. Huganir, Ph.D., Johns Hopkins University	\$100,000
3DDS: 3-D Neural Core/High-throughput Drug Screening for Alzheimer's Disease Using 3-D Human Neural Culture Systems Doo Yeon Kim, Ph.D., Massachusetts General Hospital, Harvard University	\$400,000
The Putative Role of Red Blood Cell CR1 Levels in Abeta Clearance and Alzheimer's Disease Pathogenesis Cynthia A. Lemere, Ph.D., Brigham and Women's Hospital, Harvard University	\$150,000
Extracellular Vesicle-Based Targeting of CD33-Mediated Pathology for Alzheimer's Disease Therapy Casey Maguire, Ph.D., Massachusetts General Hospital, Harvard University	\$150,000
PKC Mutations and Alzheimer's Disease Alexandra Newton, Ph.D., University of California, San Diego	\$220,000
G2T Research Models and Materials Taconic Biosciences Inc.	\$305,069
3DDS: Microglial Core/CD33 and Alzheimer's Disease: From Biology to Therapy Rudy Tanzi, Ph.D., and Ana Griciuc, Ph.D., Massachusetts General Hospital, Harvard University	\$400,000
Alzheimer's Genome Project Rudy Tanzi, Ph.D., Massachusetts General Hospital, Harvard University	\$1,500,000
Functional Characterization of GGA3 Mutations Associated with Alzheimer's Disease Giuseppina Tesco, M.D., Ph.D., Tufts University	\$150,000
BIN1 in Alzheimer's Disease Neuropathology Gopal Thinakaran, Ph.D., University of Chicago	\$150,000
Studying the Functional Consequences of Alzheimer's Disease Risk Variants in the CLU and ABCA7 Genes Using Both Human and Mouse Models Li-Huei Tsai, Ph.D., Massachusetts Institute of Technology	\$250,000
Molecular and Cellular Mechanisms of ACE1 Variant in Alzheimer's Disease Robert Vassar, Ph.D., Northwestern University	\$250,000
G2T: Centralized Research Core Operations Management Wilma Wasco, Ph.D., Massachusetts General Hospital, Harvard University	\$185,350
3DDS: High-Content Drug Screen Using a Novel 3-D Cell Model of Alzheimer's Disease Stephen Wong, Ph.D., Houston Methodist Research Institute	\$150,000
3DDS: Alzheimer's Disease Drug Discovery in 3-D Weiming Xia, Ph.D., Boston University	\$150,000
PICALM Gene Therapy and Drug Screening for Abeta Clearance Berislav Zlokovic, M.D., Ph.D., University of Southern California, and Beverly L. Davidson, Ph.D., University of Pennsylvania	\$375,170
The Role of PICALM Mutations in Alzheimer's Disease Berislav Zlokovic, M.D., Ph.D., and Zhen Zhao, Ph.D., University of Southern California	\$100,000

Project/Researcher	Distribution Amount
Identification and Early Detection	
Discovery of Alzheimer's Disease Blood Biomarkers Using Phage Display Technology, Year 2 Yueming Li, Ph.D., Memorial Sloan Kettering Institute	\$100,000
Innate Immunity	
Abeta Expression Protects the Brain from Herpes Simplex Virus Robert D. Moir, Ph.D., Massachusetts General Hospital	\$325,000
Targeting Beneficial Innate Immunity in Alzheimer's by IRAK-M Deletion Terrence Town, Ph.D., University of Southern California	\$150,000
Microbiome	
Role of the Gut Microbiome in Alzheimer's Disease Pathology and the Potential of Probiotic Therapeutic Strategies Deepak Kumar, Ph.D., Massachusetts General Hospital, and Robert D. Moir, Ph.D., Massachusetts General Hospital, Harvard University	\$150,000
The Role of Microbial Immune Responses in Alzheimer's Disease Sangram S. Sisodia, Ph.D., University of Chicago	\$250,000
Pathological Pathways and Systems	
Development of Novel APP Dimerization Inhibitors That Lower Abeta Levels Carmela R. Abraham, Ph.D., Boston University	\$100,000
Identification of Reactive Astrocyte-Secreted Neurotoxic Protein Responsible for Neuronal Apoptosis Ben Barres, M.D., Ph.D., Stanford University	\$150,000
Optimization of Pharmacologic Properties of Molecular Tweezers Gal Bitan, Ph.D., University of California, Los Angeles	\$100,000
Genetic Targets to Block Tau Propagation Marc Diamond, M.D., University of Texas Southwestern Medical Center	\$150,000
Long Abeta, Intraneuronal Amyloid and an Alternative Amyloid Hypothesis of Alzheimer's Disease Charles Glabe, Ph.D., University of California, Irvine	\$100,000
Uncovering Determinants of Neuronal Vulnerability in Alzheimer's Disease Paul Greengard, Ph.D., The Rockefeller University	\$250,000
Cell Cycle Re-entry in 3-D Human Neuron Cultures John S. Lazo, Ph.D., and George Bloom, Ph.D., University of Virginia	\$100,000
Tau Misrouting in Alzheimer's Disease—Causes and Consequences Eckhard Mandelkow, Ph.D., and Eva-Maria Mandelkow, M.D., Ph.D., German Center for Neurodegenerative Diseases	\$150,000
Systemic Inflammatory Networks in Alzheimer's Disease Matthias Nahrendorf, M.D., Ph.D., and Filip Swirski, Ph.D., Massachusetts General Hospital, Harvard University	\$200,000
Regulation of RNA Translation by MAPT in Alzheimer's Disease Benjamin Wolozin, M.D., Ph.D., Boston University	\$100,000
Rejuvenation of Microglia in Brain Aging and Neurodegeneration Tony Wyss-Coray, Ph.D., Stanford University	\$150,000
Stem Cell Models	
Stem Cell Approach to Investigating the Phenomenon of Brain Insulin Resistance Sam Gandy, M.D., Ph.D., Icahn School of Medicine at Mount Sinai Hospital	\$100,000
Therapeutic Strategies	
Evaluation of AMX0035, a Neuroprotecting and M1-Deactivating Therapeutic, in an Immunological Model of Alzheimer's Disease (Part 1) Amylyx Pharmaceuticals Inc.	\$150,000
Evaluation of AMX0035, a Neuroprotecting and M1-Deactivating Therapeutic, in an Immunological Model of Alzheimer's Disease (Part 2) Amylyx Pharmaceuticals Inc.	\$150,000
Lead Optimization and Lead Evolution of Potent SGSMs for the Treatment of Alzheimer's Disease Steven Wagner, Ph.D., University of California, San Diego, and Rudy Tanzi, Ph.D., Massachusetts General Hospital, Harvard University	\$476,988
Whole Genome Sequencing and Epigenetics	
Analytical and Statistical Tools for Sequence Analysis for Alzheimer's Disease Christoph Lange, Ph.D., Harvard T.H. Chan School of Public Health	\$150,000
Total Distributed to Research	\$10,022,815

2015 RESEARCH ABSTRACTS

Genes to Therapies™(G2T)/Drug Screening

Finding mechanisms of actions by which Alzheimer's genes affect risk and identifying targets for potential drug intervention



ABCA7 in Brain Homeostasis and Alzheimer's Disease

\$200,000



Guojun Bu, Ph.D.

Professor of Neuroscience, Mayo Clinic Jacksonville

Takahisa Kanekiyo, M.D., Ph.D.

Assistant Professor of Neuroscience, Mayo Clinic Jacksonville

Genetic and environmental risk factors contribute to the etiology of late-onset Alzheimer's disease (LOAD), which accounts for the vast majority of disease cases. In addition to APOE, recent genome-wide association studies have identified novel susceptibility genes for LOAD. Among them, gene variants in ABCA7 coding ATP-binding cassette transporter A7 (ABCA7), which is a homologous transmembrane protein to ABCA1, have shown strong association with the increased risk for LOAD. Given that loss-of-function variants in ABCA7 have been demonstrated to significantly increase AD risk, suppression of ABCA7 level and/or function is predicted to contribute to AD pathogenesis. Despite strong genetic evidence on the role of ABCA7 in AD pathogenesis, our knowledge on ABCA7 function in neurons and microglia in the brain is limited. Our recent studies using knockout mice have implicated novel functions of ABCA7 in regulating APP processing and lipid metabolism. Thus, we hypothesize that ABCA7 regulates BACE1 expression and APP processing in neurons, and lipid metabolism and immune responses in microglia. Our overall goals are to investigate how cell type-specific ABCA7 loss-of-function impacts known AD-related pathways and to identify novel ones through nontargeted approaches. Specifically, we will examine ABCA7 function using primary neurons and microglia/macrophage from ABCA7 knockout mice, as well as those derived from human induced pluripotent stem cells (iPSCs) where the ABCA7 gene can be modified by CRISPR/Cas9 technology to generate disease-associated mutants. We also will use the Cre-lox system to delete the *Abca7* gene in neurons or microglia in mice. Our innovative approaches should lead to a new therapeutic strategy targeting ABCA7 in AD.

