1 demonstrate that your oncogene is the only thing you
2 need to get your cell transformed, I would probably go
3 along with what you say then. I have, despite what
4 Harry said earlier on, I find it a bit strange that
5 you would have a highly aneuploid cell that had been
6 going along in culture for a long, even if it
7 initiated as a specific oncogene transformed cell
8 line, that it would still be the same thing at the end
9 as it was at the beginning. Provided that's actually
10 a correct statement, that seems okay to me, but I have
11 my doubts about whether it would actually be true for
12 all cells that you've actually been looking at.
13 DR. LEWIS: Yes, there's been some debate,
14 you know, among our group as to the stability of the
15 genotype and certainly that was one of the major
16 issues that we had in our initial discussions as to
17 possible risks as to what genomic instability would
18 mean.
19 Now I think when you look at stability of
20 a cell that's already tumorigenic, for example, there
21 have been very few studies on trying to determine what
22 that stability really means. Certainly, we know if
23 the cell is not tumorigenic and you carry it serially
24 for a long period of time and it's immortalized, the
25 chances of it becoming tumorigenic are very high. But

1 once it becomes tumorigenic, what does that mean in
2 terms -- does that phenotype vary? Can it become
3 quote more tumorigenic? And in fact, the way the
4 tumorigenicity studies have been done for the history
5 of science is that you take 10^7 cells and 10^6 cells
6 and you put them into animals and you say do they make
7 a tumor? It gives you no idea at all about the
8 quantitative relationship between the number of cells
9 that it takes to make a tumor and the fact that they
10 make a tumor. So without quantitative data, you have
11 no way of knowing whether the phenotype is going up or
12 down or staying the same.
13 Now we did a limited number of -- we did
14 a fairly comprehensive study on some adeno 12
15 transformed cells some years ago and we found that the
16 capacity of those cells to induce tumors over 52 or 56
17 tissue culture paths was identical in terms of the
18 number of cells it took to make a tumor. So with that
19 particular cell line, this is an adeno 12 transformed
20 hamster cell line, no, mouse cell line, the number of
21 cells it took to make a tumor was identical between
22 passage 4 or 5, after it was actually transformed and
23 after it had been carried in culture for a year. So
24 if the capacity to make a tumor is fairly stable, then
25 certainly that variable can be controlled. But what

1 is producing these other variables then is still open
2 for discussion.
3 DR. MINOR: I think that's a discussion
4 worth having which I think comes under number 2.
5 DR. LEWIS: Yes.
6 DR. MINOR: The third point was the point
7 that I raised after you made your presentation which
8 is about cell lines transformed as specific oncogenic
9 viruses. I think that needs to be pulled apart and
10 looked at a bit more closely because from my own
11 perspective, I would not be happy with a human diploid
12 cell that had been transformed with an SV40 as a
13 substrate with the SV40 history that's going around at
14 the moment, so it does seem that that needs a bit more
15 consideration perhaps.
16 CHAIRMAN GREENBERG: I've lost track. I
17 know Dr. Kohl.
18 DR. KOHL: Phil, Phil Krause, Phil, I
19 wanted to get back to your comments. It seems that
20 your hierarching the risk by transforming event and I
21 think what you're hearing, the risk of adventitious
22 agents and I think what you're hearing from at least
23 some of us is that we're concerned not with the risk
24 related to the transforming per se, but the risk
25 related just to where it comes from, what its history

1 is, etcetera and in that regard there really is no
2 difference between 2 and 5 and I think that's what
3 you're hearing. And that has implications in terms of
4 then your recommendations on the far column. Why
5 discourage 5 so severely?

