Today, we're going to be talking about Alzheimer's disease. I have two special guests,
the first one is going to be Doug Galasko. He is a neurologist, he is heavily involved with the UCSD,
Shiley-Marcos Alzheimer's Disease Research Center, ADRC.
Doug has been the Director, and now he's serving as Associated Director of the Center with an outstanding leadership of this very important program here in our community.
In clinical practice, he provides care for patients with memory and cognitive disorders including Alzheimer's disease, dementia with Lewy bodies and frontotemporal dementia.
He works at the UCSD memory disorders clinic. He is also a staff physician at
the VA Medical Center here in La Jolla. Doug Galasko has contributed to several clinical trials.
He also contributed to biomarker research, this is including analysis of CSF and
blood tests to screen for markers for Alzheimer's disease for example, he is also involved in other therapeutic research
for Alzheimer's and other related disease. He has authored hundreds of scientific articles,
book chapters, and he is the co-editor of the journal of Alzheimer's Research and Therapy.
He has received many research funding from NIH,
including from the National Institute on Aging, State of California, the Alzheimer Association, Michael J Fox Foundation,
Alzheimer's Disease Drug Discovery Foundation, and many others. He has been an investigator in the numerous industry funded clinical trials. He's our first guest. Our second guest is our local hero, Larry Goldstein.
Larry is a distinguished professor in both Department of Cellular and Molecular Medicine and Department of Neurosciences
here at UCSD School of Medicine. He is currently the Director of the Sanford Consortium for Regenerative Medicine.
Early this year, he was appointed to be on the board of the California Institute
for Regenerative Medicine, CIRM. We're all very grateful that he accepted to be on board.
Larry has used to wear many hats, he is a cell biologist,
a geneticists, and a neuroscientist. He worked with many colleagues to launch the UCSD Stem Cell Program,

the Sanford Consortium for Regenerative Medicine and the Sanford Stem Cell Clinical Center.
I have no idea how he done that at the same time. He has received the Public Service Award
from the American Society of Cell Biology, and he has had Public Policy Fellowship name
it for him by the International Society of Stem Cell Research. He's a member of the
American Academy of Arts and Science, and last year he was named a member of the prestigious
National Academy of Science.
Larry has also done a tremendous amount of work on the basic science,
mostly focusing on the molecular mechanisms of intracellular movement and the role of
transport defects in neurodegenerative diseases. He then turned more into
human pluripotent stem cells to create models and technologies to discover new disease therapies
and testing
disease mechanisms using pluripotent stem cells. Over the years you can see that
he has moved from basic research in genetics and cell biology to research on Alzheimer's disease,
to the point where he has been doing now translational studies, and we hope that some of them
will lead to clinical trials, so we're going to hear more from him soon. He has many interesting
awards
including Scientist of the Year, Lifetime Achievement, and I mentioned
the ISSCR Fellowship Program that was named on his behalf.
We're going to start by hearing from Doug first. Welcome Doug Galasko.
Thank you very much Alysson. It's a great pleasure to be here today.
I'm going to give you some background and introduction about a clinical perspective on
Alzheimer's disease.
I will cover some of the basics of Alzheimer's as well as some of the clinical and translational
research directions
we're involved in. I have some disclosures which are listed on this slide. I consult for a number of
companies and this is my research finding. Before we talk about Alzheimer's disease,
let's start by talking about dementia. Dementia is a clinical syndrome
and it covers a host of different conditions with many causes.
The formal definition is that it is a set of conditions resulting in
acquired intellectual decline that is severe enough to interfere with

somebody's social or occupational performance. When we test somebody with dementia, we are able to demonstrate that they have impaired cognitive abilities in areas such as memory, judgmental reasoning, language, visuospatial abilities, or calculation.

There should be an absence of delirium, in other words, the patient or person should be aware and conscious of what is going on. Clearly a lot of different things could meet this description. If we think about different causes of dementia in the elderly, this is one summary of a number of different studies. Alzheimer's disease is the most common cause, but it's not the only cause. Vascular dementia, vascular changes in the brain, strokes, mini strokes, and things like that, may cause pure dementia or may contribute together with Alzheimer's disease to the clinical picture. Dementia with Lewy bodies is a relative of Parkinson's disease and sometimes contributes to dementia all on its own, or it can accompany Alzheimer's disease.

Then these other small pieces of the chart relate to rare disorders, but nonetheless important ones, frontotemporal dementia, other kinds of unusual sorts of dementia, some of which are transmissible or due to genetic or other disorders.

Let's focus a little more closely on Alzheimer's disease. The pathology that was first described by Doctor Alzheimer's consisted of two problems or lesions that we could see under the microscope. One of these are plaques which occur outside of nerve cells, and plaques we now know are due to the accumulation and aggregation of a protein called amyloid Beta protein or Aβ. Tangles are the other marker of Alzheimer's disease, and they look like what a tangle would conceivably look like.

They consist of clumps or aggregates of a protein called tau, and they occur within nerve cells not on the outside.

That's the basics of Alzheimer's disease, but in fact, if we study the brains of people who die with dementia, we often see associated pathology. Some vascular pathology may be

present in as many as 60 percent of people, Lewy bodies may be present in up to 20 percent, and another protein called TDP43 can form aggregates or clumps in about 5-20 percent of people with what we thought of as being Alzheimer's disease.
The overall picture can be a little more complicated, but before we can make progress on all of these different conditions, we probably should focus on the basic abnormalities or as much as we understand about pure Alzheimer's disease on its own. From a clinical perspective,
if we think about cognition and function as being things that can be readily measured, there are some changes that occur during normal brain aging. They're measurable, they're relatively mild.
Somebody who's going to develop dementia doesn't start off one day waking up with dementia, they start out with normal cognition and function, and over time, they gradually progress to develop mild condition called mild cognitive impairment, where there may be subtle impairment of memory or other aspects of cognition. But the person can generally function in day-to-day life.
With progression they may develop dementia. Under the surface, plaques, tangles and vascular disease are developing and so as you can see, they've been developing all along for years in somebody who is later going to materialize to have MCI or dementia.
We're able to now detect these and we can now think of somebody who actually has normal cognitive and functional abilities as having pre-clinical dementia. There have been moves to try to define Alzheimer's disease as a biological entity by actually measuring these abnormal proteins.
This was one conception of many years of work in which the idea is that if we're able to define amyloid pathology, and top pathology, and some of their consequences which would be neurodegeneration or changes that are happening to nerve cells and their connections. If all three of these are present, then this is clearly Alzheimer's disease.
If both of these amyloid and tau present without neurodegeneration, there may be some Alzheimer pathological changes.

Without necessarily the full-blown picture, there may be other pathologies,
and then cognitive impairment can occur or can be absent depending on the staging of the pathology.
Can we actually bring this to life and not be reduced to simply looking at brains under the microscope?
The answer is, over the last decade there have been some exciting research breakthroughs in biomarkers to be able to map this amyloid,
tau, and neurodegeneration framework. If we think of plaques and tangles,
we actually have ways to measure these in the brain in living people. We can measure plaques through doing
a PET scan which can detect the amyloid buildup or we can measure the spinal fluid that surrounds the brain.
We can do a lumbar puncture and measure levels of CSF amyloid-Beta protein.
We can now measure the accumulation of tau by doing a tau PET scan.
Then there are many ways to look at the impact of these on the brain. This bottom slide shows a brain of somebody who died with normal cognition versus Alzheimer's. You can clearly see that there are differences.
There is atrophy or shrinkage. We can detect these through MRI through
a PET scan called an FDG or fluorodeoxyglucose PET scan,
which looks at regional glucose use. Or we can try to measure biochemical effects of damage to the brain,
again, in cerebral spinal fluid. Here are some examples of what these might look like.
These are a series of amyloid PET scans taken from a number of different people.
This would be a negative scan in someone with normal cognition, a positive scan in someone with normal cognition illustrating the point that the pathology begins to build up long before the clinical picture.
Here's someone with MCI with a negative amyloid PET scan, so they're MCI probably is
not caused by Alzheimer's disease. Here is MCI with a positive PET scan, MCI with a markedly positive PET scan,
and Alzheimer's disease dementia. We know that amyloid PET scans detect clocks and they