3DDS: A 3-D Human Neural Cell Culture System for Studying Neuron-Microglia Interaction in Alzheimer's Disease

\$150,000



Hansang Cho, Ph.D.

Assistant Professor, University of North Carolina at Charlotte

In this proposal, we aim to dramatically improve the current microglial chemotactic model into a hybrid brain model that recapitulates pathological cascades of Alzheimer's disease, including beta-amyloid deposits, microglial recruitment/clearance of beta-amyloid, neurofibrillary tangles and possibly neuronal death. To do this, we have been collaborating closely with Drs. Doo Yeon Kim and Rudolph E. Tanzi (Massachusetts General Hospital) to combine their novel three-dimensional Alzheimer's in a dish model with our microglial chemotactic model. Drs. Kim's and Tanzi's 3-D AD human neurospheroid successfully recapitulates Abeta and tau pathology in their system. By combining our microglia model and their 3-D AD neuron model, we can analyze the impact of human microglial cells on Abeta and tau pathology. Furthermore, we can assess a hypothesis that human microglial cells are recruited to Abeta deposits in a 3-D human neural culture model, and therefore contribute to AD pathogenesis.

Role of Blood-Brain Barrier Function in Alzheimer's Disease Pathogenesis Investigated Using a 3-D Microfluidic Platform

\$291,056



Se Hoon Choi, Ph.D.

Instructor of Neurology, Massachusetts General Hospital



Roger D. Kamm, Ph.D.

Cecil and Ida Green Distinguished Professor of Biological and Mechanical Engineering, Massachusetts Institute of Technology

Alzheimer's disease (AD) is the most common form of dementia among older people. The blood-brain barrier (BBB) is a highly selective permeable barrier that separates the brain from circulating blood. It is formed by brain endothelial cells and prevents harmful materials in the blood from entering the brain. Evidence identifying BBB dysfunction in AD or patients at risk (i.e., those with mild cognitive impairment) continues to escalate. In cerebral amyloid angiopathy (CAA), which is a unique form of AD, a toxic molecule generated in AD brain, called Abeta, deposits within the blood vessels of the brain. The deposition of Abeta leads to BBB impairment, including microhemorrhages, which contribute to AD pathogenesis in CAA. However, little is known about the role of the BBB function in AD pathogenesis. We propose to elucidate the function of BBB in AD progression and investigate whether toxic molecules generated in AD brain cause BBB impairment.

Discovery of CK1 Activators for Inducing the Autophagic Degradation of APP Beta-CTF

\$450,000

**Paul Greengard, Ph.D.**

Head of the Laboratory of Molecular and Cellular Neuroscience
Vincent Astor Professor, The Rockefeller University
Nobel Prize in Physiology and Medicine, 2000

Alzheimer's disease (AD) is a neurodegenerative disorder that affects more than 5 million people in the United States. One of the hallmarks of AD is the accumulation of amyloid plaques in the brain of patients. The amyloid plaque is composed of Abeta peptide, which originates from an amyloid precursor protein (APP). Multiple lines of evidence suggest that a defective clearance mechanism is involved in the pathogenesis of AD. Our laboratory has discovered a novel molecular pathway regulating protein clearance, which represents an attractive therapeutic target for developing drugs for AD. We propose to screen a chemical library of small molecules to identify compounds that can modulate the clearance of the Abeta peptide. We already have developed an assay to screen a large chemical library available at The Rockefeller University. After we identify and select modulators of Abeta clearance, we will examine their effects *in vitro* and *in vivo* using cell lines and mouse models of AD.

Investigation of sTREM2 in CSF as a Potential Biomarker for Neuronal Cell Death

\$144,182

**Christian Haass, Ph.D.**

Professor, Department of Metabolic Biochemistry,
Ludwig Maximilians University of Munich

There is strong evidence that inflammation occurs in different stages of Alzheimer's disease (AD), and understanding this process can help us to design new therapeutic approaches. TREM2 is a protein directly related to the inflammation process that occurs in the brain of patients with AD; mutations in this protein increase the risk to develop AD up to threefold. A fragment of this protein, namely soluble TREM2 (sTREM2), can be detected in biological fluids like the cerebrospinal fluid (CSF). The function of sTREM2 in health and disease currently is not known. Our aim is to investigate the function of sTREM2 and any mutation-specific effect on its function by the use of novel mouse models. Importantly, we will establish the temporal expression of sTREM2 in the CSF of patients with or without familial history of the disease. By doing so we may provide evidence for a novel biomarker for neuronal cell death.

The Biological Impact of TREM Locus Mutations in Alzheimer's Disease

\$250,000



David Holtzman, M.D.

Andrew B. and Gretchen P. Jones Professor and Chairman of Neurology; Professor of Neurology and Molecular Biology and Pharmacology; Associate Director of the Alzheimer's Disease Research Center, Washington University School of Medicine

Marco Colonna, M.D.

Professor, Pathology and Immunology, Professor of Medicine, Washington University School of Medicine

Whole genome sequencing has identified certain polymorphisms affecting genes encoding triggering receptors expressed on myeloid cells (TREMs) with increased risk of nonfamilial (sporadic) Alzheimer's disease. TREM signaling is known to be important in the innate immune response, particularly in the inflammatory response. However, the relationship between the function of TREM receptors and Alzheimer's disease pathology is largely unresolved. This project will investigate the relevant polymorphisms to determine how they facilitate Alzheimer's disease and how they might be targeted to improve brain immune function.

The Role of the KIBRA Gene in Abeta Regulation of AMPAR Trafficking

\$100,000



Richard L. Huganir, Ph.D.

Professor and Director of Neuroscience, Johns Hopkins University School of Medicine

Modulation of AMPA receptors (AMPA Rs), the major excitatory receptors in the brain, is thought to underlie learning and memory, as aberrant AMPAR regulation contributes to impaired memory and cognition. Several studies suggest dysregulation of AMPAR modulation plays a central role in Alzheimer's disease (AD). We recently identified a protein implicated in human memory performance, KIBRA, and its binding partner PICK1 as critical modulators of synaptic regulation of AMPARs. Additionally, genome-wide screening studies have suggested a strong association between single nucleotide polymorphisms in the KIBRA gene and AD. We propose to investigate the molecular and cellular mechanisms that underlie the genetic association of KIBRA variants with AD progression. Results from these studies will elucidate the role of KIBRA in AD and may establish novel targets for therapeutic treatment of early cognitive decline in AD.

3DDS: 3-D Neural Core/High-Throughput Drug Screening for Alzheimer's Disease Using 3-D Human Neural Culture Systems

\$400,000

**Doo Yeon Kim, Ph.D.**

Assistant Professor of Neurology, Harvard Medical School;
Genetics and Aging Research Unit, Massachusetts General Hospital

The Abeta cascade hypothesis of Alzheimer's disease (AD) has provided a major framework for AD drug discovery and has led to many current clinical trials. However, to date, no single *in vitro* or *in vivo* AD model has been able to recapitulate the presumed patient pathophysiology: Abeta deposition directly leads to tangles and neurodegeneration. Recently, we created a novel three-dimensional (3-D) human neural cell culture model of AD using genetically engineered human neural stem cells. Using this unique model, we showed that expression of APP and PSEN1 with familial AD mutations is sufficient to induce extracellular Abeta deposits and robust tauopathy, including hyperphosphorylated tau and detergent-resistant, silver-positive neurofibrillary tangles for the first time (Choi et al., 2014).

This human 3-D culture model has great potential to innovate and accelerate the current AD drug screening process. We now propose to use high-throughput drug screening in combination with our 3-D human cellular AD model to identify and characterize novel AD drugs and drug targets that can reduce both Abeta and tau pathologies, which is not feasible using current AD mouse models. In Aim 1, we will develop a high-throughput screening (HTS) system based on 3-D human cellular AD models (3-D AD-HTS). In Aim 2, we will carry out 3-D AD-HTS using FDA-approved drug libraries and validate the primary hits that reduce Abeta and/or tau pathologies and finally, in Aim 3, we will explore whether the validated candidate drugs rescue the neuronal injuries and functional deficits in 3-D AD culture model.

The overarching goals of this study are to establish a 3-D HTS AD drug screening system based on human 3-D neural cell culture models; find potential AD drug candidates among the FDA-approved drugs (drug repurposing); and identify novel cellular pathways that can regulate both Abeta and tau pathologies. Since no current AD mouse model of Abeta deposition leads to tangles and neurodegeneration, which are both critical aspects of the disease, the human neural cell culture model could serve as a novel, crucial drug discovery platform for AD.