6 DR. KRAUSE: I guess that's true. On the
7 other hand there's also no difference between the
8 recommendations between any of these, for instance,
9 new diploid cell line, you know, if one were to
10 develop, derive a new MRC5 or WI38 like line and so I
11 think what we're trying to dissect out here is given
12 the fact that it's presumed that we have an idea how
13 to handle diploid cells that having been done already,
14 and of course, the desire to apply current technology
15 to the best available limits to those, but the
16 presumption is if a manufacturer were to come in with
17 a brand new diploid cell line that were derived and
18 studied the same way that WI38 or MRC5 were, that we
19 probably would end up accepting something like that.
20 And so the question then is what is there in addition
21 to the concerns that are raised by something like that
22 that one would have to worry about if one were
23 thinking about a cell that were also transformed. And
24 clearly, in all of these cases some of the major
25 issues come out of factors that have nothing to do

1 with the fact that the cell is immortal. It depends
2 on what tissue the cell is derived from, if it's a
3 neuronal cell, you know, you may have additional PrP
4 issues. It depends on what -- who the donor is,
5 what's known about the donor, it's also in many cases
6 for cells that may have been derived a long time ago,
7 one might not have documentation about exactly what
8 happened to those cells in the laboratory and how they
9 were passaged and so forth. So there are all of those
10 other issues which potentially could affect any cell
11 and I guess what we were struggling with is how do you
12 dissect out from those issues, the specific issues
13 that are related to the neoplastic nature of the cell.
14 DR. KRAUSE: And I want to reiterate
15 something Harry raised a while ago. We've got all
16 these gigantic human experiments floating around with
17 people who have received various different vaccines
18 and various different cell substrates and somehow
19 there's got to be a way to get a handle on that and
20 the only agency I know that can do that is the CDC
21 possibly, so maybe Dixie could put more money in their
22 effort, $6 million.
23 CHAIRMAN GREENBERG: Who had the next
24 comment? Okay, so I see, just trying to take stock of
25 where we are.

1 Dixie, good.

2 DR. SNIDER: I just wanted to say that I

3 understand, I think, the reasons to separate 3, 4 and

4 5 and intuitively it makes some sense to me because

5 basically what we're trying to say is that we want to

6 have more knowledge about what our -- where our

7 vaccines come from and how they're grown and so forth.

8 And if we have a cell line that's transformed by a

9 particular viral or cellular oncogene, to me that does

10 make sense that I would have some preference for that,

11 actually, as opposed to an entire oncogenic virus or

12 a spontaneous transformation where I have no idea

13 where it came from.

14 But I think what you're hearing, at least

15 from me and maybe from some others is that there are

16 other factors here in terms of where did that cell

17 line come from. Is it human derived? Does it come

18 from some other animal? And what are its

19 characteristics in various model systems and so forth.

20 So it's just much more complicated than the table

21 would suggest.

22 CHAIRMAN GREENBERG: So Dixie, if I get

23 you right, if you transformed with a single gene,

24 WI38s, or MRC5s, you would feel different about that

25 than some cell line that there was no history?

1 DR. SNIDER: Absolutely.

2 CHAIRMAN GREENBERG: And if you can build
3 that into your -- so the more knowledge about what you
4 transform, the higher degree of comfort you're going
5 to have and I'm sure you feel the same way.

6 DR. KRAUSE: Sure, on the other hand, I
7 would add to that if you -- or I guess what you're
8 saying is you think that's the major issue, but I
9 think the mechanism of transformation still is a very
10 important issue in this context and maybe the best way
11 to drive this home is to give an example. Suppose
12 somebody 5, 10 years ago proposed to use Kaposi's
13 sarcoma cells to grow something. Well, at that time
14 we didn't know that Kaposi's sarcoma was caused by a
15 virus. In the meantime, somebody has figured out that
16 human herpes virus A causes it. The virus doesn't
17 grow in tissue culture, would not have been detected
18 by any of the methods that are traditionally used to
19 screen vaccines. But the hint there, essentially the
20 only clue is this cell was transformed. And we know
21 that viruses can sometimes be transforming agents.
22 And so at least from my perspective, the fact that you
23 don't know why a cell is transformed does put you in
24 a different category from a cell where you know why
25 it's transformed, although I also agree with you that

1 these other factors play very important roles as well.

