1 what I would say.

2 Either one of those studies is probably okay to

3 do. You could measure plaques. You're saying, okay,

4 there's a way to measure plaques. It's not invasive

5 presumably to measure the plaque. It may be, it may not

6 be. There may be more invasive ways. What's the gold

7 standard? And you start to notch up. You change the

8 characteristics of the trial in each case and each of those

9 calculi that you need to go through are related to that.

10 But I would like to just encourage these kind

11 of conversations to go forward and realize that each person

12 coming to the table in that conversation is going to have a

13 different reason for selecting an outcome measure.

14 DR. SIEGEL: I just wanted to put some of the

15 last set of comments into a perspective in the context of

16 clinical development from observations in many fields,

17 which is that to the extent we're talking about first

18 introduction into humans in early phase I trials, with some

19 extraordinarily rare exceptions, there aren't treatments

20 that you can measure efficacy in those trials. You need to

21 have control groups. You need to have large numbers of

22 patients. There may be, as I said, a rare home-run

23 treatment in a disease of entirely predictable course where

24 you can tell that in a small number of patients.

25 We're talking about the distinction between

1 what's measurable and what's meaningful. It's compellingly
2 important in this sort of therapy in particular, I would
3 think, to have a measure of something that at least you
4 hope and have reason to anticipate may predict efficacy
5 because as these products develop, there are so many
6 potential variables, many of which we've heard about.
7 Well, what happens if I give a few more cells, culture a
8 few more passages, change the concentration of this
9 differentiation factor, change the cell source, or
10 whatever? Am I going to have a better product or a worse
11 product? What happens when I up-scale to treat larger
12 numbers of patients if I culture at a denser or a less
13 dense -- and on down the line in many more ways than with a
14 simple chemical molecule, many questions about variations.
15 The notion that each and every one of those
16 questions can be answered by a multi-year controlled
17 clinical trial comparing efficacy outcomes is, of course,
18 ridiculous on its face. So, if you don't have a measure
19 that is measurable and you have some reason to hope will
20 predict clinical efficacy, you're going to be doing a
21 guessing game and you're going to be down the line trying
22 to guess what's the right way to do it in a very expensive
23 and a very large trial and probably have a lot of trouble
24 finding people even willing to support or participate in
25 such a trial.

1 MS. SONTAG: My name is Jordana Sontag. I'm a
2 patient advocate. My son has a leukodystrophy called
3 Canavan's disease. He was part of a protocol at Yale
4 University and successfully received gene therapy. He's
5 now part of a new IND that's being reviewed currently.
6 When you started your presentation, you said
7 that the general consensus or the general public trusts the
8 oversight, and I'd like to say that is true as a parent of
9 a patient.
10 But I'd also like to say that I feel that the
11 oversight process perhaps has lost some trust in the
12 patients and the families of patients. I feel that
13 sometimes we're perceived as hysterical, desperate people
14 that are willing to do anything and put anything to try and
15 save our families. But the truth is we do want safety. We
16 don't want to harm. I already have a fatally ill child. I
17 don't want to do worse to him. So, I wanted to state that
18 fact.
19 I also wanted to state that I feel that there
20 needs to also be some definition in ethical lines as we
21 move from gene therapy. Now we're going into stem cells.
22 At what point do we as patients have the right to say we
23 funded this research? It has been worked on for two years.
24 We have all the safety data. At what point does safety
25 then turn into political pressures, media frenzies, where

1 the FDA succumbs to that and loses site of the patients?
2 So, what I'd like to say is that I feel like
3 the oversight committee should have more trust in us as
4 patients.
5 DR. SALOMON: Can I ask you a question then?
6 We're talking about outcome parameters, measurable,
7 meaningful. So, in your own experience with a clinical
8 trial on your child -- I mean, who would know their child
9 better than the mother -- do you have an impression about
10 what would be a meaningful outcome parameter that you would
11 see as meaningful versus what the doctors who did the trial
12 on your son thought were meaningful outcome parameters?
13 MS. SONTAG: I'm lucky enough to have both. My
14 son showed improvements through MRI. He regenerated new
15 myelin, which he couldn't do without the intervention of
16 gene therapy. So, I had the scientific proof, but then, on
17 the other side, I have a child that is doing dramatically
18 well. He vocalizes. He talks. He's not on a feeding
19 tube. He's a happy child. His quality of life is
20 drastically improved. He's gained the ability to use a
21 communication device. I took him to a symposium and a
22 bunch of the researchers looked at my son and said, he has
23 Canavan's disease? If they're saying it without me even
24 saying anything, then I know something is happening.
25 I'm not willing to just put snake oil in my

1 son. I'm funding research. We have not received any
2 federal funding. This is all parent-run. I don't want to
3 put my money into something that I don't believe in or that
4 I believe won't have a benefit. I don't have that much
5 money to spend. So, I feel that we're objective and I
6 think that we need to gain the respect of the oversight
7 process as objective, passionate people that are looking to
8 move science forward for everyone's benefit.
9 DR. SALOMON: Thank you.
10 DR. CHAMPLIN: As somebody who does phase I
11 trials in cancer patients, I think there are sort of two
12 levels here. One is the patient's hopes for benefit, and
13 every once in a while, a new drug does come along that
14 actually is a major advantage and provides clear-cut,
15 symptomatic benefit for those patients. Unfortunately,
16 it's the minority of the time rather than the majority.
17 But from a drug development standpoint, one is
18 generally treating patients usually with far advanced
19 disease where the prognosis is very poor. In those
20 situations, you're looking for some biologic effects that
21 would then justify taking that trial into a better
22 prognostic setting where, in fact, it may have more
23 efficacy. So, you need to have some endpoints, obviously,
24 to measure that biology to determine is the drug promising
25 to take forward into a more definitive population.

1 DR. WALKER: Dr. Sugarman introduced an
2 important concept of "the gold standard," and then we
3 didn't discuss it particularly any further. It is critical
4 that we have a gold standard of measurement that we think
5 is important. It started out years ago in cancer being
6 solely death, and that's a pretty unsatisfactory kind of
7 gold standard but sure is a standard. It's easily
8 measurable.
9 We then move into better and better ways of
10 doing it. I think here is the place we have a tremendous
11 problem. We do not do the research into the development of
12 the better ways of measuring appropriate outcomes, be they
13 biochemical, be they looking at plaques, or whatever it is.
14 But we need to do that kind of thing. It will lead to
15 better trials, smaller sample size, greater efficiencies.
16 DR. SALOMON: Let me pose a question then to
17 continue on this. What I see happening whatever time in
18 the future, but not the long future, is there's going to be
19 a group of sponsors stepping up with a specific trial.
20 Today we have the chance to be generalists, but soon we'll
21 be faced with a specific trial in "fill in the blank."
22 So, in that each disease process will have very
23 different kinds of outcome parameters, if there were two
24 trials considered at the same time, both of which are
25 devastating diseases -- I don't want to compete the

1 diseases against each other, but one which just happens to
2 have very well-defined outcome parameters and measures and
3 one that maybe doesn't. The Alzheimer's disease is an
4 interesting one already posed by this group. What do you
5 guys think of that? Should you do both? Or should you
6 only do the one with the better outcome parameters given
7 that this is a new field?
8 DR. MACKLIS: I had a question or a few naive
9 questions that relate to that. So, maybe I can throw my
10 questions out on the table, and then everybody can discuss.
11 Dr. Sugarman repeatedly used the baseball
12 analogy of hitting a home run. Sitting here listening, I
13 thought that was a great way of telling me how to think
14 about things. As scientists, one likes to think one is
15 hitting a home run.
16 But Mark Noble earlier raised a point about
17 commercial pressures that you reinforced and we just heard
18 about patient and disease driven pressures. I wonder if
19 there is a broader either societal or governmental or field
20 set of ethics -- is it at odds or is it the same -- to
21 deciding whether we might want to go for a double or a
22 single or even a bunt.
23 (Laughter.)
24 DR. MACKLIS: And do we have just a certain
25 number of people to go at bat, to bring the analogy maybe

1 four steps further than it ever should have been taken? I
2 apologize. And do we limit that? How many studies can we
3 do, and are we willing to go for a single or a double more
4 broadly than just a few home run hitters?
5 DR. SUGARMAN: I think one of the struggles
6 with this set of conversations is what we're talking about.
7 I'll change the analogy slightly. If I bring my ethics
8 hammer to bear here, the problem we're talking about are
9 all problems related to what we call justice, fairness in
10 allocation, and fairness in access. These are all justice
11 problems and they've been a problem in ethical theory since
12 Aristotle. So, it's no surprise that we're still chewing
13 on them.
14 In research, what you're seeing as a reflection
15 here I think are multiple problems. The whole regulatory
16 scheme was built on the notion that patients were
17 vulnerable and needed to be protected. The reasons for
18 that were a series of scandals in the '60s and early '70s
19 that came out of disadvantaged subjects being used without
20 consent for research. We can go through each of those
21 examples. So, when the National Research Act was put into
22 place, the notion there was to protect folks and to make
23 sure that there were even more protections on anyone who
24 might be perceived as vulnerable in any way.
25 Once the regulatory approach came into play --

1 and we have separate regulations for prisoners and kids and
2 pregnant women, and we have this very restrictive approach.
3 With the onset of the AIDS epidemic and advances in cancer
4 chemotherapeutics, we've seen a change in the calculus.
5 It's still a problem of justice, but the claim is in
6 protect me as someone who is vulnerable but let me have
7 access to something that could potentially help me. There
8 was sort of a corrective to what was an overly protective
9 initial scheme.
10 So, what you've seen as a result of the
11 lobbying efforts of folks and patients speaking loudly is a
12 change in the regulatory structure. You've seen different
13 pathways for drug approval processes in FDA. You've seen
14 inclusion criteria in clinical trials to enhance
15 generalizability in women, minorities, kids. So, this is
16 all a reflection of the same piece.
17 Now, what has happened as a result of that is
18 we're recognizing the multiple players in the field as we
19 go from protecting folks to access and the right answer is
20 somewhere in between. We don't know how to divvy up all
21 the resources the right way. Sometimes those resources are
22 the science itself for trials to move forward. Sometimes
23 it's which patients will be in a trial. Sometimes it's
24 which disease will be appropriate. These are all criteria
25 on justice that we are all really just learning how to talk

1 about.

2 So, I think, yes, a bunt or whatever that would
3 mean. The home run may be a short question. It may be
4 that my home run is decreasing tangles, that my home run
5 here isn't curing Alzheimer's disease or whatever the
6 question is. But that needs to be visualized. If you
7 share that goal with the same enthusiasm that we would have
8 scientifically with patients and family members, this is
9 where we are, this is the next question, this is the
10 scientific question, and unfortunately the science isn't
11 good enough for us to promise more, but do you want to help
12 out? Uniformly people will help out if that's where the
13 science really is.

14 I think we just need to think through those
15 again explicitly to try to answer those. I think they're
16 all home runs. If we could achieve any of them, we could
17 move forward.

18 DR. NOBLE: I want to make one concrete
19 proposal to this often too abstract discussion. If there
20 is any way in a study to use imaging to determine whether
21 or not you have had any kind of successful cell
22 replacement, it appears to me that this has to be mandatory
23 because we need a scientist to know whether or not we may
24 be getting cell replacement in the absence of clinical
25 improvement. And if we simply use outcome measures that

1 can be measured simply in the clinic, we don't even know
2 whether the experiment worked because it might have failed
3 because we didn't replace any cells at all, or it might
4 have failed because we didn't replace enough, or it might
5 have failed because we replaced them and it doesn't matter.
6 So, I think that's a critical component.
7 The ethics questions -- I'll never forget what
8 one of my friends with a glioblastoma told me. He said,
9 Dr. Noble, your ethics are a luxury that I don't have time
10 for. This is a pressure that we have for many of the
11 patients. We know a lot of spinal cord injury patients.
12 They're going to go and get shark embryo transplants in
13 Mexico. If we had some means of making sure that these
14 patients were directed into therapies that might be more
15 beneficial that maybe are not ready for the prime time that
16 we would want, but they're better than shuffling off to
17 Tijuana for a shark embryo, then maybe we could use this
18 process to learn something also.
19 DR. TROJANOWSKI: With respect to whether there
20 are measures that are adequate to indicate a response to a
21 therapy or not, I think that is a very critical question.
22 I recall, Mark, the Motulsky Orkin report on gene therapy
23 four or five years ago which said that too many of the
24 clinical trials for gene therapy were not powered
25 sufficiently, didn't have outcome measures to know whether

1 the experiment would or would not benefit the patient. And
2 that's a horrible place to be, and I'm glad that they spoke
3 out so eloquently and strongly about more basic science,
4 more animal models before rushing to clinical trials.
5 Although I mentioned that Alzheimer's disease
6 has some perplexing conundrums about what the most toxic
7 lesion is, I think we have many potentially informative
8 measures for a response to therapy, both biological and
9 cognitive. So, I would argue that we should go for the
10 clinical trials that have the potential to do something
11 where the measures are in place.
12 I'm very much in favor of bunts and base hits
13 because we in the Alzheimer's field, although I work in
14 Parkinson's as well, would love to be faced with the
15 dilemma of an L-dopa-like therapy that carried people for
16 10 or 15 years when the disease begins at age 75 to, let's
17 say, age 85 or 90 so that they could enjoy their retirement
18 or be cognitively intact enough to enjoy retirement years
19 rather than being in a nursing home. So, it doesn't have
20 to be a cure for Alzheimer's.
21 MS. WOLFSON: I think one thing that has to be
22 thought of, when we talk about the ethics of new therapies,
23 is not only the quality of life that a patient might enjoy
24 if this was successful, but the quality of the patient's
25 death. I think that in any informed consent procedure, the

1 possibility of what will happen to the person without the
2 therapy and the effect of the therapy on a person's death I
3 think is equally as important.
4 DR. SUGARMAN: I would like to underscore the
5 points. I don't want to send the false message that I want
6 be a Luddite about the whole thing and stop science. I'm
7 doing science all the time, what I consider science,
8 empirical work, and like to move forward and work in
9 clinical trials. I am the biggest fan of moving forward on
10 clinical trials and advancing things, and if that wasn't
11 clear from my comments, I'm sorry about that.
12 But I'm a fan of moving forward not for any of
13 this right now. When safe, when appropriate, when
14 alternatives have been explained. And I hope that the
15 emphasis is when it's right, I'm all over it. I sit on
16 IRBs and DSMBs and love to see them move forward.
17 I think it's critical to bring in these other
18 points about what the alternatives are. Just because we're
19 not the folks with the alternatives when we are at major
20 medical centers and the like doesn't mean that there aren't
21 other alternatives out there that might be good for this
22 patient or this patient's family. It may be a death. It
23 may be going on vacation instead of spending their time in
24 parking decks and waiting rooms. It really depends on the
25 state of the science, and I just think we need to be up

1 front and forward about what we can to offer.