The Putative Role of Red Blood Cell CR1 Levels in Amyloid Beta Clearance and Alzheimer's Disease Pathogenesis

\$150,000

**Cynthia A. Lemere, Ph.D.**

Ann Romney Center for Neurologic Diseases, Brigham and Women's Hospital;
Associate Professor of Neurology, Harvard Medical School

The immune system uses complement proteins and receptors to “coat and clear” pathogens and proteins from the body. Complement Receptor 1 (CR1/CD35) is found on the surface of red blood cells in humans and helps shuttle cellular debris to the liver for degradation. Recently, specific genetic variations, called polymorphisms, in the CR1 gene were found to be associated with an increased risk of late-onset Alzheimer's disease. We hypothesize that people with AD-risk CR1 polymorphisms have low levels of CR1 protein on their red blood cells and, therefore, are less efficient at clearing Abeta throughout life, gradually leading to Abeta aggregation and deposition in the brain. To test this hypothesis, we will examine Abeta and CR1 in archived human brain and measure the amount of CR1 molecules on red blood cells in individuals with and without AD-risk CR1 polymorphisms.

Extracellular Vesicle-Based Targeting of CD33-Mediated Pathology for Alzheimer's Disease Therapy

\$150,000

**Casey Maguire, Ph.D.**

Assistant Professor of Neurology, Harvard Medical School

Alzheimer's disease (AD) is a devastating disease for patient and family alike. Unfortunately, there is no effective treatment and conventional, drug-based therapies have failed. An alternative approach called “gene therapy” is showing clinical promise for the treatment of several diseases and involves using genes to directly target the molecular basis of disease. In this proposal, we will test a new type of gene therapy that is specifically tailored to manipulate the outcome of “pathological” genes associated with AD. The proposal combines the expertise of AD researchers who have identified the pathological genes and that of gene delivery researchers. In a mouse model of AD, our objective is to test the efficacy of gene therapy to prevent/reduce disease progression. This work is an important step in developing a therapy to treat AD in patients.



PKC Mutations and Alzheimer's Disease

\$220,000



Alexandra Newton, Ph.D.

Professor of Pharmacology, University of California, San Diego

The goal of this project is to analyze how Alzheimer's disease (AD)-associated mutations in a key signaling molecule, protein kinase C α (PKC α), contribute to disease pathogenesis. PKC α plays a pivotal role in tuning the signaling output of cells and, as such, is frequently mutated in human cancers. The Alzheimer's Genome Project™ led by Dr. Rudolph Tanzi and colleagues has identified unique mutations in PKC α that co-segregate with AD in families with the disease. In the preceding funding period, structure/function and cellular studies revealed that a rare variant in PKC α identified in seven members of four unrelated families results in gain of function. This is in marked contrast to the mutations in human cancers that result in loss of function. These data reveal that this Alzheimer's disease-associated mutation increases the signaling by PKC α , identifying PKC α as a novel therapeutic target. The proposed research aims to examine other variants of PKC α that co-segregate with Alzheimer's disease, use genome editing to examine how one mutant allele affects cellular signaling and disease outcome in animal models, and analyze whether PKC α signaling is amplified in brains from Alzheimer's disease vs. non-disease humans.

G2T Research Models and Materials

\$305,069



Taconic Biosciences

Taconic Biosciences, a global provider of genetically modified mouse models and associated services, is providing customized mouse models (transgenic, conventional/conditional knock out, conventional/conditional knock in) for each specific gene and type of mutation that will be studied in the Genes to Therapies™ project.

3DDS: Microglial Core/CD33 and Alzheimer's Disease: From Biology to Therapy

\$400,000

**Rudolph Tanzi, Ph.D.**

Joseph P. and Rose F. Kennedy Professor of Neurology, Harvard Medical School;
Director of the Genetics and Aging Research Unit, Massachusetts General Hospital

Ana Griciuc, Ph.D.

Instructor in Neurology, Harvard Medical School; Assistant in Neuroscience,
Massachusetts General Hospital

Our current inability to prevent or delay Alzheimer's disease (AD) and the expected increase in the prevalence of AD are predicted to give rise to a global AD pandemic. We recently have identified a novel pathway for Abeta clearance in the aging brain that is highly relevant to AD pathogenesis. In a very large family-based, genome-wide association study, we identified CD33 as a novel late-onset AD risk factor. CD33 encodes a transmembrane sialic acid-binding immunoglobulinlike lectin that regulates innate immunity.

We found that CD33 is specifically expressed in microglial cells and exhibits an increased expression in AD. Using microglial cell cultures, we showed that CD33 inhibits uptake and clearance of Abeta42, a process that requires the sialic acid-binding domain of CD33. CD33 knockout led to a marked reduction in insoluble Abeta42 levels and amyloid plaque burden in mouse models of AD. We also found that CD33 knockout in AD mice results in skewing of adult microglia from the M1 (pro-inflammatory, neurotoxic) to the M2 (pro-phagocytic, neuroprotective) activation phenotype. Thus, CD33 activity in microglial cells promotes Abeta pathology and CD33 has emerged as a novel target for drug development in AD.

Here, we propose to inhibit CD33 activity to induce uptake and clearance of Abeta42, and to enhance skewing of microglia from the pro-inflammatory M1 toward the pro-phagocytic M2 activation state. We will identify and validate effective CD33 inhibitors by performing an unbiased high-throughput screen of 1,280 small molecules in microglial cells. These compounds are from Prestwick Chemical and were approved by the U.S. Food and Drug Administration and other agencies. We also will develop CD33 inhibitors by screening CD33-specific antibodies for their ability to inhibit CD33 function in microglial cell-based assays. Successful compounds and CD33-specific antibodies that inhibit CD33 activity in Abeta clearance and M1/M2 cytokine release assays will be further tested in mice. These studies might result in a novel and powerful therapeutic approach for AD.



Alzheimer's Genome Project™

\$1,500,000



Rudolph Tanzi, Ph.D.

Joseph P. and Rose F. Kennedy Professor of Neurology, Harvard Medical School;
Director of the Genetics and Aging Research Unit, Massachusetts General Hospital

Funds will be used for whole genome sequencing analyses, including family-based association using new algorithms being developed by Cure Alzheimer's Fund grantee Christoph Lange of the Harvard T.H. Chan School of Public Health, targeted re-sequencing efforts, high-throughput replication genotyping, statistical analysis consulting and computer costs, transgenic and knockout animal costs, and molecular biology and biochemical reagents for translational studies. Funds also are used for routine equipment maintenance and service contracts. Functional variants discovered in our whole genome sequencing and targeted re-sequencing efforts are confirmed by Sanger sequence confirmation and replication genotyping using Fluidigm's nano-fluidic genotyping arrays. For functional studies, we will continue to use cell-based (including neural precursor cells and iPS cells) and animal-based AD models. As part of the non-AGP pilot studies, we will continue to investigate the role of neurogenesis in AD.

Functional Characterization of GGA3 Mutations Associated with Alzheimer's Disease

\$150,000



Giuseppina Tesco, M.D., Ph.D.

Associate Professor of Neuroscience, Tufts University

Neurons, highly organized brain cells, are characterized by specialized projections called dendrites and axons. The axon is the longest neuronal projection where proteins move like cars along a highway, in two different directions and at different speeds. Scientists demonstrated that in the brain of subjects affected by Alzheimer's disease (AD), a disorder characterized by memory loss, this coordinate traffic doesn't work properly, so neurons start to be unhealthy and die. Our goal is to try to understand why this traffic no longer is functioning in order to find a way to prevent the neuronal traffic disruption and possibly find a treatment for Alzheimer's disease. The aim of this study is to determine the extent to which mutations in a trafficking molecule called GGA3 may lead to disruption of protein movements in the axon, ultimately causing neuronal death.

BIN1 in Alzheimer's Disease Neuropathology

\$150,000

**Gopal Thinakaran, Ph.D.**

Professor, Neurology and Neurobiology, University of Chicago

The goal of this proposal is to investigate how one of the recently identified late-onset Alzheimer's disease risk genes, namely BIN1, contributes to neuropathology. BIN1 is an adaptor protein that regulates membrane dynamics in a variety of cellular contexts. Only limited information is available on BIN1 expression and function in the brain. As such, there is much to be learned about the precise biological and mechanistic connection between BIN1 and Alzheimer's disease. We propose to use an integrated approach employing cultured cells and BIN1 transgenic mice to test specific hypotheses regarding BIN1 function and dysfunction in Alzheimer's disease.