become positive more than 10 years before the onset of symptoms. About 30 percent of people over the age of
70 have a positive amyloid PET scan. Clearly this would be something that we could use
in diagnosis and therapy. Tau PET imaging has been
somewhat more recent development. Taus tends to spread through the brain
along various anatomical pathways. This illustrates a series of different top head scans
from people across the spectrum of Alzheimer's disease, starting off with someone who is clinically normal.
Some of the tau PET agents bind a little bit non-specifically to the brain. This is just background binding.
This would be one of the earliest stages with some of the memory vulnerable circuits are starting to show some binding,
cognition is still normal. Again, someone with normal cognition with a bit more tau build-up.
Here's someone with MCI, with more extensive tau pathology affecting the temporal lobe, higher buildup,
but still MCI and more extensive tau that spread throughout the brain that can be detected.
Again, tau PET is a helpful tool potentially in diagnosis and
to evaluate outcomes of therapy. There are different ways of looking at MRIs and we have ways to digitize the brain and measure how big things may be. This is a comparison of someone who is cognitively normal versus Alzheimer's, looking at areas of the brain and
color coding in the cortex and some of the structures deep within the brain. Clearly, you can see that there's a difference.
There is shrinkage and atrophy, there is enlargement of fluid spaces within the brain in someone with Alzheimer's disease.
Cerebral spinal fluid allows us to look at brain biochemistry and to actually dig quite deeply.
If we look at the basic biomarkers, which would be amyloid and tau, starting off with amyloid,
we can measure the amyloid-Beta protein, the one that accumulates in the brain in plaques,
but here we're trying to detect something that isn't getting into CSF.
A particular form of the amyloid Beta protein called A Beta 42 accumulates in the brain in

Alzheimer's disease and therefore less of it escapes into the CSF. If we look at people who have had
CSF analysis and amyloid PET scans, if you have a positive amyloid PET scan,
you're accumulating amyloid in the brain, CSF A Beta 42 is low. If you have a negative amyloid PET scan in general,
CSF A Beta 42 is high. In fact, the CSF A Beta 42 may be more sensitive,
it may start to decrease before the PET scan becomes positive. Tau is this other protein
that builds up within nerve cells, and we don't see tangles in the CSF because they can't get out of nerve cells.
However, we see fragments of the tau protein. This is a study that actually characterized all different fragments.
We can set up particular assays to measure specific fragments, and in general,
levels of tau or different forms of tau called phospho tau or increased
in CSF and Alzheimer's disease. This is a helpful suite of diagnostic tools.
But it's inconvenient to have a PET scan or to undergo a lumbar puncture.
Is there any prospective about trying to develop blood tests. The big challenge here is,
can we detect proteins that originated in the brain, where detected in the CSF,
for example, but actually had pretty low levels. Now they're getting into the blood and they're being diluted and the liver and
the kidney are trying to get rid of them. We know that levels of A-Beta and tau in
plasma are extremely low and for the longest time, we couldn't show anything meaningful.
In the last few years, new assays using sensitive methods have been developed and there's
been a remarkable burgeoning set of publications showing us how we can use blood to
measure amyloid Beta protein tau, and phospho tau and some other proteins of interest.
I'll show you just a little data in this area. One of the forms of tau is called phospho tau 217.
This is one of several published studies. In this study, people had undergone blood draw while they
were alive and their brains were examined autopsy.
These were people in orange with non Alzheimer diagnoses, so no real tangles showing up at post mortem.

These were people with Alzheimer's disease with different degrees of tangle accumulation and buildup.
In general, the more Alzheimer top pathology in the brain, the higher plasma phospho tau wants.
On the right there is an illustration showing people who are cognitively normal or didn't have Alzheimer's,
and these were levels of plasma tau barely detectable, but it was detectable non the less.
These are people along the Alzheimer's spectrum, MCI, and people with Alzheimer's dementia showing that there
are increases in the level of this blood biomarker, and people with non Alzheimer disorders.
Some of these rather uncommon brain disorders that cause dementia, but they do not bump up the tau.
Plasma P-tau 217 is both sensitive and specific for Alzheimer's.
We have the promise of a suite of plasma biomarkers that are going to be able to help us enormously
with diagnosis and therapy in future. Another study on Plasma P-tau 217, for example,
showed that measuring this biomarker, this is in living people,
over a period of years, showed that if you happened to have a positive amyloid biomarker,
so you're accumulating amyloid in your brain, P-tau 217 actually increased over time.
Even if you were cognitively normal, appeared to a greater extent in people who had a diagnose of MCI,
and in those who converted from MCI to Alzheimer's disease. Again, we could imagine using this to try
to track progression over time. We have a very nice suite of emerging biomarkers.
How do we apply these to trying to understand and make a difference to Alzheimer's disease?
This is a 30,000 feet picture of why do people develop Alzheimer's.
There are a number of factors, and putting words to them doesn't mean we understand them fully.
But in general, Alzheimer's has something to
do with genetics, the aging process, and a number of factors that we call environment,
but that involve things like lifestyle and health. Here's a snapshot of some of
the genes that have been involved in Alzheimer's disease. It's a complicated looking diagram,

but I'll break it down into a few takeaways. There are some rare genes,
that's if they are abnormal, have an extremely high risk of causing Alzheimer's disease.
They've taught us a lot about the disease, and we've actually developed cellular and animal models using these genes.
They're called PSEN1 and PSEN2 and APP. There's some intermediate risk genes,
the APOE4 gene is probably the most common. If you have two copies, you have an intermediate lifetime risk
of Alzheimer's disease. Then there are a bunch of low-risk genes. Some of them are pretty unusual or rare,
but they may teach us something about mechanisms of disease. Some of them are more common APOE4,
and there is a host of other genes that have been discovered more recently that collectively impact on Alzheimer's risk,
although to a very small degree. But again, putting all of these genes together may teach us about some of the mechanisms
that underlie Alzheimer's disease. What about environment? It's a really complicated thing to try to study.
If we think about the environment as being a host of conditions that either make brain health better or worse,
a number of people have tried to summarize decades of work in the area. The Lancet Journal has put
together a commission of experts who've delivered several reports to try to estimate whether there are factors in early life,
midlife, or late life that could have an impact on the lifetime risk of dementia,
and maybe we could do something about some of these. Without going into all of the details, some of these certainly are things
that are modifiable or actionable, and in particular, vascular risk. Factors like obesity, high blood pressure,
increased cholesterol, diabetes, and smoking are things that can be addressed and modified.

Physical inactivity can be countered, and there are many studies looking at activity or exercise to try to improve brain health.
Hearing loss and social isolation may have a small impact individually,
but they are things that are quite common, and so they are things that in principle it would be worth trying to do something about.
Again, these are factors that have come out of a number of studies. Whether attacking them makes
a huge difference or not remains to be seen, but they certainly are worth investigating.
If we're going to try and make a difference to Alzheimer's, we need treatment. At the moment, this is a rather embarrassing list
of more than 35 years of work that have led to a small number of medications
being approved by the FDA for use in Alzheimer's disease. These include the cholinesterase inhibitors,
donepezil Aricept, galantamine, and rivastigmine, and then more moderately to severe Alzheimer patients,
an NMDA antagonist called memantine has been approved. For all of these medications,
there are benefits that have been shown in clinical trials, but the benefits are small. These medications may help to stabilize cognition,
sometimes only temporarily, and none of them slows disease progression.
The question is, how do we use our knowledge that has been accrued over many years and decades of work about mechanisms and biomarkers to develop new and effective disease-modifying treatment?
If I had to try to put together a summary of
pathways related to amyloid and tau, and these are not the only pathways of interest, but these are the ones that have
received the most attention. Perhaps these can give us some treatment targets.
Again, through lots of research, we know that the A beta or amyloid protein is produced
by a bunch of enzymes that cut up a parent protein called APP. Normally we're very good at getting rid of
the A beta protein from the brain. It can be broken down by enzymes, taken up by different cells.