2 I think there's a lot of good to be had here

3 and would like to see that we also focus on the outcomes of

4 benefit as well to know that what we're doing isn't the

5 snake oil. Is it that we put something in a part of the

6 brain that we think is going to be functional, it's not

7 functional, but has a therapeutic effect? We've heard this

8 kind of conversation before. We can fool ourselves too by

9 the state of the science of what we think we're doing. So,

10 we need to have a little humility here about how good it is

11 so that we can be honest and appropriate as we go forward.

12 DR. SALOMON: I know this is a great

13 discussion. I just want to do Dr. Mulligan in the back,

14 Dr. Freeman and Dr. Kurtzberg, and then we'll be done for

15 this and we'll move on.

16 DR. MULLIGAN: I'd like to come to the snake

17 oil issue. It seems that there's somewhat of an

18 inconsistency between your principles of consent and the

19 very low bar that I think you've represented to support

20 going ahead for a clinical trial. So, as I was looking at

21 your bullet points, you're saying a possible benefit is

22 desirable but not necessary. The clinical equipoise is a

23 sufficient metric. Risk/benefit is not really an issue in

24 the phase I.

25 If you're giving consent -- let's say we all

1 know clinical trials that we think are not very interesting
2 and let's say that there's one that manages to pass that
3 very low bar, who is then appropriate to give consent to
4 the patient? One person, for instance, might say in his
5 consent this is a pretty bad trial, do you want to do this?
6 Most people think this is not going to work. It's really
7 kind of ridiculous. As opposed to the investigator who
8 says, well, this has a chance, et cetera, et cetera.
9 I raise this because it forces you, I would
10 think to have to deal with what's necessary in terms of
11 preclinical information. As you set the bar lower and
12 lower, then the consent I would think becomes more and more
13 an issue about to what extent you have to get across that
14 there's great difference in thinking about whether there's
15 enough science to support going ahead.
16 DR. SUGARMAN: I think one of the emphases is
17 that in proposing these criteria for the first in humans
18 kind of trials, bench to bedside kind of argument, is that
19 it's safe. Safety is the key as number one, that we have
20 as much information as we have about safety preclinically
21 that we know it's okay to try this or we think it's okay to
22 try it. We're not going to know until we try it in folks.
23 So, the bar is really high on not hurting people in the
24 process that are willing.
25 The possibility of benefit is honest about

1 where we are with science, and that starts to become part
2 of the consent process.
3 You're right. I think the consent process does
4 play an important role here. It's arguably hard to do.
5 We're collaborating with some folks at Hopkins about trying
6 to enhance consent for phase I trials in oncology. We
7 recorded about 100 conversations with investigators talking
8 to patient subjects about enrolling in phase I. We're
9 trying to enhance that process not necessarily changing the
10 recruitment rates at all. We saw no nudge. But what we
11 see is greater satisfaction, greater understanding, and
12 greater knowledge about what's happening in the context of
13 this trial.
14 So, I do think the consent process is critical,
15 and whether it's appropriate for the investigator to give
16 the consent or the nay-saying research nurse, I don't know
17 who the right person is to do that in each case. But it's
18 something that a thorough look at the ethics of the
19 research would say don't just outline your consent
20 document, say this. Who's going to get consent? How are
21 people going to be approached? What kind of materials are
22 they going to be given? Just deliberate about what's the
23 most balanced way to give that information that's fair.
24 I don't know if I evaded your question or
25 answered it.

1 DR. MULLIGAN: You very effectively evaded it.
2 (Laughter.)
3 DR. SAUSVILLE: I have to interject. You
4 created sort of a polar situation by presuming that the
5 clinical investigator is basically going to talk it up and
6 another person, whether a research nurse or the next
7 investigator, is going to talk it down. Again, I speak as
8 someone who just yesterday participated in two informed
9 consents for a phase I trial.
10 You've got to be able to say to the patient
11 that, yes, as an investigator, I think this is a reasonable
12 idea, but you also have to say to the patient that it's
13 perfectly reasonable to consider no particular treatment
14 and to use palliative efforts to relieve symptoms as
15 potentially having no difference in outcome. So, maybe
16 that's part of an equipoise.
17 So, I don't think you have to have this -- what
18 I read into this -- a polarization of points of view. I
19 think to make the consenting process totally informed, you
20 have to open the possibility that supportive care and
21 specifically not going on this trial is a perfectly
22 reasonable thing.
23 DR. MULLIGAN: Yes. I'm attempting to really
24 get a better definition of how much effort ought to be put
25 into evaluating the science from the point of view of going

1 ahead. I guess I'm saying that it seems like reasonably
2 the bar is set very low, but that there's a very arbitrary
3 point of view put to the patient, almost by definition,
4 let's say, because generally there are different points of
5 view, and the physician that's carrying out the trial
6 clearly believes in what they're doing and think that there
7 is some hope. But should the consent process somehow take
8 into account, say, the prevailing scientific view? Often
9 there is a prevailing scientific view that something is not
10 so hot.

11 DR. SAUSVILLE: I would submit that both points
12 of view need to be offered.

13 DR. SALOMON: Okay, in the back.

14 DR. FREED: Curt Freed, University of Colorado.

15 With the issue of outcome and if we're talking
16 about cell therapy, there are two issues that always have
17 to be addressed I think. First of all is has the cell done
18 what you think it was going to do, and second is has the
19 patient had a response. Because we're dealing with cells,
20 not drugs, the cell has to go in, survive, and flourish.
21 In our neural transplants for Parkinson's that variable has
22 been less variable than the patient outcome in which you
23 have a whole different set of factors. What is Parkinson's
24 disease? What is the range of actual underlying disorders
25 that manifest itself as Parkinson's across age range and so

1 forth? So, we have two distinct parameters: one, cell
2 survival; second, patient response. Both have to be
3 considered as two endpoints, not a single endpoint I would
4 say.
5 DR. SALOMON: Dr. Freeman and then Dr.
6 Kurtzberg.
7 DR. FREEMAN: I think there is one set of
8 special challenges for stem cells, in particular, when it
9 comes to trials. Although these are pharmaceuticals, they
10 are pharmaceuticals that are delivered surgically, which
11 brings up a whole set of special considerations which fall
12 outside of the realm of standard pharmaceutical trial
13 designs.
14 First of all, how do you adequately control for
15 your outcome, and what are the ethics of placebo-controlled
16 surgical trials?
17 Secondly, the costs of a surgical trial are
18 prohibitive in comparison to pharmaceutical trials which,
19 first of all, will favor product-driven trials over, say,
20 organ transplant, fetal tissue, or kidney transplants.
21 Anything that's product-driven in a surgical trial will,
22 therefore, be favored by that.
23 Secondly, it influences the size of the trial
24 and therefore limits your ability to power adequately on
25 primary clinical endpoints which is, of course, what is

1 favored in a trial design and lends itself more to
2 surrogate marker endpoints and radiologic outcomes.
3 Finally, from a trialism point of view, many
4 surgeons are not adequately trained or prepared
5 psychologically to deal with placebo-controlled trials.
6 Then from the surgeon's point of view, surgeons generally
7 get paid to do surgical procedures which will limit their
8 equipoise.
9 (Laughter.)
10 DR. FREEMAN: And finally, if they do a
11 placebo-controlled trial where you bill for the active arm
12 by you don't for the placebo arm, of course, that's
13 unblinding, and not many surgeons are willing to operate on
14 people in either arm for free. So, there's a whole
15 constraint of issues which really are specific to surgical
16 trials that really need to be thought out carefully.
17 DR. SUGARMAN: I appreciate that. Also, any
18 other comments people have along the way, I'm happy to
19 revise and analyze and change, and throw things at me, as
20 long as they're words. I would love your thoughts and
21 input on this as we think forward about this together.
22 DR. KURTZBERG: I don't think we've really
23 addressed the topic of resource allocations. I think
24 there's really a societal disconnect between what we want
25 to do as scientists and tests and what we have the

1 resources to look at. I think the FDA is going to be in a
2 unique position of being able to control, in a way, how
3 some resources might be allocated. I think that
4 responsibility has to be discussed and that systems and
5 committees and whatever have to be put in place so that
6 it's done fairly.
7 I don't think our society realizes they need to
8 do research in certain illnesses until somebody they know
9 gets sick, and then it's too late. Then the resources get
10 emotionally allocated instead of objectively allocated. I
11 think this agency has the power, if you will, to maybe
12 change that and put direction on how we approach some of
13 these diseases and how we allocate the resources.
14 DR. SALOMON: I think that was a very good
15 discussion. I think it's probably the first time since
16 I've been associated with FDA advisory committees that
17 we've had that much productive discussion on an ethics
18 thing --
19 (Laughter.)
20 DR. SALOMON: -- which may be a statement on
21 previous committees or previous presenters. But I mean
22 that as a sincere form of compliment.
23 So, what I came away with as sort of key issues
24 that came out of this discussion from the group is that
25 clearly this idea of outcome measures, measurability and

1 meaningfulness, is really a big thing. Picking up on Dr.
2 Kurtzberg's last comment, I think bringing it around, these
3 are issues for the FDA -- and for the NIH I might add -- in
4 terms of where funding priorities are put in order to
5 address which diseases we should start with, what resource
6 allocation is going to be done and make sure that we do
7 address the issue that it's not done emotionally.
8 The second thing is clearly I think everybody
9 is sensitive to informed consent. I didn't cut that
10 conversation off even though it was a little more generic
11 informed consent than neural stem cell informed consent.
12 If people want to get back to talking about informed
13 consent, I would ask you only to integrate some specific
14 issues related to neural stem cell transplantation if the
15 topic should come back up again.
16 Jay, given your status --
17 DR. SIEGEL: Thank you. I know you want to
18 move on, but I just want to quickly say for the sake of Dr.
19 Freeman, Sugarman, anyone else who might be interested, in
20 1994 and 1995, the FDA did have a two-day advisory
21 committee discussion on issues in intraventricular
22 therapies of neurological disorders, many of the same
23 disorders, and many of the treatments involving placement
24 of catheters, reservoirs, and pumps, and issues of sham
25 surgery and issues of ethics in dementing conditions.

1 There was some very fruitful discussion there, and those
2 interested in the issue may want to check back on that
3 record.
4 DR. SALOMON: Well, given a technical glitch,
5 maybe we've got a minute here. Go ahead.
6 DR. TROJANOWSKI: Could I ask for one more
7 thing to be put on the future agenda of these discussions?
8 That is the role of media in reporting science.
9 I've been appalled at their reluctance to
10 declare conflicts of interest, what their hidden agenda
11 might be in some of their press reports. The St. Jude's
12 Hospital report of potential contamination of HIV fragments
13 in a gene therapy trial that then were retracted five days
14 later I think did enormous damage to the public's
15 confidence in this.
16 Although at the University of Pennsylvania
17 we're very sensitized to the conflicts of interest with Jim
18 Wilson -- I'm not part of his institute at all.
19 (Laughter.)
20 DR. TROJANOWSKI: I'm part of the institution,
21 but not the IHGT. I think there were accusations in the
22 press that were completely unethical and, for reasons that
23 I don't understand, they were allowed to go forward.
24 I don't know if members of the press are here,
25 but I hope they do pay attention to what Dr. Sugarman said,

1 and I would hope that -- this is a free country. You can
2 say whatever you want, of course, but I think you can say
3 things in print that have an enormously damaging effect and
4 there's no accountability.
5 DR. SALOMON: I think one comment, though, that
6 needs to be followed up on that is that, yes, the press has
7 to be responsible and irresponsible reporting -- you gave a
8 good example of it.
9 But the other side of it was, it was really a
10 black eye. There was not just Dr. Wilson, but there were
11 several prominent gene therapists with ridiculous conflicts
12 of interest that could have been dealt with in a proper
13 way. There's nothing wrong with a conflict of interest if
14 it's up front. So, I think it's definitely a two-edged
15 sword here.
16 Our next speaker -- and then we'll take a break
17 -- is Dr. Fred Gage, Rusty Gage, giving us an overview on
18 animal models.
19 DR GAGE: So, I took the charge pretty
20 literally, and when you sent me that list of questions, I
21 took the questions out of the text and pasted them onto
22 slides and put my answers onto them so we could keep it
23 fairly focused.
24 I wanted to start by harping again on this
25 concept of the definition of stem cells and the fact that

1 there are no prospective markers for stem cells that would
2 allow you to go in and look at a cell and say, that is a
3 stem cell. Rather, what we have are populations of cells.
4 At best we can say within that population we believe that
5 there is a cell that's a stem cell.
6 The other point is that there really are two
7 different definitions that we've been talking about here.
8 One is in vitro definitions which involve saying that the
9 cell has self-renewing properties and is multipotent. In
10 most cases, at best what one will do is say that twice
11 self-renewal -- you'll say that one population can be
12 derived from a single cell, expanded, differentiated into
13 multiple cells. In that population, again you can take one
14 cell out of it and give rise to that same property of self-
15 renewal and multipotentiality again. But all you've really
16 demonstrated there is twice. You've said self-renewal
17 twice. What we really invoke with self-renewal is
18 perpetual self-renewal or for long periods of time.
19 In terms of multipotency, certainly in the
20 nervous system, most people are satisfied with looking at
21 three markers: some marker of glia, some marker of
22 oligodendrocyte, and usually an early neural marker. And
23 then they say that they have a stem cell and maybe a
24 complete stem cell population.
25 The other definition related here is the in

1 vivo definition, and we really have no definition of an in
2 vivo model of stem cells in the central nervous system
3 because of the absence of ability for self-renewing
4 properties.
5 So, one of the things that this gives rise to
6 then in this little schematic is the idea that of
7 totipotency, pluripotency, and the general multipotency.
8 As we go forward, rather than be pessimistic about this,
9 I'd like to be optimistic that, in fact, while we don't
10 know very much, we have the tools presently to begin
11 answering these questions very systematically. It's the
12 answer to these questions which will help us progress
13 further rather than throwing up our hands without
14 knowledge.
15 So, I think one of the important questions that
16 we have to address that has already been brought up is the
17 relationship that exists between what we're calling stem
18 cells and progenitor cells and whether or not there is a
19 progressive unilinear progression of differentiation or
20 whether or not there's a capacity for dedifferentiation or
21 reversion back and forth between the cell types. That I
22 think will be a very important part for talking and
23 discussing things.
24 One of the ways in which we've tried to address
25 this in our lab by calling cells stem cells -- I'm going to