Studying the Functional Consequences of Alzheimer's Disease Risk Variants in the CLU and ABCA7 Genes Using Both Human and Mouse Models

\$250,000

**Li-Huei Tsai, Ph.D.**

Director of the Picower Institute for Learning and Memory; Professor of Neuroscience, Massachusetts Institute of Technology

The vast majority of people with Alzheimer's disease (AD) suffer from the sporadic, or late-onset form, the causes of which remain completely unknown. From studies involving thousands of people, researchers have identified a number of genetic variants that may increase one's risk for sporadic AD. However, little is understood regarding why these small changes impact one's AD risk. In this work, we will use the cutting-edge genome editing technique CRISPR/Cas9 to use homology-directed DNA repair to introduce AD-associated genetic variants of ABCA7 and CLU into reprogrammed human stem cells. Dr. Rudolph Tanzi has conducted whole-genome sequencing of 2,000 human DNA samples from control subjects and AD patients. We will determine whether these genetic variants interact with ApoE in the etiology of AD. We will differentiate human stem cells harboring these variants into various cell types populating the brain, including neurons, astrocytes and microglia, and study the effects of these variants in these different cell types. The proposed study will provide mechanistic insights on why some genetic variants found in the population may predispose some individuals to an increased risk for AD.



Molecular and Cellular Mechanisms of ACE1 Variant in Alzheimer's Disease

\$250,000



Robert Vassar, Ph.D.

Professor of Cell and Molecular Biology, Northwestern University Feinberg School of Medicine

Alzheimer's disease (AD) is a complex genetic disorder that is the leading cause of dementia in the elderly. Dr. Rudolph Tanzi and the Cure Alzheimer's Fund Alzheimer's Genome Project™ have identified a new mutation in a gene called ACE1 that is associated with increased risk for AD. How the ACE1 gene causes AD is completely unknown. The goal of this project is to study the role of the ACE1 gene in the disease process in genetically engineered mice. The information gathered from this study is expected to provide greater insight into the causes of AD in people and could lead to new AD therapies.

G2T: Centralized Research Core Operations Management

\$185,350



Wilma Wasco, Ph.D.

Associate Professor of Neurology, Harvard Medical School;
Associate Geneticist, Massachusetts General Hospital

Wilma Wasco, Ph.D., is responsible for the day-to-day organization of the Genes to Therapies™ (G2T) Centralized Research Core. She meets routinely with Dr. Tanzi as well as the members of the G2T Steering Committee and Meg Smith of Cure Alzheimer's Fund to outline and discuss progress with timelines and investigations as well as reagent generation and budgets. She will be responsible for determining what reagents are available from investigators or commercial sources while investigators are being recruited. In addition, she is the point person for Taconic and all other commercial or academic sources that will be used to generate reagents, as well as for the investigators who have been and will be recruited to work on each gene. It is envisioned that this will require, at a minimum, weekly email and phone interactions with each commercial or academic source and investigator. Dr. Wasco will travel to appropriate scientific meetings to meet with G2T investigators. If necessary, Dr. Wasco will be involved in any experimental work that is carried out within the Genetics and Aging Research Unit at Massachusetts General Hospital—which may include cell culture and reagent testing and confirmation. Dr. Wasco has longstanding expertise in Alzheimer's disease genetic studies; she played a significant role in the original discovery of the presenilin genes and is familiar with the techniques that will be used for the gene investigations. She also has a history of administrative project management; 80 percent effort will be devoted to this proposal.

3DDS: High-Content Drug Screen Using a Novel 3-D Cell Model of Alzheimer's Disease

\$150,000

**Stephen Wong, Ph.D.**

John S. Dunn Sr. Presidential Distinguished Chair in Biomedical Engineering, Professor of Systems Medicine and Bioengineering, Houston Methodist Research Institute

Taking advantage of several recent research progresses, we propose to carry out a compound screen to find potential drugs to treat Alzheimer's disease (AD). The screen will use a revolutionary 3-D stem cell model, recently developed by Drs. Rudolph Tanzi and Doo Yeon Kim at Massachusetts General Hospital, which for the first time faithfully recapitulated major pathological hallmarks of AD in a dish. The screen also will use the neuronal image processing software packages we developed previously, which are able to automatically and accurately assess the cell phenotype to evaluate the compound effect. Finally, we specifically will screen compounds that are known to be bioactive, including the compounds already used as drugs in clinics. Such a "drug repositioning" strategy will greatly reduce the cost of drug development, enable faster time to market and quickly translate the scientific discovery to patient bedside.

3DDS: Alzheimer's Disease Drug Discovery in 3-D

\$150,000

**Weiming Xia, Ph.D.**

Acting Associate Director of Research, Bedford VA Medical Center;
Boston University Alzheimer's Disease Center

We propose to examine biological fluids from cells treated with individual drugs that have been previously approved by the U.S. Food and Drug Administration for the treatment of numerous diseases and disorders. We will determine whether these drugs reduce the levels of the toxic proteins known to cause Alzheimer's disease. Newly identified drugs or similar modified compounds will be developed as Alzheimer's therapeutics.



PICALM Gene Therapy and Drug Screening for Abeta Clearance

\$375,170



Berislav Zlokovic, M.D., Ph.D.

Director of the Zilkha Neurogenetic Institute and Director of the Center for Neurodegeneration and Regeneration; Professor and Chair, Department of Physiology and Biophysics, Keck School of Medicine, University of Southern California

Beverly L. Davidson, Ph.D.

Professor of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania
Arthur V. Meigs Chair in Pediatrics, The Children's Hospital of Philadelphia

PICALM is a highly validated genetic risk factor for Alzheimer's disease (AD). Here, we report that PICALM reductions in AD and murine brain endothelium correlate with Abeta pathology and cognitive impairment. Moreover, PICALM deficiency diminishes Abeta clearance across the murine blood-brain barrier (BBB) and accelerates Abeta pathology that is reversible by endothelial PICALM re-expression. Using human brain endothelial monolayer, we show that PICALM regulates PICALM/clathrin-dependent internalization of Abeta bound to the low-density lipoprotein receptor related protein-1, a key Abeta clearance receptor, and guides Abeta trafficking to Rab5 and Rab11, leading to Abeta endothelial transcytosis and clearance. PICALM levels and Abeta clearance were reduced in AD-derived endothelial monolayers, which was reversible by adenoviral-mediated PICALM transfer. iPSC-derived human endothelial cells carrying the rs3851179 protective PICALM allele exhibited higher PICALM levels and enhanced Abeta clearance. Thus, PICALM regulates Abeta BBB transcytosis and clearance that has implications for Abeta brain homeostasis and clearance therapy.

The Role of PICALM Mutations in Alzheimer's Disease

\$100,000

**Berislav Zlokovic, M.D., Ph.D.**

Director of the Zilkha Neurogenetic Institute and Director of the Center for Neurodegeneration and Regeneration; Professor and Chair, Department of Physiology and Biophysics, Keck School of Medicine, University of Southern California

Zhen Zhao, Ph.D.

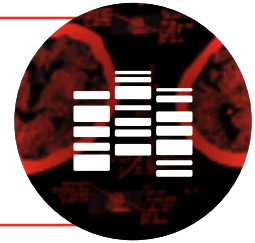
Assistant Professor of Research Physiology and Biophysics, Keck School of Medicine, University of Southern California

Alzheimer's disease (AD), the most common form of dementia in the elderly, is now the most expensive disease in the United States. The search for biological understanding of AD has expanded toward new risk factors, particularly genes. With the breakthrough of human genetics and high-throughput sequencing, PICALM, the gene encoding phosphatidylinositol binding clathrin assembly protein, has been identified as a new risk gene for AD. As a key player of the clathrin-mediated endocytosis and intracellular trafficking, PICALM critically regulates Abeta brain metabolism and neuronal toxicity. Our group recently reported that reduction of PICALM in cerebral vasculature was strongly associated with AD progression in patients, and accelerated disease progression in animal model of AD. However, it still is unclear whether genetic variations or mutations of the PICALM gene directly contribute to the disease. Fortunately, the Alzheimer's Genome Project™, led by Dr. Rudolph Tanzi at Harvard University, discovered three novel PICALM genetic mutations that are strongly associated with AD through whole genome sequencing (WGS). Therefore, the biological functions of these rare mutations need to be examined urgently, especially in a cell type-specific manner, in order to determine their directly contributions to the pathogenesis of AD.