It gets out through the CSF, through blood vessels, and eventually appears into the blood. But in Alzheimer's, something goes wrong.
Either on the production side or the parent side, and it aggregates, forms fibrils, and eventually plaques.
Somehow these promote a toxicity, and there are a number of different terms I've put out here; excitability,
neuro inflammation with astrocytes and microglia cells responding and eventually neurodegeneration,
and tangles are one of the possible outcomes. In terms of where we're at with a lot of research and a number of different organized clinical trials, some of the large-scale efforts have involves
trying to decrease A beta production. Some of the drugs that have been used are called base inhibitors or gamma secretase inhibitors or modulators. Then clearance has been very difficult to deal with.
The main method that people have tried is to deliver antibodies that bind to the amyloid-beta protein.
These either try to bind through large amounts of different forms of A beta or maybe
to bind exclusively to forms that are aggregating and maybe fall on toxicity,
maybe that will make a difference. Then there are a number of efforts to try and deliver therapy aimed at tau.
Again, many of these have involved antibodies. I'll show you a summary or snapshot of a number of
clinical trials that have either been recently completed or are in progress. Regarding amyloid antibodies, there have
been therapeutic efforts for probably 20 years by now. These are some of the more recent trials.
Solanezumab Madison antibody that binds to all forms of the A beta protein,
and its has had a mixed history. It does decrease CSF levels of A-Beta,
and it's currently being studied. After it didn't work in some early trials,
it's being studied at a very high dose in some prevention trials. There are a number of different antibodies listed here,

all of which bind to abnormal forms of the A beta protein, bind to aggregates,
and are aimed at removing them from the brain. All of these have undergone either phase 1,
2, or in some cases, phase 3 trials, typically in people with mild Alzheimer's disease.
These have been shown to be able to reduce amyloid buildup in the brain by doing PET scans.
At least they do hit a target and they do have an impact. Whether they make a difference on
clinical progression is still under a little debate.
Aducanumab has shown some hint of signals, and the data has been filed with the FDA.
Some of these other trials actually also have shown a clinical signal from trials,
and how meaningful this is will remain to be determined. Decreasing production has not had a
good track record.
Base inhibitors have been developed. This is one of the key enzymes that cuts
the A-beta protein from its parent protein, and five different drugs went into trials and all of them
work
beautifully at decreasing CSF levels of A-beta protein. However, there must have been something
else that these drugs were doing. In every one of these trials, there was a faster cognitive decline
in people
on drug treatment than in placebo, so this avenue has been discontinued.
Therapeutic so much newer, and antibodies are being tested.
In a number of different studies, there are at least five different antibodies in trials that have been
shown to
be able to decrease CSF tau, but of course, the CSF tau is a set of fragments and not necessarily
the toxic form of tau.
One or two of these have read out in phase two studies as being negative, but a lot of therapeutics
are in play.
Getting an infusion of an antibody every month is very expensive, and so there actually are
several attempts
to do active immunization, the equivalent of what we would do for COVID,
only giving people an antigen to immunize them against tau.
There are two reported phase 1-2 trials, and they have resulted in anti-tau antibodies in a
reasonable tau tier.

We will see what happens as patients are followed further over time.
If we had to try and make predictions about where Alzheimer's therapeutics is going to go,
some of the predictions are that we need to try and figure out who to treat and when to start treatment.
It is likely that anti amyloid treatment may be most effective the earlier that we start,
maybe in a prevention type of study, before people have symptoms. If we're trying to target tau,
perhaps we could start a little later, and if we're trying to do other things, we may be able to start a little later on too.
Some of the messages, and these have been very expensive messages learned over decades,
are that we need to think about starting treatment as early as possible. We may need to think about combination therapy
as we continue to develop novel approaches, but we have an exciting suite of biomarkers that we can
now use to help us to conduct and interpret clinical trials. I'll stop there. That's a fairly rapid overview
of an enormous amount of research. We will come back and take
questions and have a discussion later. Thank you very much. Thank you there. That that was a fantastic summary.
I'm amazed how much information you were able to pack. The next talk is Larry Goldstein.
Dr. Galasko just gave you a really terrific summary
of some of the basics of what we think about Alzheimer's disease and what we've observed.
Of course, you can see from Doug's talk that
one of the biggest problems we have is not being able to describe the disease,
it's been described very well, but we desperately need drugs that are effective.
What I want to tell you about in the next 20 or 25 minutes is how we've been using
stem cell technology to try to search for different types of Alzheimer's disease drug candidates,
different from the types that Doug has already beautifully told you about.
Just to give you the quick recap of what you just heard,
Alzheimer's is common, progressive, incurable. There's only a few treatments that are known
and they have minimal and temporary effects. I do want to stress something that

Doug alluded to in passing, and that is a very important part of
Alzheimer's disease in addition to the plaques and tangles, is that the connections between
brain cells are lost at early stages of disease, and these are the so-called synapses,
these are the connections between brain cells and these are lost early in disease.
Then of course, eventually, as you just heard, there's massive brain cell death, in particular death of neurons.
Now the major hypothesis in the field is the so-called amyloid cascade hypothesis.
Shown diagrammatically here, based on these images of brain pathology,
the notion is that these amyloid plaques, which as you heard are composed of
the A-beta fragment of protein, these amyloid plaques are thought to lead
to the neurofibrillary tangles, and these tangles as you just heard,
and we'll have more to say about this, are composed of a modified version of the tau protein.
That modification, in the field, we refer to as a phosphorylation event,
and for simplicity, I'm just going to refer to this modified form of tau as P-tau.
The question ultimately is, what is controlling
the amyloid plaque and A-beta formation, that is what's happening early to lead to these changes?
Similarly, how do these plaques and A-beta peptides lead to these tangles containing P-tau?
Of course, the important question that all of us wonder about is
what's ultimately the right drug target? I'll tell you about some efforts to get a handle on that.
Now what I've done here is, in a sense, redrawn the proposal for
how Alzheimer's disease develops. I'll refer to it as the single pathway proposal,
it is the simplest possible way of looking at how the defects in the disease are caused.
What this imagines is that either some altered biochemistry,
and Dr. Galasko gave you a few examples of that, or rare genetic mutations.
Dr. Galasko mentioned one of those presenilin mutations or other genetic and environmental risk factors
such as traumatic brain injury, or APOE, or the others. These are all thought and
proposed in this simple single pathway proposal to lead to formation of
amyloid plaques made of this A-beta fragment, and that this collection of plaques in turn
leads to modified tau or P-tau and then neurofibrillary tangles,

and then ultimately this all leads to the loss of connections and ultimately neuronal death characteristic
of Alzheimer's disease. But there's a zoo of other possibilities in
the literature and in experiments that have been done on this disease that is in fact rather complicated.
We can think of these as multiple pathway proposals. To be honest, this is often
the way biology and neuroscience in fact, usually work, is that there are lots of
alternative ways for getting from one event to the other. So what this multiple pathway idea
here imagines is that the factors and changes that can lead to elevation in A-beta and
amyloid plaques can also lead to other types of neuronal misbehavior.
I'll show you a couple of examples of those in a few moments, and that either of these can lead to the formation
of phosphorylated P-tau and tangles, and all of these things can lead to
the loss of connections characteristic of disease. Of course, you can see here in the middle
there's even a view that the plaques and the A-beta proteins
can lead to neuronal misbehaviors, which in turn leads to tau tangles and you can see how this
can become fairly complicated fairly quickly. But I unfortunately have to assure you
that biology is frequently this complicated, and part of our tasks as
scientists and clinicians is to understand and dissect how these kinds of pathways
work and what ultimately leads to disease pathology.
Now we've taken a somewhat different approach to this problem,
and let me just introduce it to you quickly and then give you a definition.
The notion is this, plaques and tangles are in fact
relatively late events in disease. People frequently don't develop them until they're 50s,
60s or 70s as you just heard from Dr. Galasko, or they certainly don't become abundant until people are in
their later elderly stages of life. But it's hard to believe that there
aren't very early changes that lead to the formation of the proteins and
modified proteins that ultimately form plaques and tangles. There's quite a bit of evidence
in the literature that in fact, there are a number of early changes happening early in life,