1 give you demonstrations mostly from our lab, but also
2 showing you the down side of what we're doing.
3 We take adult tissue. We isolate the tissue on
4 various isolation procedures, and we use retroviruses which
5 have the capacity for single integration into individual
6 cells as a marker. We then take colonies and clone the
7 individual cells manually. But after we have what we
8 believe to be a clonal population derived from presumably
9 one cell, we do Southern Blots to make sure that that
10 single integration -- it defines genetically that every
11 cell in that culture that expanded from the retroviral
12 integration is derived from a common cell.
13 You can then use another retrovirus with
14 another marker to then test for self-renewal, but then
15 again, at the end of the day, all you've got is being able
16 to say that within that population there are stem cells and
17 within that population there is some evidence that at least
18 some of them have self-renewing properties. So, even under
19 these very rigorous conditions, we do not have prospective
20 markers that allow us to identify the individual cell.
21 However, if you can then use these procedures
22 to isolate repeatedly, using the same procedures, cells and
23 ask about the potentially different cells, here's an
24 example of what you can get. So, here's the parent
25 population and these are 6 different clones derived from

1 the bulk population that are then differentiated. While
2 every one of the clones can give rise to a neuron -- a
3 quote/unquote neuron -- an astrocyte and oligodendrocyte,
4 the ratio that each one of the clones all derive from a
5 single population have in differentiating down different
6 lineages is quite variable. But I think the good news is
7 -- one has to look for the good news in the noise -- that
8 each of the clonal populations is multipotent.
9 Now, just to give you an idea of one of the
10 things we're trying to think about in terms of these
11 concepts of defining what is a stem cell, this is a film of
12 an individual cell in a culture dish. So, here's a cell
13 moving around. It's under conditions. This cell can
14 divide. So, is that a self-renewing property or is this a
15 committed cell?
16 Well, if we look carefully, you can see that
17 first this cell rounds up and divides, and now this cell
18 rounds up and divides. These cells then can each
19 individually divide and differentiate. This is over a
20 period of 2 or 3 days, and if you monitor these cells over
21 longer periods of time, this population will grow up to be
22 a multipotent stem cell population. But even by tracking
23 the cells individually in the culture, without prospective
24 markers, we can't identify which one of these cells, other
25 than the very first cell, was in fact a stem cell.

1 So, the next question of the questions that
2 were asked was production characteristics. What are the
3 objective, qualitative differences that distinguish stem
4 cells derived from different sources, namely, embryonic,
5 fetal, adult, and how might these distinctions impact
6 safety and potential efficacy?
7 Well, to date there has been no head-up
8 comparison between cells of different origin, species, or
9 age, in part because different methods have been used to
10 isolate and expand and differentiate the cells. But this I
11 think is certainly a challenge and one that should be met.
12 Examples of the differences that are existing
13 in the culture dishes today are, we've heard, EGF versus
14 FGF.
15 Some people grow cells as monolayers. Others
16 grow them as spheres.
17 We don't know the difference between an
18 embryonically defined cell and an adult cell.
19 How many passages does it take to change the
20 cell and how important is that?
21 We hear about cells that are immortalized.
22 Actually rationally immortalized. They're meant to
23 immortalize the stem cells. What's the difference between
24 that cell and a non-immortalized cell?
25 Then, of course, rat, mouse, and human. Many

1 of the discussions we have are interchangeably using the
2 results that we obtain from one species and generalizing
3 both in behavior experimental models and in the behavior of
4 the cells.
5 Then again, the source of the cells.
6 So, the bottom line is, the answer to the
7 question is it hasn't been done, but these are the head-up
8 kinds of experiments that certainly could be done.
9 So, we assume in some sense that there's a stem
10 cell here that's dividing down into a progenitor cell, one
11 of the other lineages and that these are some sort of
12 linear track.
13 I want to show you a recent observation by
14 Sophia Calimari using a marker that you heard from Steve
15 Goldman earlier. This is a population of cells all derived
16 from a single cell in a culture dish, and they're moving
17 around. It's very hard to tell from this who is the stem
18 cell or who is the progenitor cell and who is undergoing
19 cell division at any one particular time.
20 If we infect the same population with a marker
21 that uses the alpha tubulin promoter driving GFP, you can
22 see that in that culture, here's the cell with nice
23 branches and it's a committed neural progenitor cell, as
24 we've used these terminologies. But if we look closely,
25 follow that cell, it rounds up and undergoes cell division.

1 So, here we have a situation where we're using markers to
2 define cells as committed down a neural lineage. That same
3 cell -- it's not fusing back together. Don't worry.
4 (Laughter.)
5 DR GAGE: That cell can actually undergo cell
6 division and give rise to a cell at the same state of
7 lineage, showing here that it's expressing the alpha
8 tubulin promoter.
9 So, in addition to the propagation of cells in
10 the most primitive states, it looks like at different
11 stages along the commitment, cells can be expanded and
12 propagated. I think this is going to be a valuable tool
13 and a piece of information for us to consider in this
14 process of isolating functional cells.
15 Similarly, will the stem cell source influence
16 the robustness, the durability, the longevity of an
17 intended therapeutic response following transplantation?
18 Well, yes.
19 So, how do we compare, measure, and follow
20 them? Well, these are just a list of some of the things
21 that we need to do with our cells when we consider -- and I
22 think that the spirit of the question was how can we begin
23 to characterize cells in some systematic way so that
24 ultimately we can make comparisons between the different
25 types of cells that people are bringing forward as being a

1 potential cell source for a therapeutic intervention.
2 Certainly issues related to isolation efficiency, what
3 factors are used to propagate the cells. Do we need serum
4 for this? Most of these are fairly straightforward, but
5 what again it comes back to is making comparisons between
6 all of these variables between different cell types.
7 One of the methods that we've used to isolate
8 these cells as a method of isolating the cells from the
9 adult nervous system and enriched for certain cells has
10 allowed us to look at cells directly isolated from the
11 tissue in the absence of propagating them in conditions of
12 mitogens. One of the concerns that we all have in this
13 field is when you propagate cells for indefinite periods of
14 time or for any period of time with mitogens as potent as
15 EGF and FGF, are you changing the potentiality of the
16 cells.
17 So, we've developed a procedure where you can
18 take adult tissue and isolate it on a Percoll gradient. We
19 have a pretty good idea of the density of the cells that we
20 propagated. Then when we isolate those cells, we can ask
21 what percentage of those cells that are freshly isolated
22 from the brain are able to give rise to neurons in a dish.
23 As Steve Goldman told you, the hippocampus has this
24 potential for neurogenesis, as does the subventricular
25 zone. Here with 0 passages and induced differentiation, we

1 do in fact get neurogenesis out of these cells.
2 But if we look at other areas of the brain,
3 like the cortex where no neurogenesis normally occurs in
4 the adult, or even the optic nerve, which is extended out
5 into the rostral brain cavity, there is, we know, no
6 neurogenesis occurring in those cells. If we exposed them
7 for merely 3 days to FGF in vitro, we can get those cells
8 to begin to express neuronal markers. So, here's a
9 population of cells existing in a non-neurogenic site, when
10 exposed to FGF, can in fact give rise to cells.
11 We believe that this, in fact, suggests that
12 either there are quiescent populations of cells -- this is
13 one interpretation -- that exists within the adult brain
14 that can be activated by these mitogens or, alternatively,
15 this idea of reprogramming emerges again. Do mitogens
16 reprogram the adult or any kind of stem cell to broaden its
17 propagated capacity?
18 So, here's the question again. Please describe
19 specific markers used in the isolation, characterization of
20 stem cell preparations.
21 Well, if you read through the literature, some
22 people will say if a cell is responsive to FGF or EGF, it's
23 a stem cell. Others will say that if it forms a sphere, a
24 sphere-forming cell is a stem cell. These are in the
25 published literature. The ability to passage a cell we've

1 read may be used. Again, I'm being a little bit tough
2 here, but I think that the concept of using stem cells is
3 being used in a much broader sense than original
4 assumptions were, and I think we should return to those to
5 some extent.
6 Nestin is another marker that has been used as
7 a marker of stem cells.
8 Then in terms of differentiation, as I said,
9 usually three markers are adequate.
10 I argue that in order for you to say that a
11 single cell can give rise to a neuron, as several of the
12 talks today have already documented, we need to be more
13 rigorous in our definition of whether or not the cells are
14 in fact neuronal. So, markers needed for functional
15 characterization would be the ability of the cells to
16 actually myelinate in vitro. Can they form myelin and
17 myelinate axons? Do they form dendrites, axons, and
18 synapses? Do they make neurotransmitters, and are they
19 electrophysiologically active? These are all methods that
20 are presently available. So, it's not that difficult.
21 Here is a stem cell derived from a single cell
22 infected with GFP so you can mark it. This is a double
23 labeling with synaptophysin, a marker for synapses. MAP-2
24 is a dendritic marker. What you can see here is that
25 synapses are formed on the surface of these stem cells.

1 If you look at the action from this neuron
2 derived from stem cells -- and this again are the synapses,
3 and this is the dendrite from another cell, not this one --
4 we can say that the synapses from this cell are making
5 synaptic contacts on the dendrite of another cell. So,
6 merely by using antibody markers and collocalization, we
7 can begin to get the features which give us a little more
8 confidence that the cells are differentiating down
9 different lineages.
10 Using really pretty standard
11 electrophysiological characteristics now by taking those
12 same cells, voltage clamping so you're looking only at
13 current, you can see that a normal primary neuron in
14 culture gives rise to EPSPs -- IPSPs and stem cells or
15 cells derived from a single cell that differentiated down
16 lineages also can give rise to similar kinds of IPSPs and
17 EPSPs that are blocked by GABA and blocked by glutamatergic
18 stimulation.
19 As has been reported before by some of the
20 other speakers, one of the things we'd like to know is can
21 these neurons actually spontaneously fire an action
22 potential as a neuron. So, if you put them on a current
23 clamp and just ask whether or not the cell has the
24 capacity, in the absence of stimulating with potassium, but
25 does it have the potential for generating an action

1 potential, you can measure these properties in the cell and
2 gain confidence that the cells have actually differentiated
3 or can differentiate down fully functional neurons.
4 Are there selected genetic or biochemical
5 markers that accurately indicate the differentiation status
6 of stem cell preparations or that assure the acquisition of
7 correct functional therapeutic -- and this is a tough one.
8 Not from certainly any in vitro monitoring that is
9 available.
10 If in the standard cell preparation, all cells
11 can give rise to a defined therapeutic cell phenotype in
12 vitro and in vivo, then I think you're feeling a little bit
13 more comfortable from your animal models that it might work
14 in a patient.
15 If the cell implant can reverse the functional
16 deficits reliably in an animal model, I think that's
17 another evidence of support.
18 In addition, if the functional recovery
19 observed in the animal model can be causally linked to the
20 phenotypic differentiation of the graft itself, then I
21 think you're close to being able to say that that cell that
22 you're grafting is differentiating into a phenotype that's
23 responsible for the functional outcome that you're looking
24 at.
25 If you use these sorts of criteria in the

1 animal models, at least it begins to say that, well, we
2 believe that it's not the cell secreting a factor or dying
3 and it's that response that's causing the behavioral
4 result.
5 Alternatively, have specific markers been
6 identified that could relatively predict transplant failure
7 or indicate the likelihood of untoward events to occur,
8 such as inappropriate -- yes. I think that there are
9 things we can look at in vitro and in vivo in animal models
10 that are pretty bad predictors. One would be that the cell
11 that you're looking at can't differentiate down the
12 appropriate lineages either in vitro of in vivo. If you
13 can't get your cell to differentiate to a large extent with
14 some control down the appropriate lineages both in vitro
15 and in vivo, that does not bode well for a cell.
16 Cannot be induced for all the cells to stop
17 dividing. If in vitro or in vivo, there is any evidence
18 that these cells are continuing to divide in culture, that
19 all of your differentiation schemes -- you may have, well,
20 just 1 or 2 cells are undergoing mitosis later on -- I
21 think that that is a marker that we need to be absolutely
22 aware of. Cells need to be karyotyped to make sure that
23 there are no abnormalities, and if there are abnormalities,
24 then this needs to be evaluated.
25 And cells die following differentiation in

1 vitro or in vivo. I don't mean just that they can
2 differentiate appropriately, but some will survive and then
3 as soon as they differentiate, they die.
4 Here's some of our own data of our passaged
5 clones. These are all clonal lines that you saw before.
6 After 30 passages, we karyotyped our cells, and while 7 of
7 the 9 had basically normal karyotype, we had two abnormal
8 cells that were out 40 or 50 passages. When we grafted
9 this cell, it actually acted just like a normal cell, but
10 when we grafted this cell, we got tumors. So, I think
11 that's a beginning of at least a minimal amount of
12 characterization that one needs to do for your cells and we
13 should see that for every cell that is used.
14 So, preclinical. To what degree do particular
15 experimental animal models mimic disease conditions in the
16 human and to what extent are animal models predictive in
17 terms of evaluating safety prior to initiating studies in
18 humans?
19 I'd just like to be a little pedantic here and
20 say that there are three different kinds of animal models
21 that many of us who work in animal models think about and
22 what you can expect out of an animal model.
23 There are homologous animal models, and that is
24 a model that has common etiology to that which exists in
25 the human. The pathology is the same and the behavioral

1 outcome the same. It's not even a model. It's the animal
2 has the same disease as humans. Treating that will be a
3 lot more informative than any other model. But most of the
4 models that we actually look at are isomorphic models.
5 While the pathology or the behavior may be the same, the
6 actual cause of it, the initiating cause of it is unknown
7 or is induced by the experimenter. Most of our models,
8 even our transgenic animals that we're generating now that
9 have over-expression of certain genotypes, are really not
10 homologous to the human form of these diseases. Given
11 that's the case, one needs to be aware of that whenever one
12 is modeling these and looking at behavioral outcomes and
13 anticipating moving on for a clinical application.
14 There are obviously certain models that have a
15 greater relationship or closer to the homologous sort of
16 thing. Having said that and being critical of isomorphic
17 models, there are a lot of correlation models that are
18 actually quite good. There are a lot of in vitro models.
19 If you're modeling for one element of the human disease,
20 then in some cases in vitro models are okay if the only
21 thing you're looking for is to reverse that one thing. So,
22 being aware of the model that's being used.
23 This is my list of all the diseases. When I
24 looked through the literature to see what people have begun
25 modeling for and spoken about in terms of cell therapy --

1 this isn't something I would even think about -- there's an
2 amazing number of diseases that people are attributing to
3 as potential models for cell therapy at present. They
4 don't always say that they're going to be using stem cells,
5 but it's striking.
6 So, then they asked me, so what are the
7 relative strengths and weaknesses of any animal models
8 described in your presentation? I'll talk about a few
9 things.
10 Most of the models we deal with are isomorphic.
11 They're not real homologous models. The timing of implant
12 is almost never taken into consideration relative to the
13 disease. The size of the target is minuscule relative to
14 the clinical problem. The number of cells that we're
15 grafting have nothing to do anywhere near the cell number
16 that we're going to be using in human. Even when we go on
17 to primates, the scale-up is quite big.
18 We work to reduce variability. That's why we
19 use animal models. It allows us to make extrapolations a
20 little bit better.
21 Unfortunately, little attention is given to in
22 vivo measures to monitor the survival function.
23 This relates to what Mark just said, which is
24 we need to get working on and perhaps demanding the
25 development of imaging techniques to monitor these cells if

1 we're going to go forward in an aggressive way.