Our recent study, using both transgenic models and CRISPR (clustered regularly interspaced short palindromic repeat) mediated genomic editing in human-induced pluripotent stem cells (iPSCs), has demonstrated that PICALM plays a key role in mediating the clearance of Abeta at the blood-brain barrier (BBB), as well as mitigating Abeta toxicity in neurons, providing molecular and cellular bases to investigate the Abeta-dependent and independent functions of novel PICALM mutations. We hypothesize that these mutations may represent loss-of-function (LOF) or gain-of-function (GOF) mutations that affect Abeta metabolism and toxicity in different brain cell types, particularly the brain endothelial cells and neurons. In this proposed study, we will investigate the functions of the novel PICALM mutations using both endothelial cells and neurons derived from CRISPR-engineered iPSCs, and examine their impact on trans-vascular clearance and neuronal toxicity of Abeta. We expect to generate unique new insights into AD-related PICALM mutations that will have important impact on our understanding of the disease and implications to the development of new PICALM-targeted therapies for AD.

Identification and Early Detection

Improving our methods of detecting Alzheimer's in its early stages, opening the doors for treatments that stop the disease before symptoms begin or progress



Discovery of Alzheimer's Disease Blood Biomarkers Using Phage Display Technology, Year 2

\$100,000



Yueming Li, Ph.D.

Professor and Director of Graduate Program in Pharmacology,
Memorial Sloan-Kettering Institute

Absence of biomarkers has posed a formidable challenge in the development of effective treatment for Alzheimer disease (AD). Blood-based biomarkers could offer advantages that allow for early AD diagnosis and are critical in monitoring efficacy in clinical studies. Proposed studies aim to identify a set of novel blood biomarkers and examine their potential application as diagnostic agents. Phage display is a powerful approach to engineer peptides or proteins for binding to targets of interest. Therefore, we will apply phage display technology to identify peptides that selectively interact with molecules in AD blood samples, not in the age-matched controls. In Aim 1, we will identify potential biomarkers by screening two libraries with a diversity of approximately 2 billion peptides against AD and control blood samples. To overcome the anticipated kinetic limitations with monovalent peptides, we will polymerize them by conjugation to dendrimers combined with functional moieties, including fluorescent dyes, for validation studies in Aim 2. These studies will identify a set of peptides that potentially can be used as diagnostic agents for AD. Furthermore, the proposed research is highly transformative and can be widely applied for biomarker studies in other human disorders. Overall, these proposed studies address a critical unmet medical need in AD by providing large sets of new biomarkers for rapid and accurate noninvasive diagnosis of AD using innovative approaches.

Innate Immunity

Understanding the role of the brain's immune response—including inflammation—in the development and progression of Alzheimer's disease



Abeta Expression Protects the Brain from Herpes Simplex Virus

\$325,000



Robert D. Moir, Ph.D.

Assistant Professor of Neurology,
Harvard Medical School/Massachusetts General Hospital

The hallmark pathology in Alzheimer's disease (AD) is deposition of Abeta in brain as insoluble amyloid. Soluble Abeta assemblies called oligomers generated early in the amyloid pathway are toxic to neurons and are thought to cause neurodegeneration in AD. Prevailing theories attribute the generation of oligomers/amyloid to an abnormal propensity of Abeta to self-associate. Abeta itself is typically described as a functionless accidental product of metabolism. However, a family of proteins known as the antimicrobial peptides shares Abeta's purportedly abnormal proclivity for self-association. For antimicrobial peptides, self-association is key for their normal role in defending against invading microbial pathogens. Antimicrobial peptides are natural antibiotics and play an essential role in immunity, particularly in immunoprivileged tissues like the brain. In addition to a normal protective role in infection, dysregulated antimicrobial peptides also can be harmful, causing degenerative pathologies. Antimicrobial peptides also are deposited as amyloid in at least three human diseases.

The striking similarities between Abeta and antimicrobial peptides led us to investigate possible protective roles for oligomers and amyloid. We tested infection resistance in genetically modified cultured cells, nematode worms, fruit flies and AD mice that express human Abeta. Expression of Abeta protected against infection in all four models, doubling survival rates in some cases. Most remarkably, amyloid appears to play a key role, entrapping pathogens in tough fibrous networks that strongly resemble the Abeta deposits found in AD brain. Our study used well-characterized laboratory bacteria and yeast as experimental pathogens. However, these microbes are infrequent brain pathogens and not linked to AD. In this study, we propose to extend our investigations focusing on Abeta-mediated protection against herpes simplex one (HSV-1), a virus infecting 90 percent of people worldwide and a

continued >



Abeta Expression Protects the Brain from Herpes Simplex Virus (continued)

pathogen with strong links to AD pathology. Experiments will compare HSV-1 resistance of genetically modified AD mice and wild-type controls. Amyloid entrapment of invading HSV-1 viral particles also will be characterized.

Positive findings from our proposed study would contribute to AD research in at least three ways. Firstly, confirmation of an anti-viral activity for Abeta would advance our work on the unanticipated protective roles played by Abeta in the brain. Our novel AMP model of Abeta is likely to facilitate a significant shift in how amyloidosis in AD is viewed. Secondly, confirmation that amyloid entraps HSV-1 in brain would help address longstanding unresolved issues concerning how the virus may exacerbate amyloidosis in AD. Finally, low-virulence HSV-1 may provide a new tool for modulating amyloid deposition in AD mouse models. Both basic research and drug discovery programs are likely to find applications for animal models that can be made to develop Abeta amyloid in days as opposed to the months or years currently required.

Targeting Beneficial Innate Immunity in Alzheimer's by IRAK-M Deletion

\$150,000



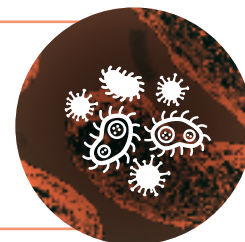
Terrence Town, Ph.D.

Professor of Physiology and Biophysics, Keck School of Medicine,
University of Southern California

A defining feature of Alzheimer's disease is brain accumulation of toxic plaques that induce memory loss. In the healthy brain, innate immune cells serve to protect; however, in Alzheimer patients' brains, these cells are inefficient at clearing the plaques. Innate immune cells express a molecule named IRAK-M that ensures immune responses to invading bacteria and viruses are kept under tight control, yet this type of immune response is dysfunctional in the Alzheimer patient brain. Our hypothesis is that rebalancing cerebral innate immunity by blocking IRAK-M will enable plaque clearance. We will use mice that develop amyloid plaques but that lack the IRAK-M gene to harness innate immune cells to clear the plaques and, hopefully, to restore learning and memory. Upon completion, this project will provide crucial data on the role of IRAK-M and the innate immune system in Alzheimer's disease. This important work represents a major step toward developing a novel immune therapy for this devastating disorder of the mind.

Microbiome

Understanding the ways in which microbes living in the body may affect brain health



Role of the Gut Microbiome in Alzheimer's Disease Pathology and the Potential of Probiotic Therapeutic Strategies

\$150,000



Deepak Vijaya Kumar, Ph.D.

Research Fellow, Massachusetts General Hospital

Robert D. Moir, Ph.D.

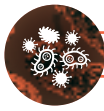
Assistant Professor of Neurology, Harvard Medical School/Massachusetts General Hospital

The human gut contains a myriad of microorganisms collectively referred to as the microbiome. We propose to investigate if the microbiome may modulate the progression of early Alzheimer's disease (AD) pathology.

More than a century ago, the Nobel laureate Elie Metchnikoff postulated that “good gut bacteria” may delay senility and have beneficial effects for the symptoms of anxiety and depression associated with deteriorating cognition. Manipulation of the gut microbiome since has become common practice, with widespread consumption of probiotics, a concentrated bacterial cocktail. While the broad array of health benefits claimed for probiotics have failed to be substantiated in controlled clinical studies, findings have shown that the gut microbiome is essential for normal brain function. Signal molecules from the microbiome can enter the central nervous system (CNS) and modulate brain activities, sometimes producing profound effects in animal models. More recently, it has emerged that fecal transplants, used to redress microbial imbalances in the gut, also can impact patient mental functions.

The complex interaction between the microbiome and brain has come to be called the microbiota-gut-brain axis. While the beneficial aspects of probiotics appear to have been overstated, what has emerged is a clear realization that an abnormal microbiota-gut-brain axis can potentiate existing pathologies or even cause new disease. Particularly germane to AD has been recent revelations that the pathways of the microbiota-gut-brain axis include a comprehensive two-way communication system between the microbiome and brain. Findings suggest that under disease conditions, the “back and forth” between the brain and microbiome may become disrupted and reinforce harmful pathways that promote pathology. Abnormal brain activity can shift conditions in the gut and lead to a rise in gut bacteria linked to neuroinflammation and poor health outcomes. Changes in the gut microbiome lead, in turn, to increases in metabolites that exacerbate neuroinflammation, anxiety and depression in the brain.

continued >



Role of the Gut Microbiome in Alzheimer's Disease Pathology and the Potential of Probiotic Therapeutic Strategies (continued)

We propose to investigate whether this pathological cycle may have a role in early AD. In preliminary studies we found that, compared with nontransgenic littermates, the gut of young, pre-symptomatic transgenic AD mice have lower levels of bacteria thought to be protective. In this study, we propose to first characterize the changes in transgenic AD mice gut microbiome in high detail. Next, we plan to test whether shifts in gut microbiota can ameliorate or potentiate the progression of pathology in transgenic AD mice. Experiments will test the effects of fecal transplants, probiotics and bacterial metabolites on young transgenic AD mice. We believe confirmation of a role for the gut microbiome in AD pathology has the potential to reshape diagnostic and treatment strategies for early AD.