possibly even as early as fetal development, that lead to these pathological changes that don't read
out until very late in life in people's 50, 60s, or 70s.
What you really want to find in a way, you can think of as follows.
If there's been a plane crash and you want to know what caused the plane to crash,
you can, I suppose, and this is initially done, examine the pattern of wreckage on the ground.
But of course, the pattern of wreckage on the ground doesn't tell you what went
wrong in the plane while it was still flying; were the pilots drunk? Did they stop paying attention
to the navigation system brake, who the heck knows? Lots of things can lead to a plane crash.
So in the case of a plane crash, what you're looking for is the black box, which records the early events
that were abnormal in the plane that lead to a crashing. Well, what we're all looking for in a sense,
is the black box of Alzheimer's disease. We want to know what happens early that leads to cells in the brain,
and neurons in particular, starting to behave abnormally leading to loss of connections and then cell death.
This is where we've turned to stem cell technology to try
to generate human brain cells in the lab. By the way, we want to have human brain cells because
while a great deal of work has been done in mouse, so-called models, or mouse
versions of Alzheimer's disease, there are two points. One, we are not just big mice.
You may have noticed that last time you looked in the mirror. Humans are not just big mice.
Second, mice actually don't develop true Alzheimer's disease,
and so treatments that work in the mouse don't necessarily work in the human,
and that's true for lots of diseases and Alzheimer's disease has been no exception. So what we want to have are
human brain cells in the lab so that we can study early stages
where genetic mutations and other risk factors that one can import into the lab can
be studied in the very earliest stages before plaques and tangles actually form,
which of course are the late stages of disease. This is where stem cells come in,
and I'll just give you a very quick super simplified definition of stem cells.

Cells grow by dividing to make more cells. These are cells that can divide to make more stem cells,
so they're self replenishing, but they can also, when you give them the correct biochemical signals, give rise to all sorts of different specialized cell types, and if you give them the right biochemical signals,
they can form different cell types in the brain, including neurons.
So there's one other wrinkle in this methodology and that is because of
the pioneering work of Shinya Yamanaka. It has now become routine in
any lab in the La Jolla area or elsewhere around the world,
to take a sample of skin cells from either a normal individual or
an individual who has a genetic form of Alzheimer's disease. You can convert that skin cell into a stem cell.
Those stem cells have the genetic architecture, that is the DNA from
the person you got the skin cells from. So can either be a control non-demented person or it can be a person with a hereditary form of Alzheimer's disease. So when you make those stem cells into neurons,
shown diagrammatically here, they can either be controls, that is, genomes, people that didn't develop disease,
or people that developed these forms of Alzheimer's disease. Then one can take
these neurons or other brain cells for example, but neurons in this case, and you can measure the proteins that
are typical of Alzheimer's disease, either A-beta or P-tau. As I'll show you momentarily, in fact,
in the neurons that carry rare genetic changes that cause hereditary Alzheimer's disease,
you indeed see elevated levels of A-beta and P-tau that are produced by those cells.
Now one other point I want to make is this diagram here is
actually a realistic diagram of a neuron. Neurons are not just a ball
or a soap bubble with cellular constituents in it. They're very asymmetric cells.
They have what's called a cell body shown here, which is the factory of the cell. It's where the DNA is and it's

where most of the materials are produced. Then they have this long structure that leads to connections to other cells.
These are both simultaneously a wire and a pipe. That's because they can transmit electrical signals
from the cell body to the connections to the synapse, but there're also a pipe through
which lots of materials are moved to get to the synapse in order to support its normal function.
So these distances can be quite large. In humans, they can be 3-4 feet or more in
the case of the neurons that control the muscles in your toes, for example.
Many of the neurons in the brain have very, very long versions of these pipes and wires,
the so-called axons of these cells. So what a student of mine,
Mason Israel did a number of years ago, was, again, plaques formed of A-beta,
tangles ultimately from P-tau was to make stem cells and then neurons that
either were control or had a rare genetic form of Alzheimer's disease,
and you can see quite clearly the controls have a low-level of A-beta, and the Alzheimer's mutations
actually have quite a high level of A-beta. Similarly, if you look at P-tau, same thing,
controls are relatively low, Alzheimer's mutations relatively high.
Then of course, the question you'd like to ask in this simplified system at least is, is A -beta leading directly to P-tau in
these early versions of Alzheimer's disease in a dish?
In fact, I won't drag you through the data. They're a little bit complex. But the point is,
we've really been able to rule this version of this kind of model out,
and Mason was able to show convincingly in at least this human neuronal version of
disease that A-beta was not what's leading to P-tau. In fact, it turns out to be a precursor to A-beta.
Now, there are some other interesting types of misbehavior that are
seen in these Alzheimer's mutant in the lab.
One essay that we developed for these cells, is to take so-called tagged lipoproteins.
You all have probably heard of lipoproteins, LDL or HDL, and the levels in the blood make
a big difference to your cardiac and other health. It turns out that the brain does not get
its lipoproteins from outside the brain, it synthesizes its own lipoproteins.

So we started to study how neurons internalize lipoproteins that are
made from other cell types in the brain. One way we could study it, is to tag the lipoproteins with little molecules that
give off light when you look at them in a special kind of microscope called a fluorescence microscope.
So I've colored them in orange here to indicate that they're giving off orange light.
They're usually really green or red, but who's counting? Neurons are capable of internalizing these lipoproteins when you apply them to the area of the cell body.
They get inside the cell and then some of them are transported,
physically moved out through this part of the cell called an axon to the synapse.
The synapse actually requires quite a bit of lipoprotein in order to function normally.
I'll just mention in passing that lipoproteins often contain cholesterol and we'll come back to that in a moment.
So here's what the data really look like. This is an image in the microscope of an axon.
You can see these little white spots in this case. Those are the little fluorescent
lipoprotein packages that have been tagged. If you look at these genetic mutation bearing neurons,
you can just see by looking that there's quite a bit less of these little white dots in the axons.
Then if you count them, you can see controls are high, mutations are low. In fact, what we can show is that there's a defect in
the cell body in the ability to internalize these lipoproteins. Then there's a second defect in
the ability of these lipoproteins to leave the cell body and to enter the axon for traffic down to the synapse.
There's quite a pronounced early defect in the internalization of materials in these neurons.
This builds off of the groundbreaking work of Ralph Nixon back at NYU,
who working with Ann Cataldo almost 20 years ago, first found this intact brains post-mortem,
and he and we have pushed ahead based on those early data.
Now the other question you might want to know the answer to is,
that's all fine and good to catalog all the things that might be wrong with these cells,
but what you'd really like is to perhaps use these cells to find drugs.

So we started that work a few years ago. We took skin cells from a patient that had
a different kind of genetic mutation in the APP gene, that is the parent molecule
to A-beta, created stem cells, made neurons, and then
plated these neurons in so-called multi well plates. A little plate that's
about two inches by 3-4 inches in size,
can hold 384 or even more, but we used 384 well plates,
and each well has neurons in it. Then each well is treated with a different drug from
a collection of every FDA approved drug that we could lay our hands-on. You wait five days and
then you measure P-tau.
What you can see here is this big morass of dots.
So just think of it as though each dot represents one drug treatment.
Dots that are clustered here in the center, that's where drugs that have no effect are.
Some drugs, lower P-tau, some drugs raised P-tau. I'm not going to tell you about
all the different drug candidates we found, but there was one very intriguing candidate drug
that we discovered called Efavirenz, originally developed for HIV,
where it interferes with the viruses polymerase that
makes the viral nucleic acid and genetic material that is. Efavirenz lowers levels of
P-tau in these neurons in very striking ways. So the question then is,
how does it do that? One of the things that came up very early
is there was previous biochemical work which suggested that efavirenz acted
by stimulating the breakdown of cholesterol into 24-hydroxycholesterol
that's then eliminated from the brain. You can see that the production of cholesterol is
this incredibly complicated pathway of synthesis steps,
branches, and then this rather simple step of elimination.
Lots of drugs that are earlier in these pathways do in fact give some changes in p-Tau
interestingly,
pointing to some role of brain cholesterol in controlling p-Tau levels.
The question of course ultimately was, is cholesterol itself the actual
target that leads to changes in p-Tau. Is it cholesterol levels or something
else that efavirenz is doing. Through a whole long series of