2 So, in the modeling, a few slides showing
3 grafting or transplantation of cells. This just relates to
4 the idea that niche is important. If we take a clonal
5 population of cells and graft them into the hippocampus,
6 what you'll see is the same cells, depending upon where
7 they end up in the granule cell layer, will differentiate
8 into different cell types. So, if you get them just in the
9 innergranular zone, you can get cells that differentiate
10 into neurons, that are calbindin positive, they receive
11 synapses, and by all criteria are indistinguishable from
12 the other granule cells.

13 If, however, you damage the granule cells and
14 you elicit a glial response, then those same cells would
15 differentiate into astrocytes. Tens of microns away or,
16 say, 100 microns away, the exact same cells are going to
17 respond differently depending upon the cues that are
18 present in the local environment. So, it's not just the
19 cell type. It is the microenvironment.

20 I'm not going to talk about Parkinson's
21 disease, but I hope that some of the people in the audience
22 who have a lot of experience will and relate the successes
23 that have been used with fetal tissue, where there's
24 defined populations of cells, albeit not enough cells, and
25 their hopes for stem cells or progenitor cells to be an

1 expanded population and the reality of what really is
2 expected out of a stem cell. I feel confident in saying at
3 present no propagated cell, CNS propagated cell, CNS-
4 derived propagated cell, that can be even closely
5 considered to be a stem cell gives anywhere near the
6 functional effects that one sees with fetal tissue even in
7 any of the most simple experimental models. At this point,
8 fetal tissue is the benchmark for both the experimental
9 model in Parkinson's disease, as well as the clinical
10 model, and the stem cell work is not even close.
11 A quasi-homologous model might be -- I think
12 eye disease has a lot of opportunities here because there
13 are a lot of animal models with genetic disorders where
14 there are selected gene mutations and retinal degenerations
15 associated with this. I wanted to show some pictures of
16 some grafts that Mike Young has done with our cells and
17 injecting in the Royal College of Surgeons rat which is a
18 rat that has degeneration of the retina.
19 If you infect the cells with GFP so they're
20 fluorescent and you select a cell that stays on once it's
21 grafted -- and this is really a selection process rather
22 than knowing what we're doing in terms of where the virus
23 inserts. We can get cells that stay on after
24 differentiation, months afterwards.
25 So, here's the degenerating eye and these green

1 cells will target to the outer region. They can send
2 processes into the synaptic region. So, this is the neuron
3 in the middle layer, projecting processes into these
4 synaptic regions. If we look closely at that, we can see
5 that in some cases synapses are being formed in the
6 processes, and they line up in an appropriate way.
7 These are being examined for functional
8 characteristics, but these are cells derived from the adult
9 hippocampus that, when grafted into a degenerating eye,
10 appear to target to these areas. They don't take on the
11 full, let's say, phenotypic characteristics of the same eye
12 cells in their present state, and we don't know if that's a
13 function of the fact that they're from the hippocampus or
14 whether or not the adult brain or damaged brain doesn't
15 have all the cues necessary to drive the cells down
16 further.
17 This is just an observation that is consistent
18 with the idea that the cells are responding appropriately
19 to the damaged cues. In addition to seeing some of the
20 cells fall into the ganglion cell area, we actually see
21 axons coming out of the ganglia and into the optic nerve
22 that are GFP positive, and you have growth counts that tip
23 as they lead into the optic nerve head.
24 So, while the cells may have the will to
25 differentiate and the degenerating target may provide some

1 of the clues, the whole process of neural regeneration and
2 recapitulation of normal circuitry down to a functional
3 neuron is going to require an orchestration of things that
4 are not just induced in vitro, but also requiring the host
5 as well.
6 A few cautionary notes about the in vivo
7 effects. So, when modeling diseases like epilepsy, which
8 is thought of as a target for cell therapy, if you pulse a
9 normal animal with bromodeoxyuridine, these are the
10 dividing cells that normally exist. If you give an animal
11 pilocarpine, as an experimental animal of epilepsy, you get
12 a massive proliferation of these progenitor cells. Many of
13 these cells migrate out into the granule cell layer and
14 form ectopic, inappropriate granule cells. So, anytime
15 when thinking about transplanting cells into an epileptic
16 host, one has to think about the fact that already in that
17 damaged tissue the local circuits are recruiting these
18 cells into aberrant locations. This is a slide from Jack
19 Parent and Dan Lowenstein in their earlier work.
20 I put this in recently because I wanted to say
21 what are some successes and what are some models that one
22 could at least think about in terms of cell therapy and the
23 rationales behind it. The successes we'll hear about in
24 the next couple of speakers.
25 We'd like to keep in mind defined populations

1 of cells and defined phenotypes. Here I'm not talking so
2 much about the cell itself as the fact that the cells can
3 be delivery vehicles for factors. This for me is a real
4 lesson that any cell that we put in there in and of itself
5 may not be acting for its intrinsic property as a cell, but
6 may be a vehicle for delivery. What's important then is to
7 understand the nature of those signals that are being
8 secreted.
9 So, in the human and all mammals, there's a
10 cholinergic system in the basal forebrain projecting into
11 the hippocampus in the septal area, and we know that there
12 are many trophins that are effective, but uniquely nerve
13 growth factor will support the survival of these
14 cholinergic neurons in aging animals and in damage
15 situations. It's a potent rescue factor but it also has a
16 lot of side effects that have been revealed as this
17 molecule would supposedly move forward into some sort of
18 clinical application because of its diffuse effects
19 throughout the system.
20 But one of the lessons we learned was that if
21 you take an aged animal and stained for markers which
22 identify what the cells are, these cholinergic neurons, we
23 concluded originally that there was a loss of cells in
24 these regions. When grafted cells that over-express nerve
25 growth factor into these target areas, we ended up getting

1 more cholinergic neurons in this region.

2 Now, one can conclude from that that if you
3 were putting in progenitor cells, that in fact some of the
4 grafted cells are differentiating into new cholinergic
5 neurons. In fact, that's not what's happening here. We're
6 up-regulating the expression of phenotypic markers on the
7 surface of the cells that were basically blank before, and
8 this could easily be interpreted as some sort of cellular
9 replacement when, in fact, it's just genetic up-regulation
10 of what's going on.

11 The other point that we face is this difference
12 of size. So, this is rat brain and this is a monkey brain.
13 Depending upon the monkey brain, the human brain is going
14 to be at least four times, if not more, and parametologists
15 in the audience will discuss this with us. But this
16 difference of size is not one that I think is easily
17 scaleable, especially when we're talking about cell therapy
18 and cell replacement.

19 One of the things that we've done with Mark
20 Tuszynski is to look in the primate and see how many cells
21 do you really have to do to mimic the effects that you see
22 in a rat in a monkey. I think that those are the kinds of
23 questions in the stem cell field which would be important.

24 The difficult thing, though, in cells like this
25 where they form an aggregate or in fetal tissue where they

1 form an aggregate and you can use various tracers like MRI
2 or PET scanning to identify the graft, this is going to be
3 significantly more difficult with progenitor cells and stem
4 cells because unlike these cells, they migrate very, very
5 far, and most of the human imaging techniques are not
6 sensitive enough to really identify where those cells are
7 going. So, this I think is once again a real challenge for
8 us.
9 So, embedded in this also, as I restate this,
10 is that we really have to consider this issue of
11 immunology, if we're harvesting our cells from autologous
12 tissues. Whereas we can take some cells from the animals
13 themselves and transplant them back, I believe it will be a
14 more difficult situation to take and perhaps an ethical
15 issue we could talk about of harvesting biopsy material
16 from humans and then growing those cells and
17 retransplanting those cells and the efficacy of doing
18 something like that.
19 Getting to the last of these questions, to what
20 extent do animal models facilitate the evaluation of
21 cellular differentiation and integration following
22 transplantation of stem cells?
23 Well, I come back to the point that rarely, if
24 ever, are we really grafting stem cells. In the central
25 nervous system, these have not been the studies. Most of

1 the cells that have gone in are progenitor cells. They're
2 not self-renewing. It's not known if the cells that do
3 survive -- in fact, migrate -- are in fact stem cells of
4 the population that go through, and many would argue that
5 it really is a committed cell that's actually the one
6 that's surviving and migrating.
7 An important question is how many species -- I
8 think this is one of the questions that is related here to
9 animal models -- and are monkey trials essential for moving
10 forward in stem cell applications?
11 There are mouse models in some cases that could
12 thought to be more homologous to the human disease than the
13 monkey, obviously, in terms of transgenic animals, but the
14 monkey provides many of the size issues that one would like
15 to have addressed for some of these trials. So,
16 nevertheless, animal models are absolutely essential.
17 And tracking. So, describe techniques
18 currently available.
19 Well, fluorodopa is being used. Olle Lindvall
20 came out with a very nice D-2 receptor occupancy, PET
21 imaging study with fetal tissue grafts giving some
22 functional index of the graft in Parkinson's patients. I
23 think we can begin to look. There are studies now being
24 looked at for BrdU PET to see whether or not there are
25 dividing cells that exist within the grafts or in the brain

1 normally, and then for some of these other grafts, if they
2 don't migrate too much, one can begin to look at MRI.
3 Highlight significant hurdles that need to be
4 overcome.
5 We need markers. We need markers for the
6 transplant of cells. We need markers that don't down-
7 regulate when they differentiate, that are easy to assay in
8 sections, and better, can be monitored in vivo and can be
9 monitored in humans to follow the cells in vivo. Develop
10 some human markers that can be put inside these stem cells
11 so that you can monitor where they are and where they go in
12 vivo.
13 You asked if there are any behavioral markers
14 in animal models. There is a ton of them. You can measure
15 every behavioral response that you want in an animal model
16 to look at safety and efficacy, but I think what's missing
17 in many cases are the appropriate controls. For cell
18 grafting, I think this is really important, especially in
19 stem cells. What is the control that you're using as a
20 control for your stem cell to say that your cell is doing
21 something different than damage or any other cell that
22 would be used. I think this issue of control and animal
23 control is a very important one.
24 Define the extent to which animal model testing
25 is useful for evaluating the impact of local environment on

1 phenotypic expression.

2 Well, to the extent that one can make a
3 homologous model of a human condition with the same
4 pathology, then I think that's one of the considerations.
5 To the extent that you have an isomorphic model that mimics
6 the local pathology of the human disease, then I think
7 you're beginning to address these issues of
8 microenvironment. But if your models have no relationship
9 to the anatomical microenvironment that's going on in the
10 human pathology, then you really can't make any prediction
11 of the fate of the cells in the human condition. So,
12 animal models, for stem cells in particular, should take
13 into consideration trying to mimic either isomorphically or
14 homologously the pathological changes.

15 To the extent that human cells behave the same
16 in animal models as they do in human disease, it's
17 obviously the gold standard. This is what you want to do.

18 I'm done. I just want to remind, as did Steve,
19 that there is neurogenesis and cell proliferation going on
20 in the adult nervous system. There's lots of it, and it's
21 not just that there's neurogenesis going on in these
22 regions of the hippocampus and the subventricular zone,
23 there are cells dividing in the cortex, there are cells
24 dividing in the spinal cord. They're not necessarily
25 giving rise to neurons, but they are dividing.

1 This is just a list. You don't have to read
2 it, but I'll just go through it. This is a list of the
3 recent studies that have shown either factors or
4 environmental stimulation that can affect the endogenous
5 rate of self-proliferation within adult animals. So, EGF,
6 FGF, estrogen, serotonin, glutamate, enriched environment,
7 exercise, learning, stress, glucocorticoids, adrenalectomy,
8 stroke, epilepsy, and aging all have shown to have effects
9 on the endogenous proliferation of certainly the
10 hippocampal neurogenesis, but many cells throughout the
11 brain. So, in any consideration of grafting cells, we need
12 to consider the fact that the environment can actually
13 influence the fate of our cells once they are grafted into
14 the host.
15 Thank you.
16 (Applause.)
17 DR. SALOMON: Well, it's getting to that part
18 of the afternoon where probably a break would be in order.
19 There was also a lot of stuff put on the table by Dr. Gage
20 here that I think is worthy of some discussion. The good
21 news is that the next three talks are also on animal
22 models. So, can we maybe have specific comments to things
23 that Dr. Gage brought up, and then we'll take a break.
24 DR. DINSMORE: Dr. Gage, you pointed out that
25 there's no one marker for any one type of these cells.