2 DR. SNIDER: I don't think we're

3 disagreeing. I think this is a terribly important

4 issue, how they're transformed. I guess all we're

5 saying is it's not the only issue and therefore we're

6 having some trouble with this simplification because

7 somebody -- depending upon the particular

8 circumstances, some of these other factors may weigh

9 more heavily or less heavily in our judgments about

10 how comfortable we feel.

11 CHAIRMAN GREENBERG: I think we're

12 beginning to get a feeling for the Committee's issues

13 on this agenda item so as you talk, remember, we have

14 to address Vero cells in a more specific way, so Dr.

15 Wolfe?

16 DR. WOLFE: Just to add another dimension

17 to the chart because the chart is in two dimensions

18 and I think if one adds a third dimension that follows

19 some of the lines what Dixie said I think we would get

20 there. But I think that without talking about Vero

21 cells because that is the special case and different

22 that the Vero cells themselves are in this other

23 dimension where we would like to be for some of these

24 other things because we know much more about it, so I

25 think that some of the things in three or four or

1 whatever else will move towards being a special and
2 more known information about kind of case with more
3 research. I think that's really what I hear everyone
4 saying.

5 CHAIRMAN GREENBERG: I totally agree, but
6 example, my example would be WI38s if they were
7 transformed by a single gene. We have tremendous
8 experiment with those cells and if we think, we're not
9 sure that that single gene that WI38s will remain
10 WI38s except for that, but at least I know a lot about
11 WI38s as opposed to some other diploid cell that
12 you've never heard of before that somebody walks in
13 the door with and transforms. That would be a very
14 big difference in comfort level for all of us.

15 DR. WOLFE: So I guess what I'm saying is
16 that we can move, I mean with more research we can
17 start moving things towards the special case.

18 CHAIRMAN GREENBERG: Exactly.

19 DR. WOLFE: That's all.

20 CHAIRMAN GREENBERG: Dr. Lewis?

21 DR. LEWIS: Yes, there were two additional
22 conditions that are not apparent in the tabulation of
23 these things. And in the first condition, special
24 regard to categories 3 and 4, the first condition was
25 that the cells would have to be -- to meet all the

1 requirements from current cell substrates and then
2 there was a second condition in which any additional
3 recommendation should be followed. The point of that
4 is to give very broad leeway in terms of what needs to
5 be thought about and what needs to be done, what needs
6 to be applied to looking at this. And I don't think
7 we are trying to give the impression here that if
8 people came up with a marginal proposal that it would
9 be any way, that it would any way go unchallenged.

10 So I think what you're hearing is an
11 appended or amended table will be helpful, especially
12 with those conditions.

13 DR. WOLFE: Those items were actually on
14 the fuller chart, the one per page and just need to be
15 out.

16 CHAIRMAN GREENBERG: Ms. Fisher?

17 MS. FISHER: I think it's really difficult
18 and must have been difficult for you to draft
19 proposals, policy proposals for the use of neoplastic
20 cell substrates in the production of vaccines and it's
21 even harder to, I think, make recommendations as to
22 what road to go down considering the fact that so much
23 is still unknown about the testing methods used to
24 detect adventitious agent contamination and as Dr.
25 Krause mentioned, no specific PCR methods to amplify

1 nucleic acids, no reverse transcriptase detection
2 method, cannot detect latent viruses, maybe other
3 viruses not detectable. And when he was talking, I
4 thought about, I believe the legal duty of the FDA to
5 insure purity of the product, not relative purity of
6 the product and that's why I'm uncomfortable with the
7 idea of thresholds for adventitious agent
8 contamination or residual cell substrate DNA in these
9 products because that puts you into the relevant
10 category.
11 CHAIRMAN GREENBERG: I think the problem
12 that we have here, Ms. Fisher, is how we assign a
13 number to nothing. The difficulty is that the cells
14 would be tested by current technology and then we
15 would strive to go below current technology to the
16 point where, as we've suggested there is less than
17 one, evidence for one infectious unit per million
18 doses. So this -- but the thing that we need to
19 realize is that all this number represents is our
20 attempt to define nothing and you can only be so good
21 at defining nothing and when you start assigning
22 numbers to nothing which we think we almost, which is
23 going to be required when we're considering risk of
24 these sorts of things. This is the best we can do or
25 some number thereof is the best we can do at this