The Role of Microbial Immune Responses in Alzheimer's Disease

\$250,000



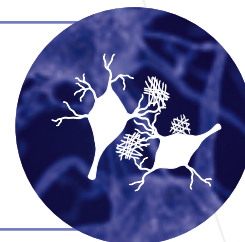
Sangram S. Sisodia, Ph.D.

Thomas Reynolds Sr. Family Professor of Neurosciences, Director of the Center for Molecular Neurobiology, University of Chicago

Animal models of Alzheimer's disease (AD) recapitulate the severe amyloidosis and neuroinflammation that is evident in the human disease. While it long has been assumed that inflammation associated with amyloid deposition reflects the activation of astrocytes and microglia into pro-inflammatory M1 states in response to injury, there is a paucity of information regarding the potential role of peripheral tissues and, more importantly, the microbiota in regulating innate immunity that in turn leads to CNS dysfunction. The notion that the commensal intestinal microbiota can influence brain function has at least one clear clinical origin: the observation that orally administered antibiotics can reverse encephalopathy in patients with decompensated liver disease (Schiano, 2010). Furthermore, psychiatric disorders frequently coexist with common gastrointestinal conditions, such as irritable bowel syndrome (IBS) that also are associated with disturbances of the intestinal microbiota. Emerging animal-based research has extended the idea of microbiota-brain interactions to other psychiatric disorders, as well as to such immunologically mediated neurological conditions as multiple sclerosis (MS) and to the exciting area of early brain development that has implications for autism spectrum disorders. For example, during vaginal delivery, the gastrointestinal tract of the newborn is colonized by the bacteria in the lower birth canal and perineum of the mother; therefore, the microbiota of infants delivered by Caesarean section differs from that of infants delivered through the genital tract. Studies in rats indicate that rats delivered at term by Caesarean section exhibit alterations (compared with rats delivered vaginally) in the prepubertal development of the prefrontal cortex and hippocampus (Juarez et al., 2008). A study on human neonates showed that the pattern of electrical activity in the brain is less complex in neonates born by Caesarean section than in age-matched neonates born by vaginal delivery (Kim et al., 2003). These latter results raise the possibility that different colonization patterns influence early postnatal brain development and also have longer-term consequences. Thus, this rapidly emerging field has the potential not only to increase our understanding of a broad spectrum of human disease, but also to generate novel therapies for these conditions based on the identification of mechanisms underlying microorganism-host interactions.

Pathological Pathways and Systems

Researching the underlying causes and mechanisms of Alzheimer's, such as Abeta accumulation, tau tangle propagation and inflammation



Development of Novel APP Dimerization Inhibitors That Lower Abeta Levels

\$100,000



Carmela R. Abraham, Ph.D.

Professor of Biochemistry and Medicine, Boston University School of Medicine

Globally, people are living longer, and Alzheimer's disease (AD), the major form of dementia among the elderly, is reaching pandemic proportions. Cognitive functions such as learning and memory are of fundamental biological importance, and diseases that affect these functions are among the most challenging biomedical problems of our time scientifically, emotionally and financially. Moreover, after decades of intense research and billions of dollars spent on clinical trials, no disease-modifying treatment for AD has been identified. In AD, Abeta accumulates in the brain and has toxic effects on neurons and their synapses. Here we propose a novel strategy that intends to lower Abeta and its associated toxicity by inhibiting the amyloid precursor protein (APP) dimerization and phosphorylation process involving the receptor tyrosine kinase c-Kit. Moreover, c-Kit is found in a complex with Gab2, a gene previously identified in genome-wide association studies to be associated with AD.

Our specific aims are to: further characterize the mechanism linking c-Kit inhibition to inhibition of APP dimerization, enhancement of APP phosphorylation and the lowering of Abeta; and optimize and characterize *in vitro* the lead Y series of Abeta-lowering compounds to prepare chemical probes with optimal pharmacokinetic and central nervous system exposure properties. These small molecules could become novel treatments for AD by reducing the levels of Abeta before the peptide exerts its toxic effects, thereby protecting neurons, their synapses and other cells impacted by Abeta.



Identification of Reactive Astrocyte-Secreted Neurotoxic Protein Responsible for Neuronal Apoptosis

\$150,000



Ben Barres, M.D., Ph.D.

Professor of Neurobiology, Developmental Biology and Neurology,
Stanford University School of Medicine

We are investigating the mechanisms that cause neurodegeneration in Alzheimer's disease. Our recent studies have led us to realize that a toxic protein unexpectedly is secreted by a class of brain cells called astrocytes in the setting of Alzheimer's disease. Our goal in this proposal is to identify this protein so that in future studies, we can test whether drugs that block the production or action of this drug will be useful as new therapies for Alzheimer's disease.

Optimization of Pharmacologic Properties of Molecular Tweezers

\$100,000



Gal Bitan, Ph.D.

Associate Professor of Neurology, David Geffen School of Medicine, University of California,
Los Angeles

Molecular tweezers (MTs) are compounds that act as Misfolded-Protein Clearance Enhancers (MPCEs) using a unique mechanism. They remodel the self-assembly of amyloidogenic proteins into formation of nontoxic and nonamyloidogenic structures that can be degraded efficiently by the natural clearance mechanisms. A lead MT called CLR01 has been found to prevent the self-assembly of multiple amyloid proteins into toxic oligomers and aggregates, including the proteins involved in Alzheimer's disease (AD)—Abeta and tau. Moreover, CLR01 reduced amyloid plaques and neurofibrillary tangles in animal models of AD following subcutaneous administration, demonstrating it was capable of passing through the blood–brain barrier (BBB). With the support of Cure Alzheimer's Fund, we recently demonstrated that CLR01 had a large safety margin in mice and measured its BBB penetration levels. These levels were found to be 1 to 3 percent. In addition, measurement of oral bioavailability showed that only 1 percent of CLR01 was absorbed through the gastrointestinal system. Therefore, we propose to optimize the oral bioavailability and BBB penetration of CLR01 via formulation and pro-drug approaches, an important step closer to obtaining FDA approval for initiation of clinical trials.



Genetic Targets to Block Tau Propagation

\$150,000



Marc Diamond, M.D.

Founding Director of the Center for Alzheimer's and Neurodegenerative Diseases,
University of Texas Southwestern Medical Center

Trans-cellular propagation of tau pathology has been implicated in the progression of Alzheimer's disease and other tauopathies. We previously have determined the mechanism by which tau aggregates bind the cell surface to trigger uptake via macropinocytosis. This involves direct binding of tau to heparan sulfate proteoglycans (HSPGs) on the cell surface. HSPGs are glycolipid-anchored and transmembrane core proteins that are extensively glycosylated and sulfated by a defined set of cellular enzymes. In prior published and unpublished work, we have determined that disruption of EXT1, a gene that plays a proximal role in the extension of sugar chains on HSPG core proteins, strongly inhibits tau aggregate uptake, seeding and transcellular propagation *in vitro* and *in vivo*. We hypothesize that individual HSPG synthetic genes required for tau uptake will represent viable drug targets.

Aim 1: We will individually test each of 24 genes associated with HSPG synthesis using Cas9/CRISPR-mediated gene knockout in HEK293T cells. We will confirm hits in HEK293T cells and primary cultured neurons.

Aim 2: We will test candidate genes in an *in vivo* model of spreading tau pathology by AAV-shRNA knockdown. If we are successful, a limited number of candidates will represent important new drug targets to block Alzheimer's disease progression.



Long Abetas, Intraneuronal Amyloid and an Alternative Amyloid Hypothesis of Alzheimer's Disease

\$100,000



Charles Glabe, Ph.D.