1 There does not need to be one type of marker. I think
2 anytime you put in a population of cells, you just have to
3 know repeatedly from time to time that you're putting in
4 the same population of cells. Therefore, your marker is
5 for whatever characterizes a population, but I don't know
6 of a single stem cell that doesn't have some marker that
7 says you're putting in a population of liver-like cells or
8 you're putting in a population of a certain type of
9 neuronal cell that responds to EGF or responds to bFGF.
10 There's always somewhat of characterizing those, and I
11 think the key factor is that you can do it repeatedly and
12 have a repeatable population which you can use. The only
13 way you can develop a therapy is if you have something you
14 can reproduce.
15 DR GAGE: Yes, I think the more completely you
16 can characterize the cells the better, and I don't think
17 you'd really argue that if you had a marker that could
18 identify the cell that was the therapeutic cell, that you'd
19 want to know what that marker is, and you'd be more
20 satisfied with an identified cell than a population that
21 contains perhaps the cell that you want. That's where we'd
22 like to go. It's one thing to say where we'd like to go
23 and where we are. I agree where we are is we work with
24 what we have.
25 DR. BECK: Tony Beck, Tissue Engineering

1 Sciences.

2 One of the overriding themes all morning has

3 been the fact that we have stem cells which are hard to

4 define, but clearly you want to have a starting population

5 that is consistent. The other theme seems to be that the

6 final residency of the cells often dictates their final

7 disposition.

8 Now, there's a technology that hasn't really

9 been addressed, and I address it to Dr. Gage and the panel

10 in general. The field of genomics has created a tremendous

11 potential for defining the state of the stem cells you're

12 starting with, and your slide on the karyotypic variation

13 on those clonally expanded cells shows that certainly

14 genomics or genetics plays a part.

15 Is it possible that some application of the

16 EST, the expressed sequence tags, which really define the

17 genes that are being expressed in those cells at that

18 moment -- is that something that potentially could be

19 applied to this quality control for stem cells?

20 DR GAGE: Yes, I think absolutely. I think

21 it's a very, very good point. Ihor Lemischka has begun, as

22 many other people have, to define the genetic profiles or

23 the genetic fingerprint of cells that are defined either

24 through FACS sorting -- I think that that's the starting

25 point. You have to have some reference point from which to

1 make other comparisons. So, I don't think that we all have
2 to pick one cell. I think that everybody should begin to
3 genetically profile each of their cell types, bank that
4 information from which we can begin to make comparisons.
5 So, I think that I couldn't agree with you more. I think
6 that's a terrific idea and certainly one that I hope the
7 NIH picks up and does in some sort of formal way.
8 DR. DINSMORE: One has to be very careful about
9 that. This whole talk about stem cells -- we do get down
10 to semantics of what a stem cell is, and we have to get
11 down to talking about a symmetric division versus an
12 asymmetric division. In any population of stem cells, you
13 have some cells that are potentially undergoing symmetric
14 divisions and others that are undergoing asymmetric
15 divisions. It's hard to characterize a population like
16 that as being uniform, and in many ways a true stem cell
17 population is not going to be uniform because there may be
18 some that are dividing symmetrically and others that are
19 dividing asymmetrically.
20 DR GAGE: Sure, I agree entirely. But I think
21 that the technology is prepared to match that challenge at
22 present, and by using strategies like laser capture where
23 you can identify individual cells, you will begin to bank
24 this information. I don't think it really matters what
25 cell you begin with, but we definitely need to begin to

1 identify them.

2 I think what you're saying is an important
3 point, and that is, are you going to do this in a
4 population of cells, are you going to do it in somehow
5 characterized cells maybe that are FACS sorted to be in a
6 certain stage of the cell cycle or based on some marker on
7 their surface? In any case, the more discretely you can
8 define the starting population, I think the more
9 informative that technology will be.

10 DR. SALOMON: I just wanted to make a point
11 before we keep going. If you're coming from the audience,
12 can you make sure that you identify yourself when you step
13 to the mike, because it's very hard for our
14 transcriptionist to do that.

15 DR. RAO: Rusty, through your talk, you
16 emphasized the fact that human cells may be different from
17 mouse and from rat, and you also talked about isomorphic
18 and autologous sort of transplants. Do you think that
19 there's some point that can be made about which would be a
20 better model based on the fact -- what should we be using?
21 Would rat into rat be a better model in your mind than
22 human into rat in that sense, given the differences?

23 DR GAGE: I think you've hit on a very
24 important point, and I think probably some of the
25 biological questions that are being asked are going to be

1 better asked in mouse/mouse, mouse/rat, but as it moves
2 forward to a clinical application where we're really asking
3 whether or not the cell that you think you've characterized
4 is going to be of clinical use, I can't see how you can
5 avoid using the exact material that you think is going to
6 be going into the human. And it better be prepared exactly
7 the same way that it was and expanded in the same number of
8 expansions and characterized in exactly the same way.
9 That doesn't mean that there isn't a
10 significant amount of information to be gained by using
11 mouse for understanding the biology. As you can see, we
12 know there's lots to learn there.
13 DR. TROJANOWSKI: Rusty, the adult stem cell
14 work is enormously exciting, and I think it's clear that
15 our concepts were wrong about neurogenesis. But is there
16 any evidence -- I don't think there's any published, but do
17 you know of any evidence of functional benefit from adult
18 progenitor cell differentiation into neurons or glia or
19 what have you? And if not, could one perhaps think of ways
20 to ramp up that neurogenesis so that one wouldn't have to
21 transplant but could exploit the patient's own stem cells
22 as replacement therapy? Is that inconceivable? I guess
23 nothing is inconceivable, but is that a plausible strategy?
24 DR GAGE: Well, I think several of the other
25 speakers have already talked about it. Jeff Macklis has a

1 nice paper that just came out in Nature a week or ago or
2 something like that evidencing the fact that there's some
3 level of recruitment under selective damage situations
4 where the populations of endogenous cells can target in
5 some reasonable way.
6 I think there is a big theoretical question out
7 there. The brain has this capacity for generating new
8 neurons. Does this have some sort of functional
9 consequence to it that's beneficial or negative? All the
10 evidence so far is correlative. There's no causal link,
11 but there's certainly disease models where aberrant type
12 growth has been seen from these endogenous cells just like
13 in, one could say, more therapeutic minded approaches, one
14 gets an elevation or a function of function which is
15 correlated with the increase in neurogenesis.
16 But having said that, I think that this is an
17 area that's important, probably a little bit outside of the
18 goals of this meeting. On the other hand, we had to sort
19 of consider that, that these are cells that are going to be
20 activated or influenced by whatever procedure that we are
21 imposing on them from the outside. They are a responsive
22 population.
23 DR. TROJANOWSKI: I was struck by the injection
24 that was not saline but the other thing that really seemed
25 to ramp up proliferation and could one exploit that in a

1 positive way rather than having it be a confound --

2 DR GAGE: That would be the objective. But we
3 think of this whole process as a continuum. There's
4 proliferation. There's migration. There's
5 differentiation. There's function. What that sort of
6 demonstrates is that there are stimuli that can potentiate
7 stimulation, but having the entire sequence for functional
8 differentiation I think is not here.

9 DR. SALOMON: Well, then with that, we'll take
10 a 10-minute break.

11 (Recess.)

12 DR. SALOMON: The next speaker this afternoon
13 is Dr. John Trojanowski from the University of
14 Pennsylvania, from the university and from the Center for
15 Neurodegenerative Disease Research and not from the Genetic
16 Therapy Institute. He's going to talk about animal models
17 for evaluating cell therapy of neurodegenerative diseases.

18 DR. TROJANOWSKI: I realize that some of my
19 slides are small, but I'll be able to read off some of the
20 things that don't project to the back of the room.

21 The first slide has something up here that says
22 "fatal protein attractions underlying neurodegenerative
23 diseases," and it was my attempt in a cinematic way or an
24 allusion to a film to capture what's wrong in many
25 neurodegenerative diseases. I'm going to focus on

1 Alzheimer's disease. The list of diseases here in
2 abbreviations are some of the very common ones. We know of
3 Alzheimer's, Down's syndrome, Parkinson's disease; less
4 common ones, the dementia with Lewy bodies, multiple system
5 atrophy, Pick's disease, ALS.
6 All of these diseases have very different
7 clinical phenotypes, very different pathological
8 phenotypes, but they share one intriguing commonality that
9 struck us and has stimulated our efforts to understand how
10 the lesions form. And that is, they're characterized by
11 intracellular or extracellular protein aggregates. These
12 proteins don't start off in life doing bad things or
13 they're not designed to be toxic, but because of a
14 mutation, let us say, in PS1 or PS2 or APP, they give rise
15 to toxic proteins, the A-beta peptide, tau aggregates,
16 synuclein aggregates. But they have a very intriguing
17 symmetry and that is the wild type protein in the sporadic
18 disease also shows the same predisposition, perhaps
19 influenced by genetic risk factors, environmental factors,
20 to also convert from a soluble protein that performs a
21 normal beneficial function to a toxic, insoluble, and often
22 filamentous derivative that then accumulates as lesions in
23 one of the places that I just mentioned.
24 So, that offers the hope that if we can find
25 anti-fibrillogenic drugs, that we can take drugs that

1 convert bad conformations into normal conformations or that
2 are filamentous aggregate-busting or plaque-busting or
3 tangle-busting, we may be able to translate an advance in a
4 boutique disease like MSA or dementia with Lewy bodies to a
5 very common disease like Alzheimer's disease. In other
6 words, you may be able to exploit that drug to bust not
7 only Lewy bodies but plaques, tangles, and other bad
8 accumulations of proteins.
9 So, I'll just start with Alzheimer's disease as
10 the prototype of these diseases and the prototype, of
11 course, of all late onset, adult onset neurodegenerative
12 diseases. These statistics are quite ominous and
13 worrisome. 4 million Alzheimer's patients today. By 2050,
14 there may be 14 million. So, this is something that will
15 not only cause a lot of anguish and pain among families,
16 but it threatens to break the bank as well because of the
17 high cost of caring for patients for a very long time with
18 Alzheimer's disease.
19 So, the defining lesions of Alzheimer's disease
20 -- there's are lots of way to become demented and there are
21 many different neurodegenerative diseases that cause
22 dementia. Alzheimer's is about 60 percent of dementias
23 over age 65, and the defining lesions are the
24 neurofibrillary tangle and the amyloid plaque. The tangle
25 is inside neurons, the plaque is outside neurons. The one

1 inside is formed by twisted filaments largely. They are
2 the tau filaments. The ones outside are the amyloid
3 fibrils formed from short peptides called the beta amyloid
4 peptide.
5 It's not known how they're related in sporadic
6 disease mutations. Of course, NF AD kindreds will produce
7 plaques and tangles, but in most diseases, sporadic so far
8 as we know -- and this is just a schematic from the work of
9 Steve Arnold, who is at Penn, showing the distribution of
10 plaques and tangles. They pretty much collocalize but
11 they're not always in the same place, and there's been no
12 meaningful way to connect plaques as causative of tangles
13 or vice versa as yet, except in the genetic diseases.
14 There are areas of the brain that are spared,
15 however, motor cortex, sensory cortex, occipital cortex,
16 offering the hope that if at least a few neurons are smart
17 enough to escape this pathology, if we figured out how they
18 got to be that smart, in molecular terms or genetic terms,
19 we would be able to perhaps develop a therapy to help spare
20 neurons, that otherwise would be affected, the fate of
21 dying because of plaque and tangle accumulations.
22 So, these pathways are two parallel pathways so
23 far. This is the hypothetical scheme of how tangles are
24 bad for you and how plaques are bad for you. I'm going to
25 focus on the tangle pathology just as it seems more

1 intractable at this moment. I mentioned the report
2 yesterday in the Washington Post extending the findings of
3 Dale Schenk a year ago showing that you can somehow take a
4 formed plaque and clear it by an A-beta peptide vaccine.
5 That was in July of last year.
6 In December at Penn, we began a clinical trial.
7 I'm the Director of the Alzheimer's Center at Penn. I
8 didn't personally. I'm not taking A-beta myself --
9 (Laughter.)
10 DR. TROJANOWSKI: -- although it looks really
11 exciting and might want to consider it just to be sure.
12 But it is in clinical trial and it looks as though there
13 are no deleterious consequences at all.
14 So, I think we'll rapidly have in patients
15 confirmation, or the opposite thereof, of the amyloid
16 hypothesis. For the patient advocates in the group, we'll
17 also know right away whether we have a therapy that may in
18 fact be beneficial.
19 I have said that if you clear all the plaques,
20 you're going to be left with another disease that is
21 referred to as a "tauopathy", Pick's disease, CBD, because
22 I don't know that this therapy directed at amyloid will
23 necessarily clear tangles. Those are inside cells.
24 They're formed from the protein called tau, which is a
25 microtubule associated protein. I'll say a little bit more

1 about what its functions are, but one of the important
2 things it does is bind to microtubules and stabilizes them
3 in the polymerized state. These are the guide wires, the
4 train tracks upon which traffic moves from cell body to
5 processes and back again. If those ties fall off or there
6 are too few of them, it's plausible -- and in fact we've
7 shown it does happen -- the microtubules depolymerize,
8 transport fails, and that severely compromises neuronal
9 survival. That's a loss of function when you pull off tau
10 and sequester it in tangles.
11 The tangles also, which are shown here in red,
12 and dystrophic neurites also in red in these little
13 processes could have a -- the formation of the tangles
14 could represent a toxic gain in function. You'd have plugs
15 of stuff occluding conduits that should be open for traffic
16 flowing both directions. By whatever mechanism, the
17 ultimate consequence of these toxic gains of functions and
18 losses of normal functions would be the death of a neuron
19 and release of tau into the CSF, which is now one of the
20 markers that can be used to follow responses to therapy
21 potentially.
22 So, just without getting too technical, tau is
23 a microtubule associated protein, one of many that binds to
24 microtubules, and has some function related to the
25 stability of microtubules, in this case keeping

1 microtubules polymerized, and again microtubules are
2 essential for axonal transport.
3 It's a phosphoprotein and phosphorylation
4 negatively regulates tau binding. The more phosphorylated
5 tau is the less it binds. That's one of the things that
6 goes wrong in Alzheimer's disease.
7 All of us have as adults 6 tau isoforms that
8 come from a single gene that's alternatively spliced. This
9 is the biochemical profile. They have these either 3 or 4
10 microtubule binding repeats in the amino terminus of
11 unknown function, and it's by this alternative splicing
12 that one gets then what we call 4R2N all the way down to
13 3R0N. As you will see, there may be some reason to believe
14 that the ratio of 4R to 3R tau is somehow important.
15 So, what is a tangle then? A tangle was one of
16 the two signature lesions that Alois Alzheimer discovered
17 and associated with the disease that bears his name.
18 These are tangles that you see here. He used a
19 Gallyas or Bill Shofsky or some of the silver stain. These
20 are anti-tau antibodies. This is an anti-tau antibody
21 labeling of tangles.
22 I would say that what impresses me much more
23 about the tau pathology in the Alzheimer brain is not
24 what's in the cell bodies but out in the processes. I just
25 want to emphasize to everyone that you could conceivably