1 point in time. It's easy to say that or to think in
2 terms of absolutes, but when you're asking to document
3 the absoluteness, then you have a major problem and
4 there's no way to avoid that conflict.

5 CHAIRMAN GREENBERG: I would just remind
6 everybody around the table that this conundrum, while
7 quite important in the area we're looking at,
8 biologics exist in the theoretical way for all drugs,
9 so when you take a drug and you feel it's pure, nobody
10 has proven that every molecule in the pill is what is
11 said to be and it's not possible to prove that, but to
12 the ability of either atomic resolution of HPLC or
13 whatever test is done on that drug, that states how
14 pure it is, but there will always be other further
15 tests that can be done.

16 MS. FISHER: No, I understand that, it's
17 just that these vaccines are required by law and the
18 standard, it seems to me, has to be higher than
19 anything else that we apply and I think that if you're
20 going to move down this road, then there has to be
21 full public disclosure of the unknowns which I don't
22 think the public has been aware of up to this point in
23 terms of adventitious agent contamination.

24 CHAIRMAN GREENBERG: Thank you. Could I
25 remind the audience, I think Nancy has reminded that

1 cell phones are annoying and distracting and yes, I
2 know who it was. He's part of my audience here.
3 Please turn off your cell phones or put them on the
4 stun mode. Any other -- if not, I think -- do you
5 feel, Andy, that you've gotten, and Phil, that you've
6 gotten a sense -- David?

7 DR. STEPHENS: One other comment, we were
8 just discussing the use of the term neoplastic which
9 I think may be a difficult one in terms of eventually
10 selling this particular product and I think that the
11 word transformed may be somewhat better than that
12 particular word.

13 CHAIRMAN GREENBERG: I think that's a very
14 important and critical issue and I would simply say
15 that I was worried that that was a beeper, somebody
16 was pushing me.

17 (Laughter.)

18 I would say that without further
19 discussing the FDA needs to think long and hard about
20 the word that they choose to describe this entity and
21 that is very important for the public and for policy
22 and I think it's a very, very important point and the
23 best possible that is most descriptive and accurate to
24 characterize what you're doing should be used and I
25 don't know whether it's neoplastic or transformed or

1 some other word and I don't think we'll figure that
2 out at this moment.
3 Anything else? Okay, then we're going to
4 move from theory into a little bit more substantive
5 issues, equally, certainly equally important and Dr.
6 Sheets is now going to give us history and
7 characterization of Vero cells.
8 DR. SHEETS: Thank you. Is there a screen
9 saver on that or is it okay? I don't need to put a
10 password?
11 Good afternoon. I guess it's afternoon.
12 Now while all of you would rather be having lunch
13 right now instead of listening to me, I appreciate
14 your patience and I'll try to be brief but this is a
15 complicated area. I have a lot of information that
16 I'm going to try to convey and I'll try to do it in
17 the most concise way possible.
18 I'm in the Office of Vaccines. My name is
19 Rebecca Sheets and I'm here today to talk about the
20 history and characterization of Vero cells as a cell
21 substrate for viral vaccine production.
22 Next slide, please. CBER has regulatory
23 authority to regulate viral vaccines, investigational
24 vaccines, according to the Code of Federal
25 Regulations. This authority is to insure product