biochemical experiments with Van Der Kant and Vanessa Langness working in my lab figured out that efavirenz in fact is
acting to reduce p-Tau by acting at this step that was previously reported.
It does catalyze the removal of cholesterol by stimulating the production of
24-hydroxycholesterol by cholesterol breakdown. But in fact, it turns out through
a series of biochemical experiments with Rik and Vanessa were able to show is that efavirenz does reduce or does stimulate cholesterol breakdown, but the storage form of cholesterol called cholesterol
esters circled here replenishes the lost cholesterol. Ultimately, it is the level of this storage form of cholesterol that controls the levels of phospho or p-Tau.
Now, one thing you might want to know is, well, this is having an effect on
cells or neurons growing in a dish. Of course, neurons in the brain
are quite a bit more complicated than neurons in a dish. Of course, any drug treatment
has to cross the blood-brain barrier. Not a simple task for many different sorts of drugs.
We wanted to know would efavirenz work on a brain?
To do this, we took advantage of mice that make tangles in their brain,
in different regions, in the cortex, and in other parts of the brain such as the hippocampus. We did these experiments with Robert Rissman.
You can treat these mice that make lots of Tau tangles either with control vehicle or with efavirenz.
What you can see for example here is that efavirenz gives quite a pronounced drop in
these mouse brains of tau tangles. The next step for us,
and this is a project that we're working on with Rik Van Der Kant, and with Dr. Galasko is to raise the funding
needed to put efavirenz into human clinical trials, figure out how it's working in an intact human,
and then begin testing it on Alzheimer's patients. That hopefully will happen in the next few years.
I at least I'm very optimistic about this, but there have been so many disappointments in this field.
I can't let myself be too optimistic. Where does this leave us?
To wrap up, what I've told you here in this last part, is we've got good evidence that
the storage form of cholesterol on the brain, cholesterol ester, actually in other works published by the [inaudible] ,

is able to control the level of amyloid. More cholesterol ester leads to more amyloid production. It also leads to an increase in the level of p-Tau we think through some other neuronal misbehavior that we haven't identified as yet. Of course, in this multi-path way model, everything seems to influence everything else, which by the way is often how biology works. These are ultimately systems that are sometimes referred to as homeostatic. If we go back to the original multiple pathway proposal, we can add something to the altered biochemistry and disease we think, and that is the levels of storage forms of cholesterol in addition to genetic mutations and environmental risk factors. Then finally, I just want to give credit to the people who've done a lot of this work over the years. Guys like me sit in our offices and try to be helpful in giving advice to the people who work in our laboratories. In particular Vanessa Langness and Rik Van Der Kant have been very involved in drug development. Grace Woodruff and Sol Reyna on the internalization work that I told you about. We owe a great deal of gratitude to patients that allowed us to get skin samples from them by biopsy. Dr. Galasko, has helped us with that a great deal. Funding for this project has come from the California Institute for Regenerative Medicine, the state stem cell agency called CIRM, and of course also the National Institutes of Health. I will stop there.

That's fantastic, Larry. Thank you so much. I see some questions already appear here. I'll try to summarize the ones that are common. But the first one I think is more related to the genetics of Alzheimer's. Probably Doug, you are the best one to answered that one. For example, my mom has Alzheimer's or die from Alzheimer's, what are the chances that someone in the family will also develop Alzheimer's? I think the answer to all of these questions is going to be, it's complicated. With regards to this question, if a member of your family, a first-degree relative, parent, sibling had Alzheimer's,

that increases your own chances a little bit. But it may not be enough to worry about,
and it may not be enough at this stage to know what to do about, other than to think about keeping your brain as healthy as possible and reduce some of those risk factors. The family histories, we really worry about that.
I think some of the later questions may deal with this. Or when there are multiple people across generations who develop Alzheimer's disease, and in some of those rare genes,
the ones that Dr. Goldstein mentioned have helped us develop animal models and things.
In those situations, the age at onset can be between 30 and 45, which is quite catastrophic.
For those families. They are rare but very important for our understanding and to do certain kinds of research.
Yeah. I have a question regarding the antibody treatments.
If the antibodies are injected in blood or in the CSF,
do they actually reach the brain and reach the Tau and tangles in there.
That's a great question. That's one of the many reasons why antibodies
end up being super expensive for brain diseases. They don't get into the brain very well,
but a little bit does get in. For the amyloid antibodies, enough seems to get in that if we give high doses,
we are able to remove the amyloid to some extent with some of
the newer antibodies to what appears to be quite a reasonable extent over time.
Tau is going to be a different question and a bigger challenge because Tau is accumulating inside nerve cells.
One might imagine that an antibody has to get into the brain, and then it has to find
the Tau that's inside the nerve cells if that's where the abnormal forms of Tau are doing their mischief.
If there are some bits of Tau that are escaping and communicating between nerves cells,
maybe the antibodies can meet them there. But I think that's one of the bigger conceptual challenges
for Tau antibody therapy. Okay. I have another one for you.
How strong is the association between Alzheimer's disease and inflammation?
There's inflammation and inflammation. [LAUGHTER]

Inflammation occurs in the brain, for example and some of
the cells that are unique to the brain, in particular, these things called astrocytes and microglia contributes to inflammation in the brain.
Sometimes this can be a good and necessary thing. Sometimes they may be taking care of turnover.
We turnover a certain amount of membranes of nerve cells and synapses and some of these microglia and astrocytes are
helping to remove and kill them, but sometimes it can be a bad thing.
In Alzheimer's, there's some genetic and other evidence that suggests that microglia get
turned on in some ways that can contribute to the pathology.
Inflammation, the way most of us think of it actually occurs in the whole body and that's a different question.
If you get a really severe infection somewhere in your bloodstream, does this do anything to Alzheimer's disease?
The answer is, as long as whatever is going on doesn't cross the blood-brain barrier,
then we don't know that there is a direct impact. It probably isn't good to be going
around with chronic inflammation or chronic infections or things like that in your body in general, because these can maybe deprive the brain of its optimal sources
of glucose or other things. There are some associations between people who
have Alzheimer's and then have a severe infection of some sorts, get admitted to hospital,
recover from the infection and gee whiz, the Alzheimer's got worse. We don't understand all of those mechanisms,
but there are lots of potential things one could think how that play. Before we jump to Larry on more science questions,
I have another one on the clinical aspect. I think it's related to the inflammation.
Is there are clinical trials respect to investigation drugs that prevent Alzheimer?
There certainly are. If we think about it, if we wanted to do a clinical trial
to prevent Alzheimer's, that sounds like a great thing to aspire to,
but the answer is, it's not easy. We can't round up a bunch of 65 year old people

and reliably predict who's going to get Alzheimer's and when they're going to get it. For some of these folks, they may get Alzheimer's
10 years later and we don't have the time to do a 10 year or 15 year clinical trial.
There are ways to try and enhance some of the prevention trials regarding late onset Alzheimer's.
But in fact one of the most interesting prevention aspects has come from some of
these rare families that have genes that can cause early onset Alzheimer's disease.
At UCSD, we're part of one of these networks, it's an international network, studying people with mutations in APP PS-1 and PS-2,
and we are part of a group that are doing clinical trials.
Some of the trials involve people who already have symptoms. But the idea is to try and do prevention trials because here we can actually
calculate if we take a family in which 10 people got Alzheimer's disease and the average age at which they got Alzheimer's was 40. We could round up a bunch of 35 year olds, for example, or people within five or six years of the estimated age at onset and we can do a lot of modeling induced biomarkers to give us insights that we just couldn't get from late onset Alzheimer's disease.
There's a lot of exciting research going on in that area in prevention. Very nice. Larry, I will start with some of the questions here. Is there any correlation between
the brain cholesterol ester levels in APOE4?
Great question. I think this is going to be one of those questions where I'm going to have to just confess, we don't know. I think it's a terrific question. It's something that follow up experiments really need to be done on. Of course, part of what this question alludes to is that the normal biological function of APOE is to be a cholesterol carrier in brain lipoproteins.
You might expect that there would be some connection between the different genetic forms of APOE,
either the two allele, the three allele or the four allele, and their ability to carry cholesterol or their effects on
storage forms of cholesterol and we just really don't know. There's a lot of work going on in this area,
it's moving very quickly, so stay tuned. There's another question about the mouse treatment.