1 preserve neuron viability. You could keep all these
2 neurons alive, but if their processes are caked with tau
3 inside of them, they're not going to be talking to each
4 other and there will not be any functional discourse that
5 takes place between neurons that need to do so in order for
6 cognition and other things to take place.
7 So, neuron rescue is a laudable goal, but it
8 may not do the trick in all of the diseases that I'm
9 thinking of and that were listed on that first table.
10 Accumulations of filamentous aggregates and processes can
11 be the equivalent of a dead neuron even though you can
12 still identify it in the section.
13 So, these are the paired helical filaments.
14 They're abnormal aggregates of tau proteins and they
15 accumulate in the cell bodies and processes of neurons and
16 sometimes glial cells. After many years of controversy, it
17 was determined that these proteins are, indeed, formed by
18 tau proteins, derivatized though they are by being
19 abnormally excessively phosphorylated, and that comes with
20 a very serious functional loss.
21 These proteins used to be called A68, so just
22 forgive the old slide. It should be tau or PHF-tau.
23 This is dephosphorylated PHF-tau, adult tau,
24 dephosphorylated adult tau. You don't have to know much
25 about microtubule biology or biochemistry to realize that

1 the only guy that winds up on the left side in the
2 supernatant unbound to microtubules is the
3 hyperphosphorylated A68. And that's bad. That's not where
4 it should be. It should pellet with the microtubules where
5 it binds to and stabilizes the microtubule. So, this is
6 the loss of function.
7 The good news is it's not an irreversible loss
8 of function because if you can figure out a way to
9 dephosphorylate tau, pull a few phosphates off, we don't
10 know if you could convert it into a water soluble protein,
11 but it will then bind to microtubules again. So, that's
12 one potential target of therapy.
13 So, PHF-tau is insoluble, cell bodies,
14 aberrantly hyperphosphorylated, and unable to bind to
15 microtubules.
16 Now, I have to mention that there are other
17 diseases -- the tauopathies that I referred to that I worry
18 about will emerge when you remove plaques -- are Pick's
19 disease, corticobasal degeneration, the other
20 frontotemporal dementias, including hereditary ones,
21 frontotemporal dementia with Parkinson's disease linked to
22 chromosome 17.
23 So, in 1998 Jerry Shellaberg called me up and
24 said, I've got a mutation. No longer will you suffer from
25 mutation envy. And knowing that our proteins were bad

1 already, I was quite peeved. I said, Jerry, I didn't need
2 your mutations to tell me that. I knew it already. But I
3 was pleased because the rest of the world began to take
4 these proteins seriously as players in cell degeneration.
5 What was already emerging when the mutations
6 appeared was the fact that it was clear that Alzheimer tau,
7 where most of the studies that have been done, has this
8 abnormal banding pattern that you see here. This is a
9 cartoon, of course. When you dephosphorylate, you can see
10 all of the 6 isoforms that are seen in the normal brain.
11 Other diseases had a more peculiar banding
12 pattern, either the two lower bands here or the three upper
13 bands, which, when dephosphorylated, revealed that you had
14 a preponderance of either the 3 microtubule binding repeat
15 tau or the 4 microtubule binding repeat, suggesting perhaps
16 that an imbalance in the ratio of these isoforms could in
17 fact be deleterious. That is what rapidly emerged in
18 studies of the frontotemporal dementia with Parkinsonism
19 linked to chromosome 17 patients.
20 A flurry of papers in '98 demonstrated that
21 there were intronic and exonic mutations in tau and that
22 these caused disease. This is sort of the cartoonist tau
23 protein. This is the segment of exon 10 and the intron
24 following exon 10 where many of these mutations are. We
25 and others have shown that the mutations impair tau

1 functions. They reduce the ability of tau to bind to
2 microtubules, reduce the ability of tau to promote
3 microtubules polymerization, or they alter splicing that
4 either introduces more exon 10 or less exon 10 into the tau
5 transcripts that encode 3R/4R tau.
6 All of us in this room hopefully have a 3R/4R
7 tau ratio of 1. When it begins to deviate from that, for
8 reasons that we don't understand completely, you get
9 accumulations of the species in excess, and that can lead
10 to disease, as is clearly evident in this group now.
11 There's about 20 mutations, about 30 families, and we don't
12 know so far of any escapees. Disease can begin in the 20s
13 and the 30s and it runs a very malignant course, and
14 patients are dead in 10-15 years.
15 So, because of this data showing that
16 abnormalities in tau ratios could be an underlying
17 mechanism of disease in "tauopathy" -- this is before the
18 mutations -- we began to generate a tau transgenic mouse
19 that doesn't have exon 2 or 3 and doesn't have exon 10.
20 So, this would be the 3R0N, the smallest tau isoform. I
21 just have to tell you that, although what I've just said
22 applies to people, rats and mice only have the 4R, the 4
23 microtubule binding repeat harboring tau transcripts. They
24 don't have the 3R tau isoform.
25 So, we figured we would perturb their

1 microtubule metabolism, if you will, and their tau
2 metabolism by introducing the smallest tau isoform. Here
3 are the three lines, the copy abundance of the transgene
4 shown here, driven by a prion promoter shown to be
5 effective in previous transgenic mouse generation efforts.
6 So, we had these three lines that have increasing amounts
7 of transgene and increasing amounts of protein. We exploit
8 an antibody called T14, which is human-specific here, the
9 human recombinant tau proteins on the left. The wild type
10 doesn't have this because it's a wild type and the
11 antibody, of course, doesn't dissect it.
12 The smear is due to the variable extent of
13 phosphorylation. It's not a crisp band like this. But
14 you'll see later on you can dephosphorylate it and get the
15 same.
16 Line 7 was the one we used. Line 27 died at 3
17 months. And line 7 was the one that we selected for our
18 studies, and I'm going to focus mostly on line 7. You can
19 see here, again with this T14 antibody, the polyclonal
20 detects everything, but I'll just focus your attention on
21 what is cortex, hippocampus, brainstem, cerebellum, and
22 spinal cord. Spinal cord has about 60 percent of that
23 amount of tau found in the other regions.
24 So, we've got robust expression, and anyone who
25 is involved in transgenic mouse production efforts knows

1 that the 3 L's of mouse is levels, levels, levels, or
2 higher, higher, higher. The rapidity of disease onset and
3 the robustness of the phenotype depends very much on the
4 abundance of the transgene protein expression.
5 6-month wild type mouse. T14, nothing. 6-
6 month transgenic mouse. You begin to see a few cortical
7 tangles, in brainstem a few tangles, and here are higher
8 power views of the 6-month tangles in, I guess, brainstem
9 and cortex. Well, I shouldn't say they're tangles until I
10 show you the filaments, but they stain for Bodian methods.
11 So, here in spinal cord and in cortex are things that to
12 somebody who does diagnostic neuropathology as well as
13 basic neurobiology like myself is persuasive of being a
14 tangle.
15 We now have older mice that I'm not going to
16 discuss or present who have more cortical tangles that are
17 Gallyas positive, Congo red, thioflavin. For those
18 interested, I can tell why -- basically it says it looks
19 more and more like the tangles of human beings.
20 Since the filamentous inclusions will be more
21 abundant in spinal cord and the mice begin to develop those
22 at 3 months and then developed a motor neuron disease
23 phenotype by 6 months that progressed, we focused a lot of
24 our attention on spinal cord.
25 This is a normal nerve, and you can see there's

1 a filamentous aggregate in this normal nerve of a wild type
2 mouse, transgenic mouse, higher power view of these
3 filaments. And these are labeled with antibodies to tau,
4 as well as neurofilament and tubulin. Some tangles in
5 Alzheimer's disease brains, progressive supernuclear palsy,
6 Guam amyotrophic lateral sclerosis, Parkinson's dementia
7 complex, or Guam ALS PDC, diseases that are like their
8 western counterparts, except taus, that I would have called
9 pathology, also have some neurofilament proteins.
10 I don't want to confuse you. Tau is the
11 building block of the filaments in our mouse and in humans,
12 but other filaments may co-precipitate. In the older mice
13 that we have now, 18 to 24 months of age, we're not seeing
14 that, that is, the co-aggregation of other elements.
15 So, I'll just say that there are other
16 signatures of tau pathology that may also be relevant for
17 targets of therapy and so forth that you want to pay
18 attention of and make sure in your transgenic mice. And
19 here they are.
20 There is a progressive accumulation of
21 insoluble tau with time, and you see that not only in the
22 brain but you see in the spinal cord. In fact, it's more
23 abundant in the spinal cord. So, this is RAB buffer.
24 Water soluble proteins would be present. This is a buffer
25 that is a next level of solubilization ability, and formic

1 acid is really harsh and will solubilize just about
2 everything.
3 So, you can see that in the spinal cord, we had
4 the most abundant material that required formic acid
5 extraction. And then if one thinks that phosphorylation is
6 somehow involved in Alzheimer's disease, as we do, you
7 would want to see that there is an accumulation of
8 phosphates on these molecules, which is indeed the case,
9 and that that is in the insoluble pool as well.
10 So, here's the ADPHF tau. The mouse tau
11 doesn't migrate at the same level because, remember,
12 there's only one, the lowest, the smallest, the 3R0N tau
13 isoform. But the principle is that it's winding up in the
14 insoluble fraction, and it is detected by antibodies, for
15 example, PHF1, that don't see normal tau, see PHF tau, and
16 then this increasingly phosphorylated mouse tau.
17 This paper appeared in Neuron last year and we
18 thought we had persuaded them for what we thought where the
19 bar was set at that time on a phenotype due to the
20 accumulation of tau proteins. And they insisted that we do
21 something of a functional nature, which we did, and that is
22 axonal transport studies. I want to draw your attention.
23 These are the fluorograms. Basically you
24 inject P35 into the spinal cord motor neuron pool, and this
25 radioactive amino acid is incorporated into all proteins

1 that move out by axonal transport. This is the normal wild
2 type mouse here and this is the transgenic mouse. The
3 delay in transport is represented by -- these are
4 increasing millimeter distances from the spinal cord, 2, 4,
5 6, 8, 10 millimeters from spinal cord.
6 You can see the wild type mice move at a normal
7 rate and those in the transgenic move at a slow rate.
8 They're still walking around and doing things. So, these
9 are the remaining residual neurons, and there are axons
10 that are not yet dead but are not functioning at the level
11 they should.
12 We also showed -- I don't have slide for that
13 -- that the microtubules were depolymerized and lost while
14 neurofilaments and actin filaments were not.
15 So, let me just say that there are many genetic
16 factors, tau mutations, APP, PS1, PS2 mutations that can
17 produce what we call a "tauopathy." Alzheimer's disease
18 can be called a "tauopathy." Of course, there are other
19 lesions, but there are diseases, FTDP-17, in which the only
20 lesion is a tangle and that is associated with whatever
21 clinical phenotype, dementia, Parkinsonism, and neuron
22 loss.
23 I haven't shown you the data, of course, but I
24 have referred to the fact that these genetic lesions can
25 perturb the ratio of normal tau, 3R to 4R tau, or cause a

1 loss of function and/or a gain of toxic function.
2 Hyperphosphorylation is something that may be down stream
3 of all this but would certainly contribute to the failure
4 to bind to microtubules.
5 A big unknown is environmental factors, and we
6 have a lot to learn about that. But wouldn't it be nice if
7 we could prevent Alzheimer's disease by determining
8 something in the environment that was bad? And we're
9 trying to work on that.
10 Tau dysfunction then, in one way or another,
11 leads to tau aggregation and neurodegeneration. I think
12 despite the controversies between tauists and Baptists --
13 and I belong to both camps. I work on both tau and A-beta,
14 but I think there's very little reason for even the most
15 maniacal Baptist to say that tau doesn't play a role
16 anymore in neurodegeneration. So, we hopefully built
17 bridges and resolved our religious wars and can get on with
18 the real business of curing these diseases.
19 So, Nancy Bonini allowed me to use this slide
20 from some of her work on Drosophila models. We like to
21 think of mice and monkeys as being the way to go, but
22 there's a great deal of appeal to mouse models. Nancy
23 talks about late onset disease at day 9 or 10, and I hear
24 some transgenic people laugh. I lust for a number like
25 that because we have to wait for a year, 18 months. It's

1 not only expensive, but very nerve-racking to know whether
2 your investment of $50,000 or $100,000 in a mouse line is
3 going to be thrown into the trash because it didn't develop
4 a phenotype or if it's going to work.
5 So, Nancy I think revolutionized our thinking
6 about models of disease when she reported -- Woek, et al.
7 reported in Cell that she could create a mouse model of
8 Sca-3, the Machado-Joseph disease, one of the trinucleotide
9 repeat diseases, and do this in a most unconventional way
10 by getting protein expression, the transgenic protein to be
11 expressed in the eye, and could follow a disease phenotype
12 in a very straightforward, easy way just by looking at the
13 mice. They do the sectioning and all that sort of stuff,
14 but that is the real bottleneck, the rate-limiting stuff.
15 DR. SALOMON: Do you mean mice?
16 DR. TROJANOWSKI: These are flies. Did I say
17 mice? Flies, Drosophila. I'm sorry. So, these are fly
18 eyes.
19 So, this is the normal eye, and then at 10 or
20 15 days, the flies -- this is the normal ataxin-3 protein
21 with the normal length of polyglutamines. These flies have
22 an eye that's identical to the normal eye. But flies that
23 express the disease, ataxin-3 protein, develop a phenotype
24 that's very, very evident even to someone unschooled in fly
25 biology, like myself.