1 safety. I think a lot of discussion that we've heard
2 already is about how to define these terms like safety
3 and purity. These are relative terms. There's no
4 guarantees in life and nothing is safe. Air is not
5 safe. Water is not safe in an absolute. It's all
6 about relativity, unfortunately, and I know that
7 that's a difficult concept to understand. We want to
8 insure parents that their baby is safe when they get
9 a vaccine, that they're not going to get anything that
10 they're not supposed to be. Unfortunately, we deal in
11 a reality world where it's safe to the level we can
12 measure it. And that's, I think, a lot of the
13 discussion that we've gotten to.
14 So while we have the authority to insure
15 product safety, it is a relative level of safety. And
16 we do not have the authority to dictate to
17 manufacturers what product they should make or what
18 cell line they should make it in. All we can do is to
19 tell them you have to show us that it's safe. And we
20 must provide them guidance on how to demonstrate that
21 safety. If they come in with a product made in Vero
22 cells and we say we're concerned about the safety of
23 Vero cells, then they have to know what can we do to
24 make you comfortable to know that Vero cells are safe?
25 So we have to come up with guidance for them.

1 In addition, it's important to remember
2 that CBER licenses products for intended uses or
3 clinical indications. We don't license cell lines.
4 We don't approve cell lines. We get the whole meal
5 deal. We license a product and that product is made
6 in whatever cell lines it's made in and it's either
7 inactivated or it's whatever it is. That's the
8 product. That's what we license. So we don't have
9 the luxury of saying these are acceptable cell
10 substrates that are approved and licensed, so I think
11 these concepts need to be kept in mind as we have the
12 discussion this afternoon.
13 Next slide, please. Guidance for industry
14 on the characterization of cell lines to produce
15 biologicals is provided in certain documents including
16 a Points to Consider document written by CBER and
17 published by CBER. Throughout the rest of my talk
18 when I refer to the Points to Consider, there are
19 multiple Points to Consider documents. There's one on
20 monoclonal antibodies. There's one on combination
21 products and one on DNA vaccines, etcetera. But the
22 one I'm talking about all throughout this talk is the
23 cell lines Points to Consider, this 1993 document.
24 In addition, guidance is available to
25 sponsors through the International Conference on

1 Harmonization. This is an organization that assembled
2 for the purpose of trying to have more consistent
3 guidance for sponsors from the European regulatory
4 authorities, Japanese regulatory authorities and the
5 U.S. FDA. And so the guidance, much of the guidance
6 is consistent between these two documents, although
7 probably not totally one for one. These guidance
8 documents apply to all viral vaccines except those
9 made in primary cells. And you've already heard some
10 discussion about primary cells. These include eggs
11 and primary monkey kidney cells.

12 It's also important to keep in mind that
13 guidance documents are published as recommendations
14 for regulatory submissions. They are not law. They
15 are not regulations. They are recommendations. So if
16 a sponsor chooses not to follow those recommendations,
17 then that's their choice and we have to deal with what
18 information we get and decide whether we have
19 sufficient information to assess product safety.

20 Next slide, please. Now I know we've
21 already talked about it a little bit, but because Vero
22 cells are continuous cells I just want to reaffirm
23 that a continuous cell line is one that is generally
24 heterogeneous. Many of these are not cloned cell
25 lines, so most of these continuous cell lines are

1 heterogeneous mixtures of cells that have selective
2 survival potential and therefore have survived in
3 culture beyond crisis. And this is usually due to an
4 accumulation of mutations or chromosomal
5 rearrangements during the extended culture. These are
6 the ones that are not transformed by known mechanisms,
7 that are just like Vero cells, just grew out and
8 survived crisis. They're generally aneuploid. They
9 can be both hyper or hypo diploid. They're
10 immortalized by definition and they can be tumorigenic
11 or not. So depending on the passage level and I take
12 your exception about passage level or population
13 doubling, and also depending on the number, location
14 and types of mutations, this can influence the
15 tumorigenicity and usually when we refer to
16 tumorigenicity, we're talking about the ability of the
17 cells themselves to form tumors in an immunosuppressed
18 rodent.
19 Also, because of the heterogeneity, banks
20 of the same substrate may vary in this quality. In
21 fact, Vero cells at different passage levels vary in
22 their ability to form tumors in immunosuppressed
23 rodents.
24 Next slide. The concerns of regulatory
25 authorities, we've traditionally been concerned about