How the drug, efavirenz got into the brain?
What was the route, and what dose did you use compared to what would you use to treat humans?
Is there equivalence here? [LAUGHTER] Two excellent questions. First, how does it get into the brain?
Well, actually a lot of drugs do cross the blood-brain barrier and efavirenz is a drug
that not only crosses the blood-brain barrier, it seems to accumulate in the brain. Interestingly, it's used for
AIDS treatment sometimes and one of the nasty side effects when it's used for AIDS treatment is in fact neurotoxicity.
We think that that may in fact be because it's messing with cholesterol levels in the brain because it's used
at very high concentrations in AIDS treatment relative to what we think would be effective for Alzheimer's disease.
The question of whether the mouse dose is the same as a human dose or not is a hard one.
There's an area called allometry, depending on how you want to pronounce it,
that tries to estimate what's the equivalent dose of drug,
a nutrient or whatever for a mouse relative to a human.
Because we're obviously much bigger, our brains are bigger, there's just all issues with dosing.
We think based on some calculations that the dose we've been using in a mouse is way
below the neurotoxic dose in humans, and that this is a dose that we want to take into
clinical trials that we've generated by calculation. We think it'll be a low enough dose,
that it won't be toxic but you just don't know until you do the experiment.
This is why it's so important for us to get into trials. Fair enough. There is another question.
This is coming from Joe Acker. He said that there must be data on cholesterol lowering and Alzheimer's progression.
Is there any impact on folks that use statins for example? Yes. [LAUGHTER] Dr. Galasko is laughing because we have gone round and round about this topic for quite some time.
There are some epidemiologic data that suggests that there is a statin effect in some settings.
But a number of clinical trials that had been done prospectively with

statins have been disappointing. But some of them have given biochemical changes
when proteins are measured in the CSF. One of the real complications is not just age
and condition of the brain when statins start, but also some statins
cross the blood-brain barrier, some don't, and for some it's not even particularly clear what level of penetration there is.
That has just really complicated the literature on this. I'll also note.
Many people are aware that statins frequently have side effects.
You may remember that complicated branched synthesis pathway I showed.
There are branches off of the pathway and statins act at a very early step,
and so all of the downstream steps are messed up. The side effect issues
in the brain may also be complicating things. One of the reasons we like efavirenz is that it's acting at
a point very low in the synthesis pathway. In fact, we think it's in the degradation pathway,
and so it's much nearer cholesterol than statins are. Just a related question.
In your mouse model, I'm assuming you didn't see any toxicity, but did you notice any cognitive improvement on these treated animals?
These were simple pathology experiments. As you know, Dr. Muotri,
mouse behavioral experiments are a significant undertaking.
Got it. This is for Doug. This is an interesting one.
Why not use Down's syndrome as a early cohort of Alzheimer's disease?
That's a terrific question and the answer is yes, but it's complicated.
People with Down's syndrome have an extra copy of the APP gene on chromosome 21.
They make more Beta-amyloid and they invariably get deposits of plaques
and tangles in their brains with age. One would imagine we could do
clinical trials or study people with Down's syndrome. The difficult thing is measuring cognition in people with
Down's Syndrome and measuring function is really difficult. To do the clinical trials and come up with robust clinical measures and outcomes, and endpoints is not at all easy.
There have been some more recent initiatives to try and improve how people with Down's are assessed and this includes things like doing

brain MRI and biomarkers of one sort and another, but in the absence of really
having good clinical measures, it's going to be difficult to decide if
whatever biochemical impact we have is actually helping a patient with Down's syndrome or not.
There are efforts in the area and stay tuned is my response.
Larry, is there a link between high cholesterol and frequency of Alzheimer's?
I think Doug touched on that point? Yeah. Let me just do a little bit of clean up on this.
It's an excellent question, and it turns out that to the best of
our knowledge and to the best of the data available, the brain and the bloodstream are
two completely separate systems with respect to cholesterol synthesis, degradation, and use.
In the bloodstream, cholesterol is synthesized in the liver, it's packaged into primarily low density lipoproteins,
LDL, and a little bit of HDL, and then it circulates. In the brain,
the synthesis of cholesterol is probably happening in the microglia and the astrocytes to support cells in the brain,
and it's packaged into HDL, primarily, not LDL, and so brain cholesterol is primarily in the HDL form,
not the LDL form as happens in the blood, and as far as we know, there's just no correlation.
Now that said, there may well be genetic variation that leads to similar changes,
for example, in cholesterol synthesis in cells of the liver and cells in the brain,
some of the enzymes are shared. The same enzymes are used in the liver as in the brain.
So it's a little unclear, and I'll just mention finally, it's a little hard to measure brain cholesterol levels in
a living brain for obvious reasons. I think when Doug mentioned
about the environmental factors, these was probably like a multifactorial list that includes high cholesterol.
Yeah. Got it. One comment when we think of
environments and many of the studies have been done, many of these are epidemiology
population-based studies,
and the outcome is dementia not Alzheimer's disease. If you remember from one of my slides,
dementia will include a lot of people who had strokes and vascular pathology and things like that,

it may well be that some of those risk factors are going to help us to reduce the vascular damage in
the brain but not do anything to the Alzheimer pathology. It doesn't mean that we shouldn't go after these things,
it just means that we should try to figure out what we are targeting. I think you two clarified it really well.
I think Larry would like this question; is the amyloid plaque a
byproduct of Alzheimer's disease as opposed to a cause of Alzheimer's disease. [LAUGHTER]
Here we go. [LAUGHTER] This question is debated hotly at meetings,
in labs, in hallways, in coffee shops. I think you're going to get three different answers
from every scientist or a clinician you talk to about this. I personally think that plaques are
probably not the actual cause of the disease, I think it's probably earlier events caused
by those genetic mutations and risk factors. There's some evidence for
that point of view, for example, a duplication of the APP gene actually has
an abnormal neuronal phenotype during fetal development.
It's a very early change and disappears to be related to processing of the protein.
But that said, I don't actually think that having a head full of plaques is good for you.
So it may not be the cause, but boy, sure could be a complication. But I think Doug would have
a completely different view on this than what I just said.
I think I would be ambivalent, but I'm taking the point
of view that it's hard to ignore amyloid. The genetics and the pathology and everything else,
and it seems to be specific for Alzheimer's. So you don't, for example, have a stroke and then deposit
amyloid around all of those damaged nerve cells. You don't have some other kind of brain pathology and
then deposit amyloid as a reaction.
It's not a direct toxin, if you have a little bit of amyloid,
as best we know, that's not going to even cause any kind of detectable clinical effect.
That's where the debate gets a little bit tricky. In a test tube or if you grow nerve cells,
you can actually do things with the amyloid protein and you can show that it's a fantastic toxin,

but if that was the case, Alzheimer's would be a three-month disease.
I remember on my conversations with Ajit, Pascal here from the CARTA Center,
showing that other primates do show signs of plaque aggregations as well,
but they never developed dementia. I think even chimpanzees their brains are full of that when they get old,
but we don't see the signs of dementia. It might be that they just die earlier,
but I don't know, that's an interesting observation.
There is another one that I would like to hear from you both, is this idea of studying resilient brains, people who are resistant or resilient to Alzheimer's, are they missing the inflammatory component or is there
any genetic variant signal that we can take from these people? Firstly, we need to define what we mean by resilient.
I'll give you a few examples, so there's a very large extended set of
families mainly living in Colombia that carry a particular presenilin mutation.
On average, the age at onset across here, this is hundreds and hundreds of people.
The average age at onset is approximately 42.
These families have not been studied intensively, and a woman who happened to be
a carrier of the gene was found who was in her 60s with normal cognition.
So people did a big dive into her genetics and found that she actually carried an unusual variant of the
Apolipoprotein E. So maybe that was protected. Maybe that's an example of how a
very rare genetic contribution, this was extraordinarily rare, caused some sort of resilience.
The other kind of resilience people think about is somebody gets to the age of
90 and their identical twin [NOISE] also gets to the age of 90,
and the twin develops severe Alzheimer's disease, whereas the other person
has amyloid in their brain and is cognitively normal. There's something different going on there and there're a lot of different possibilities. These could range from biological ones like how do people respond to amyloid to a whole bunch of developmental ones like other ways to make brain connections
stronger and to develop cognitive reserve as it's been called.