1 She went on to show that there were aggregates
2 and she showed that hsp, heat shock protein, 70 accumulated
3 in them. And she began very creatively thinking that maybe
4 hsp 70 is there for a purpose. It's not just sucked into a
5 vortex of a crashing cell but has some productive role.
6 Again, Woek, et al. in Nature Medicine showed
7 that if you co-expressed the heat shock protein 70 with the
8 expanded polyglutamine protein in the same cells of the fly
9 eye, you could eliminate the phenotype. So, here's hsp 70
10 alone in the fly eye. Here is the diseased eye with the
11 disease protein, and when you co-express both of them, you
12 suppress the phenotype. So, the abnormal confirmations
13 that are assumed by this expanded polyglutamine stretch are
14 not formed or are smoothed out or reversed when you have
15 sufficient heat shock protein on board.
16 There's a lot more that we need to know about
17 what goes wrong inside tangle-bearing neurons as well as
18 how plaques kill neurons. I will suggest that one way to
19 go -- it was mentioned that one could use gene expression
20 profiling methods. This is work that Steve Ginsburg did
21 recently in our laboratory before going to Baylor.
22 These are normal neurons in the normal brain of
23 an elderly individual, and these are neurons, some of which
24 contain tangles in the brain of an Alzheimer's patient.
25 Steve harvested single cells, labeled with an antibody so

1 he knew what it was he was taking out of the brain. And he
2 wanted pure populations of neurons because, remember, the
3 thing that one always has to appreciate about many of these
4 neurodegenerative diseases is selective vulnerability. Not
5 every neuron is affected, and certainly many other types of
6 cells in the brain, glial cells are not affected.
7 So, if you just homogenize these two areas and
8 look for differences in gene expression, you probably see
9 lots of differences, but you wouldn't know whether you
10 should attribute them to dying neurons or something else
11 going on in glial cells. Remember, if you do a Western
12 Blot, the most elevated protein you'll see in a very
13 severely diseased Alzheimer's brain is glial fibrillary
14 acidic protein because there's a lot of gliosis and
15 astrocytes that proliferate it. So, you've got to do this
16 in a focused, intelligent way, and it's very appealing to
17 be able to have single cells.
18 So, then Steve was able to pool these cells,
19 the tangle-bearing neurons and the non-tangle-bearing
20 neurons. This was done in collaboration with Jim Eberwine
21 and Virginia Lee, Scott Hemby at Penn, and it just came out
22 in Annals of Neurology, if you're interested in the
23 details.
24 But basically Jim Eberwine's aRNA amplification
25 method allows one to amplify a million-fold. Here are some

1 controls that were done to show linearity of amplification.
2 We took two approaches. One is the "gee whiz, what can we
3 see that's different approach" and then what we called the
4 "candidate gene approach" where we knew there were proteins
5 already implicated in Alzheimer's disease and where there
6 was even some data to indicate that their message levels
7 changed. We interrogated what we call custom arrays with
8 the amplified transcripts that are labeled in the second
9 round of amplification.
10 Here are the GDA arrays, and I forgot whether
11 red is normal and green is tangle. Yellow indicates where
12 both levels are equivalent, but if you have more of one
13 transcript, it will be red. If it's down, it will be
14 green. Then you can plot and do statistics and group
15 proteins together by their function, phosphotases, kinases,
16 actin binding proteins, cell cycle proteins, cytoskeletal
17 proteins, and look for those that deviate up or down. This
18 may be away to identify other candidate proteins for
19 targeting for cell therapy or some other form of therapy.
20 Obviously, this is the beginning of an approach
21 to dissecting out the molecular mechanisms of
22 neurodegenerative disease, but potentially a very powerful
23 one. I'll just say that the one that we followed out in
24 the most detail was cathepsin D, which Randy Nixon and
25 collaborators had shown was up-regulated to lysosomal

1 protein, and we showed that, indeed, it was one of the most
2 highly elevated transcripts in our array study relative to
3 controls, and when we did in situ hybridization
4 immunohistochemistry, we showed, as Randy had -- it was
5 very pleasing to not have to struggle with data that went
6 the other way. I saw Randy recently and said, thanks for
7 making our job easier because we were able to show what he
8 had already shown so nicely earlier, that lysosomal
9 proteases, in particular cathepsin D, are up-regulated.
10 This is the second-to-the-last slide. I'll
11 come back and emphasize once again that the approaches one
12 can use to correct a disease that may be a boutique
13 condition, such as MSA or Hallervorden-Spatz or
14 neurodegeneration with brain iron type 1, could possibly be
15 exploited in other situations. What could cell therapy do
16 in these kinds of situations?
17 Well, one thing would be to make a heat shock-
18 like protein that could be secreted and taken up. So, if
19 you were able, as Evan is able to do, to inject cells into
20 the brain and get them to go everywhere you want them to be
21 or everywhere you can get them to be, they obviously -- if
22 you had more heat shock protein around, I don't think it
23 would make good protein fold bad, but it could make the
24 bad-folding proteins fold right. That would be one
25 strategy.

1 Another alternative, and far less clear-cut to
2 me at this point, would be to have the neurons go to the
3 damaged area and perhaps rewire, if they were interneurons.
4 That's really a great leap of faith and beyond my
5 imagination to work out in my own mind in detail. But
6 maybe they will function as these human neurons do in
7 stroke trials right now where they seem to reinvigorate
8 cells in the penumbra of a stroke cavity so that they
9 function better. I'm really just speculating about
10 possibilities, for which there is at this time very little
11 data.
12 So, I think that one could get a base hit in
13 one of these diseases that could be a base hit over and
14 over and over again in other diseases and have potential to
15 cure all of these diseases that are caused by fatal
16 attractions of brain proteins.
17 I'll just close by saying that these studies
18 were done with my wife and colleague, Virginia Lee. We've
19 been working together on this tau and Alzheimer's disease
20 problem for many years and have a wonderful group of
21 collaborators in our Center for Neurodegenerative Disease
22 Research that extends to many departments at Penn,
23 psychiatrists, neurologists. It takes the whole university
24 and many universities, I think, to marshal the talents to
25 make advances in this area, and there are many colleagues

1 who are at other universities, upon whose expertise we've
2 drawn. Of course, we couldn't do any of this without
3 support from the National Institute of Aging and NINDS and
4 the Alzheimer's Association.
5 Thank you very much.
6 (Applause.)
7 DR. SALOMON: So, I think one of the things
8 that we need to do in these next couple talks is to take
9 advantage of the fact now that these specific models here
10 -- this is Alzheimer's. This is a model for Alzheimer's as
11 well as -- I think you made very eloquently -- a number of
12 different degenerative diseases.
13 So, in the comments that I'd like to get from
14 the expert panel, can we talk about then how this kind of a
15 model now could be integrated into preclinical studies for
16 a clinical trial in Alzheimer's, the pros, the cons, what
17 kind of things you would want to see measured, et cetera?
18 We'll pick up on this more specifically again tomorrow, but
19 just in the short time we're going to allot to conversation
20 right now, I'd like to start in that direction.
21 DR. TROJANOWSKI: I would just add that at the
22 meeting there were crosses of the APP, the presenile, and
23 what have you. It sounds like a word salad I know, but you
24 can get all of these mice that make the different
25 pathologies to cross breed and get plaques and tangles.

1 So, that's going to be there very shortly if it's not here
2 already.
3 DR. NOBLE: It seems a fundamental distinction
4 that needs to be made is that these kinds of diseases with
5 the tremendous diffuse damage throughout the nervous system
6 are very different than the focal problems of spinal cord
7 injury. So, a different order of problem, different
8 biology entirely.
9 DR. TROJANOWSKI: I was told to make it as
10 tough on you guys as I could.
11 DR. NOBLE: You did a good job.
12 (Laughter.)
13 DR. NOBLE: So, one question that comes to my
14 mind is the extent to which one can delineate between being
15 a cell therapy problem in the contemporary world and a gene
16 therapy problem and whether one is simply using cells as an
17 enzymatic delivery system. So, for example, in the work
18 that Evan and his colleagues have done in relation to
19 storage disorders. So, I'm wondering what your thoughts
20 are.
21 DR. TROJANOWSKI: I think we needn't think in a
22 compartmentalized way, of course. You might want to try
23 several different options, and one could even conceive in
24 the same patient of more than one option. One would want
25 to start off, of course, in a very focused and meticulous

1 way looking at one at a time, but maybe you could replace
2 cells in hippocampus. If, as Brad Hyman argues, that is
3 the switch that does or does not enable memories to go --
4 remember, in Alzheimer's patients the short-term memory is
5 okay. It's the long-term memory. They can't sock new
6 memories away. So, maybe you could repopulate the
7 hippocampus and maybe local connections. That's a very
8 small circuit. The hippocampus is as big as my thumb. Is
9 it going to be that hard to restore circuits? It seems
10 like an immense task to me even though the hippocampus is
11 small. But that would be one way to go, and then you could
12 also do something more diffuse.
13 DR. NOBLE: Let's follow that along. I'm
14 trying to figure out how to get to a clinical trial. What
15 is the preclinical experiment that you would want to
16 conduct? If you showed that putting neuron-restricted
17 precursor cells or neural stem cells into the hippocampus
18 of these animals now led to a restoration of cell number or
19 a decrease in apoptosis or some parameter like this, is
20 that the point at which you would argue one should go
21 forward?
22 DR. TROJANOWSKI: If you're a purist, you would
23 say, well, it's not quite the model of Alzheimer's disease
24 I want because there aren't enough tangles up north, in the
25 brain. They're most abundant in spinal cord. But from a

1 proof of concept point of view, it's much easier to follow
2 motor dysfunction than cognitive dysfunction. I don't know
3 if you corrected a cognitive dysfunction in mouse, whether
4 you're immediately assume that that's going to make a
5 difference in people.

6 But from a proof of concept point of view, you
7 could inject cells into the anterior horn and see if they
8 would keep those that are alive functioning better. If you
9 could transfer from those cells a compound like a heat
10 shock protein to eliminate, bust up, a tangle-busting drug,
11 that might be efficacious. I don't know if you would get
12 elongation of processes to the muscle from the transplant.
13 No one has done that kind of thing, the equivalent of
14 cutting a nerve -- actually the nerves aren't cut, so you'd
15 have a better chance probably, but I don't know if the
16 rewiring would be meaningful. So, those would be the kinds
17 of the things that I would think of as trial-like concepts.

18 DR GAGE: You mentioned clinical applications
19 in stroke too. I think that you've been involved initially
20 in these studies with the N-Tera-2 cells. I wonder if you
21 could update us on that and the rationale behind
22 utilization of these because this is really the first real
23 application of human whatever cells in a --

24 DR. TROJANOWSKI: I'm a founding scientist of
25 Layton BioScience, but I'm a full-time Penn faculty. I'm

1 not involved daily in discussions of how the trial goes on.
2 I'm certainly glad in retrospect, considering Jim Wilson's
3 problems, that I was not closer to the clinical trial than
4 Pittsburgh, which is where it takes place.
5 But the underlying rationale was not that we
6 were rewiring the brain, because it was clear from the
7 animal studies that we weren't. It seemed as though the
8 cells that went into the perimeter or the vicinity of the
9 ischemic damage survived and survived very well and somehow
10 made those remaining neurons function better because we
11 clearly were not reinvigorating. We didn't do
12 bromodeoxyuridine to see if more proliferated. I guess
13 that would be an option now that we didn't think of 4 years
14 ago.
15 So, I think what the N-Tera-2 cells may be
16 doing, the neurons may be doing, in people is having some
17 sort of trophic effect on their neighbors. That is
18 completely unvalidated and unverified. It's a subject of
19 intense research at Layton, but not in my lab. I don't do
20 those studies in my lab. That is, so far, I think all that
21 we can say about what we know might be going on. But it's
22 not a rewiring.
23 DR. DRACHMAN: John, if tau is your target --
24 and certainly that was what Alzheimer described originally,
25 not really plaques -- we've got to believe that tau is a

1 very specific problem. Yet, we know that one of the most
2 nonspecific etiologies that you may think of produces huge
3 amounts of tangles, namely dementia pugilistica. A good
4 knock on the head certainly is a far cry from a very
5 specific biochemical abnormality.
6 What are you thoughts about that and whether
7 this is secondary, primary, or what?
8 DR. TROJANOWSKI: I think that this argument is
9 rendered moot by the mutations. I don't know that there's
10 any way that one could argue -- at least in FTDP-17 that
11 the cause -- we have prima facie evidence that the cause is
12 a mutation in the tau gene. It tracks with the tau gene.
13 If you have the mutation, you get the disease. I don't
14 know unless there's another mutation someplace else that no
15 one has seen -- and that happens perversely to track with
16 disease.
17 So, tau abnormalities absolutely cause disease,
18 and it causes disease that -- the Jerry Shellaberg B337M
19 family was first thought to be schizophrenic, then
20 Alzheimer's disease, and then a "tauopathy." So, these are
21 diseases that can look very much like Alzheimer's disease.
22 I think the only difference is where the pathology falls,
23 and we don't have a good understanding of that.
24 Dementia pugilistica is very interesting. We
25 are working ourselves very intently on head trauma in

1 transgenic mice to see if we can dissect out what's going
2 on. Is it inflammatory? It is up-regulation of a kinase,
3 down-regulation of a phosphatase? What is going on here?
4 Because most Alzheimer's disease is sporadic. Many people
5 sustain head trauma. I fell off a horse when I was 16.
6 So, I've had the head trauma history, as probably many
7 people in this room have, of some sort of a fall, hit on
8 the head, or what have you. If we could figure out what
9 that's all about, perhaps we would put people on anti-
10 inflammatories after they've had their head trauma or
11 recommend that everyone wear bicycle helmets. And we do
12 that already, but may soccer helmets and football helmets
13 and all the other things, particularly if they have the
14 E-4/E-4 genotype.
15 Tangles are not nonspecific. You can do a lot
16 of things experimentally and fail, as many of us have for
17 many years, to produce tangles in an animal. It's a long
18 list but it's a limited list of diseases that are caused by
19 or have prominent tangle pathology. So, it's not like
20 gliosis in response to injury. I just want to emphasize
21 that. It's real specific and linked to bad stuff.
22 DR. MACKLIS: I have two questions that will
23 tie into this morning's discussion about the
24 characteristics and appropriateness of animal models and
25 then the characteristics and appropriateness of source

1 cells.

2 The first one would be, in the tau transgenic
3 that you mentioned, or the mutant, there's anterior horn
4 cell loss, as I understand. To use Rusty's slide, homology
5 versus isotypic, is that a model for us for AD or for part
6 of ALS, for example?

7 Then the second part would be we've heard a lot
8 this morning and discussed whether neural stem cells or
9 precursors or progenitors effect their potential recovery
10 by trophin or growth factor secretion rather than by
11 connectivity. If that's the case, are we actually looking
12 at them in all of these slide talks by the wrong axes? Do
13 we really care if they turn into oligos, astrocytes, or
14 neurons, or do we really want them to stay round cells that
15 pump a lot of factor X, Y, and Z and maybe migrate to
16 wherever they go? I think we may want to ask those
17 questions in our models ahead of time so that we can answer
18 them in our preclinical studies.