1 tumorigenicity of continuous cell lines and the
2 possibility that there's a presence of oncogenic
3 agents, including previously unrecognized and
4 undetected agents. I think we've had a lot of
5 discussion about that already.
6 However, I want to point out that these
7 same concerns were actually expressed about human
8 diploid cells before they became an acceptable
9 substrate, so back in the 1960s there was concern that
10 if you use a human cell that you would actually be
11 propagating a human leukemic agent and that these
12 would be dangerous for use as vaccine substrates and
13 currently we have a lot of licensed products made in
14 these human diploid cell strains and they're really
15 actually probably what we would all like to think of
16 as a preferable substrate. So I think it's important
17 to keep this in mind that many of these concerns are
18 true for any cell substrate.
19 Next slide. I also want to make sure
20 we're all on the same page about what we mean about
21 cell line characterization. Each manufacturer must
22 characterize the cell substrates banked and used in
23 the production in their facility. What I mean by
24 characterization is they have to have documented the
25 history of the isolation and banking of the cell

1 substrate. They need to define its growth
2 characteristics so that they can look for stability of
3 that capacity. They should be looking at karyology
4 and tumorigenicity of the cells and they need to
5 assess the freedom from adventitious agents and again,
6 this is the relative freedom to the ability or the
7 limit of the detection methods that we have available.
8 Next slide. So the specific test for
9 characterizing cell banks include karyology and I'll
10 go a little more into tumorigenicity. The
11 tumorigenicity tests that are recommended include
12 tumor formation and this is assessed as progressing
13 nodules and lung metastases in immunosuppressed
14 rodents. So that's the definition of a positive
15 tumorigenicity test is one in which the cells have
16 progressively growing nodules and/or they metastasize
17 to distal sites.
18 Another way of assessing tumorigenicity or
19 rather oncogenicity is by colony formation in soft
20 agar. This is not the most highly recommended method
21 and in fact, the guidance document has some language
22 where this needs to be demonstrated to be more
23 sensitive than the tumorigenicity test if it's to be
24 applied for your particular substrate.
25 These tests are not necessary for cells of

1 rodent origin because all rodent cells tend to
2 demonstrate tumorigenicity in these kinds of tests.
3 And other cells are expected to pass the
4 tumorigenicity testing according to the current
5 guidance. Part of what we're trying to do today is
6 move beyond that, but the current guidance documents
7 expect that all other cells will pass tumorigenicity
8 testing as defined here.
9 Next slide. In addition, there's
10 extensive testing recommended for adventitious agents
11 and these include bacterial and fungal sterility and
12 I know there was a question earlier about how to
13 demonstrate that. This is a compendial test that is
14 described, the test methods are specified in the Code
15 of Federal Regulations. In addition, testing for
16 mycoplasma and in the case of insect cells,
17 sprioplasma, both cultivatable and noncultivable
18 mycoplasma should be assessed.
19 In some cases, we may want to look for
20 micobacteria and there are tests that are specified in
21 the CFR for either culture methods or guinea pig tests
22 for mycobacteria if that's a possible contaminant of
23 your cell substrate. And then finally, what we'll
24 focus a lot of time on is really talking about
25 viruses. This testing can be done in vitro or in vivo