Professor of Molecular Biology and Biochemistry, University of California, Irvine

The goal of this proposal is to test a new paradigm for the causative mechanisms of Alzheimer's disease (AD): The accumulation of amyloid aggregates inside neurons, leading to their degeneration and initiating plaque formation. The hypothesis we will test is the inverse or mirror image of the commonly held view of the amyloid hypothesis of AD, which proposes that soluble Abeta is secreted from neurons, aggregates in the extracellular space and causes neuronal dysfunction from the outside. Based on new preliminary data, we propose that amyloid aggregation begins inside neurons, leading to neuronal dysfunction and death, which then releases the amyloid to the extracellular environment as a neuritic plaque. Recent reports of clinical trials of drugs that inhibit secretion of Abeta and decrease the concentration of Abeta in the interstitial fluid indicate that inhibition of Abeta secretion may exacerbate cognitive dysfunction in humans and have no effect on plaque accumulation. These results support the alternative amyloid hypothesis and suggest that the intracellular retention and accumulation of insoluble long Abetas may be an initial pathological event leading to intraneuronal accumulation of amyloid and, ultimately, neuronal death and neuritic plaque formation. We propose two specific aims that are pilot studies to generate reagents and models to test key predictions of this hypothesis. The first aim is to develop end-specific antibodies that specifically recognize long Abeta peptides ending at residues 45, 46, 47, 48 and 49. The second aim is to develop organotypic slice models of transgenic mouse brain to test the pathological significance of intraneuronal amyloid and examine whether it is the precursor to amyloid deposited in neuritic plaques. The successful completion of this project may have an important impact on the road map plan of Cure Alzheimer's Fund and research in Alzheimer's disease by initiating a paradigm shift and providing a clearer understanding of the mechanisms of neuronal dysfunction and degeneration underlying cognitive dysfunction. An accurate understanding of the disease mechanisms will provide a focus for the development of drugs that prevent or reverse cognitive decline rather than making drugs that make patients cognitively worse.



Uncovering Determinants of Neuronal Vulnerability in Alzheimer's Disease

\$250,000



Paul Greengard, Ph.D.

Head of the Laboratory of Molecular and Cellular Neuroscience
Vincent Astor Professor, The Rockefeller University
Nobel Prize in Physiology and Medicine, 2000

Neurofibrillary tangles (NFTs) and neurodegeneration occur only in very specific regions at early stages of Alzheimer's disease (AD), while many regions remain virtually unaffected. Using the bacTRAP technology the lab developed to isolate mRNAs from specific neuron types, we molecularly profiled these very vulnerable neurons and other neurons that are much more resistant to pathological lesions of AD. We looked for genes enriched in vulnerable neurons compared with resistant neurons, and were able to pinpoint a list of these candidate vulnerability genes. Genetic analyses on these genes led by the laboratories of Drs. Rudolph Tanzi and Kari Stefansson, uncovered one of these vulnerable neuron-enriched genes as genetically associated with AD in two very different populations. Dr. Tanzi further found three rare mutations in this gene that are present in individuals from three families with AD, indicating the importance of this gene in AD.

We now want to test whether these three mutations are indeed causative of the disease. For this we will test if the candidate gene is involved in the pathogenesis of the disease by analyzing mice invalidated for this gene. We also will study the three mutations, and investigate how they interfere with the protein function. Finally, we want to elucidate the pathways the candidate gene is involved in. We think this gene is potentially a crucial novel actor of AD pathogenesis that could explain specific vulnerability of certain neurons. Understanding its function and its molecular partners will yield new targets for the AD research community, for generating drugs that would prevent vulnerable neurons from degenerating.



Cell Cycle Re-entry in 3-D Human Neuron Cultures

\$100,000



John S. Lazo, Ph.D.

Professor of Pharmacology, University of Virginia School of Medicine

George Bloom, Ph.D.

Professor of Biology and Cell Biology, University of Virginia School of Medicine

The well-known behavioral symptoms of Alzheimer's disease (AD) are caused by the loss of connections, or synapses, among neurons that control memory and cognition, and by the death of those neurons. A major goal of our labs is to unravel the seminal molecular pathways that convert normal healthy neurons into neurons that will die long before the AD patients themselves. To that end, we recently made major strides toward understanding what may be the most common pathway for neuron death in AD: cell cycle re-entry (CCR), which represents the aberrant reactivation of innate processes for neuronal cell division. Whereas normal, fully differentiated neurons never attempt to divide, up to 5 to 10 percent of the neurons in brain regions affected by AD show signs of CCR over the course of many years. These neurons, which typically have duplicated much of their DNA, evidently never divide, but instead eventually die, and may account for as much as 90 percent of the massive neuron loss that occurs in AD. During the past few years we have defined many features of a complex biochemical signaling web that causes CCR. This process is initiated by soluble Abeta oligomers, which are the building blocks of the insoluble amyloid plaques that accumulate in AD brain, and requires soluble forms of tau, the protein that aggregates inside AD neurons to form insoluble neurofibrillary tangles.

Our principal strategy so far has been to model CCR in two-dimensional (2-D) cultures of mouse brain cells, and to test the *in vivo* relevance of our findings through parallel studies of transgenic AD model mice and human brain tissue samples. Now we would like to test the hypothesis that Abeta-induced, tau-dependent CCR can be observed in human neurons grown in three-dimensional (3-D) culture. Such cultures recently were shown to accumulate plaques and Abeta-dependent tangles, and thereby recapitulate human AD features that have not been achieved by any other cultured cell model. If successful, this effort will establish 3-D cultures of human neurons as a viable platform for screening potential drugs that block CCR, and revealing new diagnostic markers for this seminal process in AD pathogenesis.



Tau Missorting in Alzheimer's Disease—Causes and Consequences

\$150,000



Eckhard Mandelkow, Ph.D.

Principal Investigator, German Center for Neurodegenerative Diseases

Eva-Maria Mandelkow, M.D., Ph.D.

Principal Investigator, German Center for Neurodegenerative Diseases

During the development of Alzheimer dementia, multiple changes occur in brain cells, which makes the search and treatment of the underlying causes difficult. Therefore, a key goal of AD research is to identify very early changes, which occur long before cognitive deficits become apparent. One such early event is the so-called “missorting” of tau protein, which is normally found in the axons of neurons, but which in AD accumulates in the “wrong” compartments, the cell bodies and dendrites. The project aims to analyze the reasons for this pathological change and to find ways to prevent it.

Systemic Inflammatory Networks in Alzheimer's Disease

\$200,000



Matthias Nahrendorf, M.D., Ph.D.

Associate Professor of Radiology, Harvard Medical School, Director of the Mouse Imaging Program at the Center for Systems Biology, Massachusetts General Hospital

Filip Swirski, Ph.D.

Associate Professor of Radiology, Massachusetts General Hospital/Harvard Medical School

A common early symptom of Alzheimer's disease (AD) is short-term memory loss. As the disease worsens, symptoms can include problems with language, disorientation, mood and behavior changes, confusion about events, difficulty speaking, swallowing and walking. AD is the most common form of dementia and worsens over time, accounting for approximately 70 percent of dementia cases. It is a neurodegenerative disease characterized by loss of normal brain function as a result of damage and destruction of nerve cells. The damage occurs when structures called plaques, which are protein deposits called Abeta, and tangles, which are fibers called tau, build up in the brain and interfere with nerve function. Brains of AD patients also contain inflammatory cells, but the relevance of inflammation to disease development and exacerbation is unknown. Inflammation is a biological response to damage, stress and infection. It is a natural defense process that manifests itself as heat, pain, redness and swelling, playing an essential role in disease. Depending on the extent, type and duration of inflammation, the process either can be helpful or harmful, because it can remove the offending pathogen, but it also can cause damage to healthy tissue. This grant will investigate how inflammation in the body and inflammation in the brain influence AD. The main hypothesis of this project is that inflammation exacerbates AD and is thus a major component of AD pathology and a potential therapeutic target.



Regulation of RNA Translation by MAPT in Alzheimer's Disease

\$100,000



Benjamin Wolozin, M.D., Ph.D.

Professor of Pharmacology and Neurology, Boston University School of Medicine

We have recently identified a new type of molecular pathology in AD that develops in concert with neurofibrillary tangles, one of the hallmark pathologies of Alzheimer's disease (AD). Neurofibrillary tangles form from clumping of tau protein, and occur as nerve cells deteriorate. In the Wolozin lab, we have discovered that a class of proteins, termed RNA binding proteins, clump alongside the tau protein and constitute a new type of pathology in the AD.

This new pathology is important for three reasons; 1) it appears to cause some of the tau pathology that occurs in AD; 2) the RNA binding proteins sequester mRNA transcripts as they clump, which interferes with the normal functions of the nerve cells in our brain; and 3) there are potentially new pathways that regulate RNA binding proteins, and can be targeted for pharmacotherapy in AD.

The research in the proposal focuses on understanding how the pathological clumping of these RNA binding proteins interferes with the process of protein synthesis as mRNA is translated into protein, and thus interferes with the functioning of nerve cells. The first part of the proposal will be to determine the extent and location of deficits in protein synthesis in the Alzheimer brain. The second part of the proposal will identify the exact mRNA transcripts that are sequestered by the RNA binding protein/tau complexes; this will possibly allow us to develop treatments or diagnostics targeted to the specific mRNA transcripts and proteins that are lost in disease.