19 DR. TROJANOWSKI: I'd say yes to the second
20 question. My early training in neuroscience was in
21 neuroanatomy, and maybe I'm just hung up with the minutia
22 of wiring diagrams, but I think it's hard to imagine
23 restoring all the complex circuits that all of us walk
24 around with before disease strikes. But maybe that's just
25 limited imagination. Maybe we'll be able to do it.

1 Your second part of putting out whatever
2 molecules that will help cells function better is I think a
3 laudable goal and a direction that we should think of.
4 For those of us who struggled with animals for
5 so long in Alzheimer's disease -- and it may seem like a
6 blink of an eye to people who are still struggling to make
7 animal models in other diseases, and there are people who
8 are struggling -- we just wanted a proof of concept that
9 this pathology is bad. So, we have shown that tangles
10 kill. Amyloid transgenic mice don't kill neurons. So, I
11 believe that amyloid is bad, and I work on that too. But
12 it was most pleasing to have an animal accumulate tau, show
13 a functional deficit, failure to bind microtubules,
14 microtubule dissolution, many functional deficits actually,
15 axonal transport, perturbation, disruption, attenuation,
16 what have you, and that these mice got sick. They were
17 weak. They had disease. I would love to move all those
18 tangles up north. They're getting older now and they are
19 getting more tangles in their cortex and hippocampus.
20 And I know others who are making other
21 transgenic mice with other promoters and other constructs.
22 It's going to happen. A year from now, we'll be talking
23 about tangles in hippocampus that look just like
24 Alzheimer's, believe me.
25 DR. MACKLIS: Just a quick follow-up on that,

1 though. There may be a dissociation where that same mouse
2 may be an incredibly good and motivating model for
3 biochemical and molecular therapies for "tauopathy." It
4 may or may not be as good a model for repopulation or
5 cellular repair. Do you agree with that?
6 DR. TROJANOWSKI: No. I think you start where
7 you can start. These things don't have to be done in
8 series. They can be done in parallel. I appreciate the
9 personal insight or contribution of one of the patient
10 advocates in the audience. I think all of us know people,
11 have relatives, what have you, who died of an untreatable
12 disease. I think we should do things in parallel. I think
13 we have the resources. I think we have the intelligence.
14 We have the people and it should be full steam ahead.
15 DR. KOLIATSOS: Can I make a comment here? I'm
16 sorry.
17 DR. McDONALD: I just wanted to say that
18 perhaps some of the neurodegenerative diseases like
19 Alzheimer's that occur over decades are really a problem of
20 turnover, more or less. That is, you're accelerating the
21 rate of death, and with the new concept of the nervous.
22 constantly turning over -- that is, neurons and glia are
23 continually being replaced -- that we might do better just
24 augmenting cell birth and survival.
25 DR. TROJANOWSKI: Hence my question to Rusty.

1 If we could up the rate of normal adult neurogenesis, might
2 we be able to repopulate some of the hippocampal neurons
3 that are maybe still alive but not functioning because
4 their axons aren't talking to each other. I would agree.
5 When I say full steam ahead, I just want to let you know
6 that means clinical trials should be done with great
7 oversight and contemplation, but I don't think we should
8 fail to exploit the models at hand. That's what I'm
9 saying.
10 DR. McDONALD: For example, do we know, in
11 those animal models where the disease is present, is there
12 reduced proliferation and survival of progenitors?
13 DR. TROJANOWSKI: I don't know that. We will
14 have that answer in a year or less.
15 These are very important questions and I don't
16 think we should hold back from addressing them. There's
17 only so much I can do in my lab, but if Rusty stops in
18 Philadelphia on the way home, I'll give him a bunch of mice
19 to take back to San Diego. I'd love to know the answer to
20 that question is what I'm saying, and I think it's
21 important to the field.
22 DR. KOLIATSOS: I strongly feel that it is
23 inappropriate to make any direct comparisons between
24 neurodegenerative models and stem cell transplant
25 approaches at this time, especially as far as molecular

1 specificity of lesions. I think it's not the job of this
2 panel to go into the depths of neurodegenerative models,
3 and it would probably be a very bad idea I would propose.
4 I think that this is just a very important
5 input from John to let us know that there are powerful
6 models out there which might be used when the time is
7 appropriate for what we're talking about in this meeting
8 not only in the sense of repopulating, but also in the
9 sense of assessing some potential side effects of grafts.
10 For example, mice which have the genetic propensity to make
11 plaques or tangles could be very well used as biological
12 models to see if the cells we want to put in Alzheimer's
13 disease are going to increase plaques and tangles by some
14 side effect mechanism. So, there is much more genetic
15 value to the models that John described than looking at
16 specific associations between circuitry and cells and so on
17 and so forth.
18 DR. TROJANOWSKI: That's true. If A-beta is as
19 toxic as it's reported to be, it may kill all new grafted
20 cells on contact. In agreement with what you said about
21 the grafted cells doing bad stuff, we thought of that and
22 did do those kinds of studies on the N-Tera-2 cells five or
23 six years ago. They do make APP. They're great little
24 factories for studying amyloid precursor protein
25 metabolism, but we showed that in over a year in nude mice,

1 they didn't produce plaque or tangles. So, that was
2 something that we felt was important to look at.
3 DR. SALOMON: I would point out that one of the
4 things that the FDA does want from us is some comment on
5 what might be perceived as gold standard models for
6 different neurologic diseases that, indeed, could be put
7 forward as models in which preclinical studies should be
8 focused. Or equally important would be to identify where
9 there are no models or where there are incomplete models
10 and that's where perhaps resource allocation from the NIH
11 or others should be put. It's certainly not meant that
12 anyone take apart John's model. I think I was only using
13 it as an example of --
14 DR. TROJANOWSKI: Just on that point, one
15 controversy that arose at the meeting -- there is
16 inflammation in many neurodegenerative diseases, including
17 Alzheimer's disease. Dale Schenk, who is from Elan and
18 really conceived of the experiment of using A-beta as a
19 vaccine, reported data saying that microglia were doing
20 good things scooping up, gobbling up A-beta and eliminating
21 it. That elicited irritated comments by the
22 neuroimmunomodulation group who thought that all the
23 inflammation was really bad and what you want to do is
24 suppress that. Well, there's a lot of heat but no light
25 here, and I think what you can do very quickly is

1 transplant microglia into these animals and see if they
2 will sweep through and scavenge up. That could be a
3 therapy.
4 DR. KORDOWER: John, if your clinical trial
5 with the vaccine shows no clinical improvement and then
6 when patients eventually come to post, you find that the
7 vaccine did clear the beta amyloid, would that be enough
8 data to suggest that we should go to non-beta amyloid
9 models and abandon the beta amyloid models?
10 DR. TROJANOWSKI: I don't think you have to
11 wait that long actually. I think if they declined and the
12 A-beta levels in CSF or blood -- I don't know that I
13 believe this completely, but Steve Younkin said plasma
14 levels of A-beta can monitor -- you're shaking your head
15 the way I do, but I believe that the CSF.
16 So, just so that everyone knows, the best
17 markers for Alzheimer's disease compared to normal controls
18 -- it gets murky when you get into the other unusual, less
19 common neurodegenerative diseases -- is diminished A-beta
20 levels and elevated tau levels. So, diminished A-beta is
21 thought to be, because it's all retained in the brain for
22 plaque formation, and tau goes up as neurons die and
23 externalize or release their tau and it goes into the CSF.
24 So, if you followed patients and showed that the A-beta CSF
25 levels rise to the normal level and they're still getting

1 demented -- you would still want to do postmortem follow-up
2 of course -- but that would --
3 DR. KORDOWER: Would that scenario be enough to
4 abandon A-beta models?
5 DR. TROJANOWSKI: That's a provocative
6 question. I think it's maybe not meant to be provocative.
7 Many people tease Dale Schenk. But, no, I think animals
8 and people still are different in many ways and you'd want
9 to look very, very closely I think and extensively at the
10 A-beta levels and so forth to make sure you weren't
11 throwing something out.
12 DR. DRACHMAN: No way. You may remove a
13 subdural hematoma from an elderly individual, remove the
14 pathology and watch that person go downhill. Now, that
15 says that you've done some primary damage. Neurons will
16 not recover. Then you've fallen below a threshold and
17 normal aging will then cause further dementia. So, even
18 though I'm not a huge fan of giving beta amyloid -- I'm not
19 sure that I believe that it will do a great deal of good --
20 the failure of that to reverse certain symptoms at a
21 certain point may not be the proof that that's wrong.
22 DR. TROJANOWSKI: There's a large Columbian
23 kindred with FAD mutations, APP. You know like clockwork
24 when they're going to get demented.
25 There is this condition called mild cognitive

1 impairments. Individuals have a measurable decrement in
2 cognition that does not equal dementia, and they convert
3 from that state to Alzheimer's disease at a rate of 15
4 percent a year. So, in five years you would know.
5 DR. SIEGEL: Just as an aside, I would hope
6 that the measure of whether that therapy or other therapies
7 in that or most other diseases worked clinically would not
8 be observation as to whether patients continued to decline,
9 but ultimately a comparison of two randomized groups, one
10 treated and one not in a controlled manner. And the
11 predictability of decline in Alzheimer's and many other
12 diseases -- there's so much variability that -- if what you
13 really meant was if they continued to decline, that would
14 be a failure --
15 DR. TROJANOWSKI: Relatively controlled. Sure.
16 The clinical trial that's underway -- and I'm not involved.
17 The vaccine is vehicle and A-beta peptide. They'll do
18 imaging.
19 DR. SALOMON: So, I would summarize what I got
20 out of this specifically with respect to the things on the
21 table would be that even though these diseases like
22 Alzheimer's are devastating diseases and the implications
23 for this really path-breaking research is obvious, the
24 complexity of these diseases, as they then translate into
25 the practical realities of setting up an animal model where

1 -- you picked looking at the tau, but that doesn't change
2 the contribution of the fibrillogenesis that's occurring
3 extracellularly. Yet, to construct a true animal model of
4 this, perhaps we're going to have to create them both and
5 then cross breed the animals. All of these are really
6 amazingly daunting scientific tasks. Just what you've
7 accomplished is several years of work, as we both know.
8 So, it's just an interesting and sobering
9 thing. We have to be very careful then in the discussions
10 tomorrow that if we take a position like, yes, you need
11 this animal model and you've got to demonstrate specificity
12 -- I think that was some of the concerns you were having --
13 and we get really into that, it may sound really good. But
14 then we've got to deal with the realities, what it really
15 is like setting up these sort of animal models and doing
16 studies in them.
17 A really interesting thing to spend the night
18 thinking about is that there may be certain diseases that,
19 if you feel compelled enough, based on whatever line of
20 reasoning you're going to take from your lab, there may not
21 be an animal model. Or you may have to construct two or
22 three animal models and pick bits of them in order to
23 generate a rationale rather than this simplistic concept
24 that you're going to have an animal model -- I guess,
25 Rusty, you called it an homologous animal model. I think

1 that really comes out very clear here in these discussions.
2 The next person who's going to speak is Evan
3 Snyder. He's from Harvard. The migration and integration
4 of transplanted stem cells within the recipient nervous
5 system.
6 DR. SNYDER: I was asked to talk about
7 migration of neural stem cells. I think it's very
8 interesting that this is what I was asked to talk about
9 because this is both probably one of the appeals of stem
10 cells, at least neural stem cells, but also probably a
11 nightmare for the FDA because this is exactly what they
12 don't want is cells moving away in transplantation
13 paradigms from their point of administration.
14 But to illustrate that what we who have been
15 working in various models of stem cell-like biology -- and
16 admittedly, we're all just working on models of this kind
17 of biology -- have illustrated I think -- and I'm going to
18 try to illustrate -- taps into what the brain may be doing
19 anyway with its own endogenous supply of progenitors or
20 precursors or stem cells, whatever we're going to choose to
21 call it.
22 So, I want to start off talking about a non-
23 transplantation paradigm, and that's illustrated over here
24 by this well-known migratory pathway that Steve and Rusty
25 and a number of the others have already spoken about where

1 throughout life, cells in the subventricular zone are born
2 and follow this stereotypical migratory pathway from their
3 birth out over here into the olfactory bulb where they
4 become neurons.
5 Well, we've wondered what would happen to this
6 stereotypical developmental migratory pattern if injury was
7 imposed up here in the opposite direction. The model that
8 we decided to us what we had been doing in the lab because
9 it actually emulated for the brain about as close as we
10 thought we were going to be able to come to what
11 hematologists can come to in terms of ablating the bone
12 marrow. Well, you can't really do that with the brain, we
13 figured, but we can come fairly close.
14 If we actually take this particular model,
15 which is actually, I should say, a model of a real
16 pediatric disease -- it's hypoxic ischemic encephalopathy,
17 which is a major cause of mental retardation, cerebral
18 palsy, epilepsy in the pediatric population. As the only
19 pediatrician I think on the panel, I feel I need to be an
20 advocate for the pediatric group.
21 Anyway, the way this model works -- and it's a
22 very devastating model -- is you take about a week-old
23 mouse, you ligate the common carotid artery, expose the
24 animal to hypoxia, and you blow away a huge area of the
25 hemisphere on this particular side, leaving this side as a

1 pretty good, intact control.

2 Well, Krucken Park in the lab wondered what
3 would happen if he took an animal like that and at the
4 exact same time as he imposed this kind of devastating
5 injury, he started pulsing these animals with two markers
6 of cells that would be proliferative at the time of this
7 injury. One way is to pulse the animals with BrdU.
8 Another way is to inject a retroviral vector into the
9 ventricles which would then label subventricular zone cells
10 going through S phase at the time of the injury. The
11 retrovirus would encode lacZ and you could follow that.
12 So, you could follow these cells either by their BrdU
13 immunoreactivity or by their lacZ immunoreactivity, and the
14 results are basically the same.

15 Let me just lead you through this very quickly.
16 Obviously, here's a parasaggital section of the animal.
17 These sections over here, right through here through the
18 subventricular zone, are shown in coronal sections over
19 here, and sections through the olfactory bulb are shown in
20 coronal sections over here. This side over here is the
21 intact side. This side over here is the infarcted side.

22 If one looks at the cells that were labeled at
23 the time of the injury on the intact side, they're actually
24 not here in the subventricular zone. They have
25 appropriately moved out here into the olfactory bulb.