It's a really complicated question and people are trying to look at different angles
because as the genetic home run would be one example of trying to find one mechanism,
but there may be much more complicated things at play. Larry, do you have anything to add?
Yeah, I was just going to add that there is one other rare genetic variant that clearly
does confer resistance to developing Alzheimer's disease. It was found in a cohort or a set of families in
Iceland where everybody has been sequenced and analyzed as near as I can tell.
It's a rare mutation in the APP gene at
the same site as mutations that give rise to disease. What appears to be going on is the mutations that give
rise to disease enhanced breakdown of the APP protein, ultimately leading to amyloid in a sequence
of steps not immediately, and the genetic mutation that's resistant is a different change there
that appears to interfere with the breakdown of the molecule. That's a very rare but interesting situation,
the fans of the amyloid hypothesis love that observation. I think there are extenuating arguments
that make it not so clear cut but time will tell. Larry, could [inaudible] block,
increase a signal by the lipid message PGE2 that has been
implicated in cognitive decline during aging. Do metabolites from the gut microbiome
alter the capacity of interferon gamma response in the meningeal NQ cells
to modify T-cell of those using the brains. This is Kate asking a complicated question.
[LAUGHTER] I'm going to say the following; one of the secrets to being
a long-lived successful scientist is to know when to say you don't know something.
I really don't know the answer to either of those questions. There have been some experiments that had been
done in the mouse that suggests that the microbiome may have an effect on
the development of the mouse version of Alzheimer's disease, but remember, these are mice with lots of
plaques and not necessarily, well, the mouse version of dementia, I'm not entirely sure exactly what that is.

Other metabolites related to cholesterol metabolism,
this is in early days and unclear where that's going to lead.
Doug, even though the biochemical pathways
are connected between late onset and the familiar cases,
does that mean that these are two different diseases that should be treated with different targets?
Again, that's a great question. The [inaudible] would say
that regardless of age at onset, genetics, and so forth, if we look at the brain of someone who dies with advanced Alzheimer's disease, they're going to have plaques and tangles. Therefore, it's one disease.
I think that if one is dealing with the mutation, so some of the really interesting ways
to do gene editing might lead us to come up with some really interesting ways to approach people with presenilin or APP mutations and try and do something to deliver
a vector to their brains to try and do some genetic correcting, for example, there actually are some very early clinical trials looking at using AAV vectors to deliver APOE e2,
which seems to protect and confer some degree of longevity and there
have been a couple of pre-clinical studies and there are actually some early phase clinical trial looking at that sort of approach.
That would be a more personalized, I think break it down into personalized treatment rather than age at onset might be something to think about. The more we know about some of
the drivers and the mechanisms, the more we might think of where do we have an opportunity to tackle something
super early and where would we have some different opportunities to maybe be intervening later in the chain of events.
I hear you too and to me, there's so many parallels with autism,
the loss of synapses, and then there is stratification of the different sub-types and
that's more or less what happens in autism. As we define better, we create a new syndrome.
Larry, I have two questions about the in vitro model, which I think is relevant.
You treated only IPS derived neurons. How about microglia, astrocytes, and oligodendrocytes?
Are they included? A related question is, how robust is the IPS differentiation?
Do you always have the same phenotype in vitro? Yes.

The first question would be, did we do any of our experiments in so called co-culture models, where astrocytes or microglia are grown along with neurons, and then neuronal behavior analyzed? Many of these experiments were done quite early in the development of this field before astrocytes and microglia were available to actually do these experiments. There's been some efforts to do these sorts of things.

But to be honest, I think simple co-culture experiments are going to be completely supplanted by organoids experiments, with, of course, the caveat that while astrocytes are derived from the same lineage as neurons, microglia, in essence, come from a blood-forming lineage and so are completely separate. Then would have to be introduced into organoids in some more controlled fashion. The consistency, generally, in the experiments, I talked about use a method called fluorescence-activated cell sorting to allow us to purify neurons to near homogeneity. The cultures were usually done with purified neurons. That actually helps quite a bit with reproducibility over the datasets.

But boy, there's a lot more to be done in these systems. Someone asked if the data with the genetic cases could be the same on the sporadic cases. But I guess we need to test if the drug will work on a more complex system. Absolutely. It's a great question.

It's something that plague all of us in this field. A lot of the work is done with the genetic mutations because they're much more straightforward to study, they're homogeneous, you don't have as much variability. When we've made neurons that were made from skin cells of people who have sporadic disease, there's quite a bit of variability in the biochemical behavior and it's going to take large numbers of different genetic types of neurons and glia to really understand how that plays out in the behavior in these systems and that work just needs to be done. We're not there yet. Nobody is. I have another epidemiological question that you might know because [inaudible] seems to be also used on HIV field in people with HIV sometimes develop dementia. Are the population taking this drug protected somehow? Do you know that? Great question. I don't know the answer to that.

I should actually hit the literature on that one and find out. Good point.
I can comment a little, and that is that firstly, HIV dementia really existed
before there was effective antiviral therapy, and florid dementia is really unusual in people
who consistently take their antiretroviral drugs.
There are some more subtle cognitive changes and that's being studied quite intensively.
But the era of rapid HIV dementia, at least in your well-developed countries where
the antivirals are given out systematically, seems to have disappeared.
An interesting question is, do people with HIV have an increased risk of Alzheimer's disease?
Again, the studies haven't been done yet. The survivors of the HIV epidemic
who have had successful treatments, some of them are getting into their late 60s and 70s, but it
may not be enough to do
a really comprehensive study of Alzheimer's at this stage.
Another clinical question is, how similar are those dementia-related or neurodegeneration
diseases?
Are they really different diseases or they share some of the biochemical pathways or neuronal
degeneration?
What would you say here? We see different, let's call them tombstone proteins,
if we're going to be derogatory, in a number of disorder. Alzheimer's is amyloid and tau,
Parkinson's and DLB is Alpha-synuclein. Some of the front and temporal dementia are TDP-43,
and just because we're seeing different markers, does this mean that these are all radically
different diseases or other commonalities?
There's synaptic loss in all of them, but I don't know if that is common in our finding.
I think some of the interesting commonalities have to do with microglial and neuroinflammatory
responses
which seem to occur, to some extent across, these diseases, maybe some shades of difference.
Then there are some hints of autophagy and lysosomal pathways that,
again, may be altered, but for different reasons across some of these diseases.
But at the moment, I think we have better reasons to split them than to lump them.
Is there any support for the pathogen "theory"
being the initiating factor of Alzheimer's? Do we have any evidence for them?

Again, that's a very complicated question. There are different kinds of
pathogens and there have been some very far-fetched claims about infectious etiologies of Alzheimer's,
ranging from people who claim they could see spirochetes in some very badly silver-stained brains way back when.
There's a burgeoning literature and there are some fans of herpes viruses, HSV,
and some of the herpes family, which can accumulate and
go latent and dormant in neurons, and there isn't really an absolutely compelling answer.
I'd say I think the majority of people say that there is
not super strong evidence that these are causative. Larry, in your opinion,
how good are the LPS-induced inflammation in modeling than no inflammation in Alzheimer?
That's a very interesting question. I think that the way I would answer it is to say,
we really aren't clear on what the inflammatory triggers are in any of the neurodegenerative diseases.
I think Doug mentioned in passing that pretty much any neurodegenerative disease you look at,
regardless of what part of the brain it affects, they all lead to astrocytic activation, microglial activation.
There are signs of inflammation. I don't know of any evidence one way or another as
to whether the LPS pathway in these cells is similar to the pathway of activation in
the actual disease and I don't think anybody really knows what the inflammatory initiators are.
I agree. Another question for you, Larry. Is there any correlation
between 2,4-hydroxylase variants and AD risk report?
Wow, I don't know that one. Doug, do you? I don't.
Okay. I'll just mention that there's a list
of 10 genes where variants have the biggest impact. As far as I know, two hydroxylase is not among that list.
There's also a list of 100 and I couldn't swear that it's not on that list of 100,
but it's certainly not among the list of the highest impact. But the genetic variants you
find are still in the population after they've gone through all sorts of natural selection
and they're not really a random set of variants. It's a little hard to suss that one out cleanly.