Rejuvenation of Microglia in Brain Aging and Neurodegeneration

\$150,000



Tony Wyss-Coray, Ph.D.

Professor of Neurology and Neurological Sciences,
Stanford University School of Medicine,
Palo Alto Veterans Institute for Research

Aging impacts nearly every tissue and function in an organism, and the associated deterioration is the primary risk factor for major human diseases, including cancer, cardiac disease and such neurodegenerative diseases as Alzheimer's disease. The underlying cause of aging is likely a multifaceted yet interconnected tangle of processes, but there is growing evidence that in the brain, microglia—which are the only resident immune cell—have a major role. We discovered that these cells show profound changes with aging and that soluble factors in the blood of young mice can rejuvenate these cells. We propose here to study how these cells age and what the mechanism of rejuvenation is. Our studies will help characterize the role of microglia in brain aging and Alzheimer's disease models, and may uncover new ways to rejuvenate these cells and slow down brain aging and degeneration.

Stem Cell Models

Using stem cells to create highly accurate models of the Alzheimer's brain in the lab, in order to observe disease pathology and test possible therapies



Stem Cell Approach to Investigating the Phenomenon of Brain Insulin Resistance

\$100,000



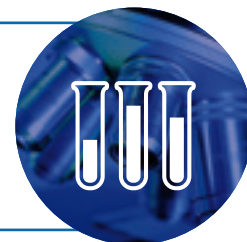
Sam Gandy, M.D., Ph.D.

Professor of Neurology and Psychiatry, Icahn School of Medicine at Mount Sinai Hospital
Director, Center for Cognitive Health and NFL Neurological Care at Mount Sinai Hospital

What is the evidence that “brain insulin resistance” is a consistent feature of Alzheimer’s disease? The National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) convened a summit in the summer of 2015 wherein the major question for new research focuses on establishing molecular and physiological criteria for “brain insulin resistance.” Several investigators have proposed that brain insulin resistance is a feature of human AD. Some have gone so far as to propose that AD be considered as “Type 3 diabetes.” The only direct study of insulin signaling in AD brain tissue was that reported by Talbot and colleagues. We propose to apply induced pluripotent stem cell technology to study insulin signaling in neurons, astrocytes and mixed cultures from subjects with Donohue’s extreme insulin resistance (DIR) syndrome, in whom mutations in the insulin receptor abrogate all insulin signaling.

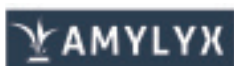
Therapeutic Strategies

Identifying, testing and perfecting small molecules that could function as therapies for Alzheimer's disease



Evaluation of AMX0035, a Neuroprotecting and M1-Deactivating Therapeutic, in an Immunological Model of AD (Part 1)

\$150,000



Amylyx Pharmaceuticals Inc.

In Alzheimer's disease (AD) and other neurodegenerative diseases (ND) such as ALS, neurological inflammation and cell death form a vicious cycle that is one of the main causes of decline. Mitochondrial and endoplasmic reticulum stresses mediate these pathways, accelerating inflammation and triggering apoptosis. Therefore, we developed a combination therapeutic of repurposed compounds to simultaneously reduce endoplasmic reticulum stress and mitochondrial dysfunction to halt the cycle of inflammation and cell death. To date, we have shown that our therapeutic, AMX0035, reduced inflammation and oxidative cellular death in cellular models and reduced soluble Abeta in a transgenic mouse model, likely by immunological clearance. While AMX0035 is a promising candidate, a series of optimization studies is needed before it will be ready for IND-enablement. We propose evaluating AMX0035 in the G93A SOD1 mouse model of ALS, a series of *in vitro* dose optimization studies, healthy rats for pharmacokinetics, and a stringent rat model of innate immune response. The individual components of AMX0035 are clinically well tolerated and easily dosed in our expected dose range, which will facilitate rapid translation to and evaluation in the clinic. The proposed studies therefore will enable us to bring this promising new drug candidate to patients suffering from AD and ALS.



Evaluation of AMX0035, a Neuroprotecting and M1-Deactivating Therapeutic, in an Immunological Model of AD (Part 2)

\$150,000



Amylyx Pharmaceuticals Inc.

With the help of Cure Alzheimer's Fund, we now have conducted a series of optimization studies, which have allowed for fine tuning of dose selection. Furthermore, Amylyx now is completing our IND-enabling studies to bring AMX0035 to clinic. Recently, a series of biomarkers of inflammation, oxidative stress and cellular death have been studied clinically as potential endpoints to make trials easier and cheaper in both AD and ALS. We propose evaluating AMX0035 in a stringent mouse model of innate inflammation that expresses these same biomarkers to make a highly translatable evaluation in this model. Using a phenotypic model that expresses clinic-validated biomarkers hopefully will solve the longstanding issue of a lack of translatability in neurology from mouse models to patients. This study should thus both provide confidence in our drugs and choice of biomarkers, as well as provide a pathway for future drug development.

Lead Optimization and Lead Evolution of Potent SGSMS for the Treatment of Alzheimer's Disease

\$476,988



Steven Wagner, Ph.D.

Associate Professor, University of California at San Diego School of Medicine

Rudolph Tanzi, Ph.D.

Joseph P. and Rose F. Kennedy Professor of Neurology, Harvard Medical School

Director of the Genetics and Aging Research Unit, Massachusetts General Hospital

This application outlines a highly focused extension of an NIH-funded Blueprint Neurotherapeutics (BPN) U01 program to create more potent, soluble, brain penetrant, nontoxic small molecules known as soluble gamma-secretase modulators (SGSMS) that act to enhance the activity/processivity of γ -secretase, thereby reducing the levels of Abeta42 and to a lesser extent Abeta40 while increasing the levels of shorter Abeta peptides (e.g., Abeta38 and Abeta37). Our application builds on the previous synthesis of a large number of SGSMS that have been optimized for potency, physicochemical properties, absorption, distribution, metabolism, excretion and toxicity (ADMET), in addition to *in vivo* pharmacokinetic (PK) and pharmacodynamic (PD) properties. The prior studies were supported through a BPN U01 award that included three years of lead optimization and lead evolution involving structure-activity relationship (SAR), quantitative structure-activity relationship (QSAR) and structure-property relationship (SPR) studies.

Whole Genome Sequencing and Epigenetics

Assessing the entire human genome, including intergenic DNA, for variants that influence risk for Alzheimer's



Analytical and Statistical Tools for Sequence Analysis for Alzheimer's Disease

\$150,000



Christoph Lange, Ph.D.

Professor of Biostatistics, Harvard T.H. Chan School of Public Health

The availability of next-generation sequencing data in large-scale association studies for Alzheimer's disease provides a unique research opportunity. The data contains the information that is required to identify causal disease susceptibility loci (DSL) for Alzheimer's disease and many other mental health phenotypes and psychiatric diseases. In order to translate the wealth of information into DSL discovery for Alzheimer's disease, powerful statistical methodology is required. So far, a large number of rare variant association tests have been proposed. However, they do not incorporate all the important information about the variants. So far, none of the existing approaches takes the physical location of the variant into account. Under the assumption that deleterious DSLs and protective DSLs cluster in different genomic regions, we will develop a general association analysis framework for Alzheimer's disease that is built on spatial clustering approaches. The framework will be able to handle complex phenotypes, e.g., binary, quantitative, etc., and be applicable to different study designs, i.e., family-based studies and designs of unrelated subjects. If the DSLs indeed cluster, the increase of statistical power of the approach will be of practical relevance, enabling the discovery of DSLs. In the absence of DSL clustering, our approach will achieve similar power levels as existing methodology. Furthermore, in order to test larger genomic regions for association, we will develop network-based association methodology. The network-based approach will have sufficient power for larger genomic regions than existing approaches and, at the same time, provide an intuitive understanding of the complex relationships between the variants that drive the association, fostering new biological insights. The approach can incorporate complex phenotypes and different design types. All the proposed methodology will be implemented in user-friendly software packages with existing user communities, i.e., PBA, NPBAT and R. We will test, validate and compare the proposed approaches with the existing methodology, using large scale simulation studies and by applications to the whole genome sequencing family study for Alzheimer's disease from the Tanzi lab.

OUR RESEARCH LEADERSHIP, BOARD AND STAFF

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Research Consortium

The volunteer members of Cure Alzheimer's Fund's Research Consortium develop and update our research areas of focus to identify the most promising opportunities for slowing, stopping and/or reversing Alzheimer's disease. Members pursue their own research projects consistent with these priorities and others whose work will hasten development of effective therapies for and prevention of Alzheimer's disease.

Rudolph E. Tanzi, Ph.D., Chairman, Research Consortium;
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