It doesn't mean that a variant couldn't, but it may not occur in the population for other reasons.
So hard to say. Any other comments on the toxicity of
the beta-amyloid entangles on neuronal cells?
Someone is giving example of the alpha-synuclein parts where we know more or less about the toxicity.
What about in human neurons in Alzheimer's disease? Well, I think that's where
some of the rubber meets the road on what's really toxic in Alzheimer's disease
with respect to the pathologies. Doug just mentioned one of the old jokes
which is that the plaques are tombstones not guns you might [LAUGHTER] say.
There's evidence of amyloid toxicity in some experiments and not others.
The experiments that have the greatest toxicity are done at doses that are way, way unreasonable relative to
how these things would have to act biologically. Changes in tau however,
those could well be very straightforwardly neurotoxic. Tau itself is a regulator of
movement along microtubules in axons. That's its day job.
If you interfere with its ability to do that properly, you will probably get neuronal abnormalities
because you'll get changes in the movement pathways between the cell body and the end of the axon at the synapse.
I'm checking the time, I think we are approaching the end. But I want you guys to
answer two questions that are related that I saved to the end. The question is, how many years away are we
from having a sensitive diagnostic panel, a blood test that we can do it early in life?
The related question is, what would be the implications of having such a screening in early life?
I think I mean ethical implications here. [LAUGHTER]
Sometimes things can have unintended consequences.
The biomarker data on plasma phosphotyrosine, this are the markers,
is really incredibly exciting and there's probably around 40 or 50 publications in
the last year and almost nothing meaningful before then. It looks like we could use
plasma biomarkers in people who have symptoms to give us
a reasonably good idea at least at the MCI stage, definitely at the dementia stage that there

could very well be an Alzheimer process going on.

Do we want to do anything in pre-symptomatic people?

I think it's really difficult. I think in the absence of having a treatment, even if you could get genetic testing done and you

had something scored out that's told you you have a three times lifetime risk of Alzheimer's disease,

that is three times higher than somebody else, at the moment, there's absolutely nothing to do with that information.

I think being able to predict things is [NOISE] not something that we should go into very likely.

We need to know how good the predictors are and really whether they're going to be actionable and if all they're going to

is give us sleepless nights and worry about things. I'll just add to that and say that the utility

of prediction does help in some cases with life planning.

Now, on the other hand, if you tell a 20-year-old that they've got a significant chance of developing Alzheimer's disease when they're 60,

if you don't have a useful therapeutic, that is not a useful thing to tell that person, I don't think.

But in the case of statins for cardiovascular disease,

having some predictive capacity along with a very effective drug

outside the brain for cardiovascular abnormalities, that of course is incredibly powerful and one could see

this field going in that direction but it's not going to take only one year

or two years to get there. We're still pretty far away from an effective therapeutic especially one that would act early, I think. Doug may disagree.

I think it's just going to take a huge amount more work to get to the point where this is useful.

But if you had a really safe drug that would work at the very early stages

of disease and prevent it, then of course that would be an incredibly powerful way to go. But boy, we are not there.

By the way, I think that's one of the [LAUGHTER] most fascinating things, is to be able to find these very early stages using like

the stem cell tools that we have for things that will happen really late in life.

It seems to me like a catastrophic thing that we'll build it up and then at
one point it crashes the system. It does it over the years.
It is amazing to find and it's not only to Alzheimer's. We hear about other conditions,
late onset diseases that seems to start even in the embryo which is [LAUGHTER] amazing.
I have other questions here.
Someone asking that patients are always asking for non prescribe medications to prevent memory loss.
If there is anything to support any of those in the literature
and what are things that can be done specifically to prevent
the major things that you are doing. [LAUGHTER]
I think if you read the advertisements and the labels very carefully,
nothing actually says this will prevent dementia. That's because there would be a law suit as a result.
Doctors was bangs magic elixir, when you read about what the claims are,
it says this may strengthen the brain in aging or it has something that's really not very easy to do directly interpret.
There are almost no well-conducted clinical trials actually have been some decent clinical trials of supplements and vitamins, all of which had been negative. The products that are claimed to boost brain health,
for the most part, haven't really been tested in in an appropriate way.
There are a couple of things that are nodding along and look a little bit interesting, but not to a degree that I would
start handing them out in great profusion to people.
Brain health is like heart health, doing and looking after your body in general.
Not smoking and getting some exercise, watching your sleep,
eating a reasonable diet at some evidence for a Mediterranean diet. I think some of these are reasonably
basic kinds of things and they don't sound like much, but they actually aren't always so easy for all of us to stick to virtuously.

For now, I think that that would just be the absolute core while we wait for real targeted treatments to come along.

Larry, any comments on this one? I believe in the health benefits of exercise,

and I don't take any of those off the shelf things. I'm voting with my feet for what it's worth.

[LAUGHTER]

Good. Chronic traumatic encephalopathy, with dementia.

How is that related to Alzheimer's? Is there a role for that in Alzheimer's? Quite frankly chronic encephalopathy has

gotten a lot of publicity from two areas. The one is the retired NFL players and help

form of boxers who would be the largest contributors to this type of literature.

The interesting thing in CTE is there a tangles and they look like the exact same kinds

of tangles that we see in Alzheimer's disease, except that they have a different distribution in the brain.

There is not necessarily amyloid presence. So something related to the chronic repetitive trauma does is setting up the development of tangles. Whether these tangles spread and

progress and trigger things is very difficult to be sure about and very difficult to model.

But in principle, something that might be used in

a treatment trial for tangles in Alzheimer's could in theory

be used for the treatment trial in CTE. Except I don't know that we could round up enough people to do a treatment trial in

CTE because we really don't know who is at definite risk right now.

The other area that got a lot of attention are people who had shock blast type TBI exposures during the various Gulf Wars. It's not clear that this is the same pathology.

It's not fair that they get the same kind of top pathology. They do get some other things happening with neuro inflammation and so forth.

But it's not clear that these have super common features. We don't know is again,

because folks who were exposed were too young is where the people who were exposed to some of these shockwaves

and so the Gulf War exposures, whether that might somehow, maybe increase the risk of Alzheimer's intellect life.

Got it. Someone is asking to clarify the results
of Aducanumab which seems
to be confusing regarding effectiveness.
Aducanumab was one of the antibodies that I mentioned with
regards to being able to bind to amyloid and help to remove it from the brain.
Different doses of Aducanumab were studied in different trials.
In general, the finding which isn't too surprising is that the higher the dose,
the more effective it was at removing amyloid. There was a dose of 10 milligram
per kilogram dose was used. In the clinical trials seem to be the
most effective at removing amyloid. It seemed to be the one that was more likely to show some clinical signal.
It turned out in these trials there was a potential downside. That is that one thing we haven't talked about is that
amyloid can also deposit in blood vessels in the brain. Sometimes as the amyloid is removed,
it maybe does some damage to blood vessels or the antibody binds to the vascular amyloid.
There can be some degree of local bleeding and inflammation in the brain. In the clinical trials,
we've learned how to manage.
This is a little bit of a limitation in terms of how high we can dose.
I think that the final word obviously isn't that on the antibodies and removing amyloid.
I think that now that we have the possibility of four or five different antibodies out there in
trials that all maybe can do this. It will be possible to explore whether this is helpful and how helpful it is.
All right, we have no more questions. I think this is a very nasty condition,
horrible for the families and so glad to hear on the progress here, Larry, we need to take this drug
to the clinical trials [LAUGHTER] this is so important. I am glad to hear you say CD is
moving with another clinical trial, think BDNF gene therapy approach with Mark Tuszynski
So this is ours are always good news. I'm just hoping for a bright future
here for those families. I wonder if any of you has any last word. What you need is lots of shots on goal.
Absolutely. The cancer field, people point to lots of therapeutic breakthroughs.

But in fact, there were thousands of cancer clinical trials. At the moment, the Alzheimer clinical trials barely make the hundreds. We just have to keep going. As long as the ideas are good and we know how to identify what we think we're doing, then a trial is worthwhile. Yeah.

I'm very happy to see that Sam is really committed with the neurological conditions as well.

This is also good news, not only for California but for everybody. All right.

If no further comments, I think we will stop here. Thanks, Larry. Thanks Doug.

I hope you all enjoyed it.