1 and you can be looking for acute viruses that either
2 lyse the cells which is also called cytopathic effect
3 or CPE and for hemadsorbing or hemagglutinating
4 viruses, so at the end of the culture period the cells
5 will be exposed to red blood cells and look for
6 hemagglutination. In addition, testing is done to
7 look for latent viruses, for example, retroviruses or
8 other oncogenic viruses.
9 Next slide. The in vitro tests include
10 exposing monolayers of at least three cell types to
11 the supernatant fluids from the production cell
12 culture and one cell type should be of the same
13 species and tissue as a substrate. Another should be
14 human diploid cells and a third is the monkey kidney
15 cells. And again, at the end of the culture period
16 tests for hemadsorption and hemagglutination should be
17 performed as well as looking for CPE throughout the
18 culture period.
19 Animal-derived raw materials should be
20 tested according to the USDA regulations and they
21 should be certified to come from herds that are
22 believed to be free of the bovine spongiform
23 encephalopathy agent. This is generally based on
24 country by country. In other words, the U.S. and
25 Canada is believed to be free of these agents and so

1 if you certify that your serum is from cattle from
2 these countries, that's the sort of thing we're
3 expecting to find.
4 Next slide. The in vivo tests include
5 inoculating adult and suckling mice, embryonated hens'
6 eggs and when appropriate, guinea pigs, rabbits or
7 monkeys.
8 Next slide. For rodent substrates,
9 testing should be performed by looking for antibody or
10 rather sero conversion of mice, rats or hamsters, two
11 agents that are known to affect those animals by
12 taking specific pathogen free animals, exposing them
13 to the cell supernatant or to the cells and looking
14 for antibody production.
15 In addition, a test for lymphocytic
16 choriomeningitis virus is requested to be performed.
17 Now for human cell substrates, testing that's
18 recommended includes for Epstein-Barr virus,
19 cytomegalovirus, for hepatitis B and C viruses and
20 these tests are by in vitro techniques such as PCR.
21 But this depends, the recommendation is that this
22 depends on tissue source and donor medical history.
23 Next slide. Also, if appropriate, the
24 cell substrate should be assessed for papilloma
25 viruses, adeno viruses and HHV6. The more recent

1 guidance document includes HHV7. And retro virus
2 testing is recommended for all cell substrates by
3 transmission electron microscopy. This is a very
4 general assay. It's not specific for retroviruses,
5 but it's also an insensitive method, but it can detect
6 contamination of all sorts. And also, by reverse
7 transcriptase assays. Both the conventional test and
8 PCR based tests may be utilized and more recently the
9 Office of Vaccines has recommended that PCR-based
10 tests be applied to viral vaccines. And for rodent
11 cell substrates infectivity assays for retro viruses
12 is recommended.
13 Next slide. Now I want to get into the
14 Vero cells themselves. Vero cells are derived from
15 the normal kidney of an adult African green monkey,
16 the Cercopithecus monkey. This was performed in Chiba
17 University in Japan in 1962 and these cells were
18 passaged with a well documented history and they were
19 brought to NIH in 1984 at passage level 93. They were
20 subsequently submitted to the American Type Culture
21 Collection or the ATCC which established a bank of
22 them at passage level 121. This is referred to the
23 ATCC catalog as certified cell line 81. The vaccine
24 cell substrates that are proposed for use or are used
25 for the licensed product are all derived from this

1 ATCC bank. Therefore, vaccine banks are at passage
2 levels in the 130s and 140s.
3 Next slide. Initial characterization of
4 this cell substrate as a vaccine cell substrate was
5 performed by the Institut Merieux and they initially
6 characterized these cells for production of
7 inactivated polio vaccine, oral polio vaccine and
8 inactivated rabies vaccine. And here are the
9 publications where the information I'm about to
10 present is published.
11 Next slide. The reason for some of this
12 testing was to address these concerns of regulatory
13 authorities that we might be causing tumors in vaccine
14 recipients if there were contaminants in the vaccine
15 which may have come from the Vero cells and the
16 contaminants we were concerned about are unknown
17 oncogenic viruses in cellular DNA. So to address such
18 concerns, sponsors have undertaken characterization of
19 their Vero cell banks for tumorigenicity.
20 Next slide. The Institut Merieux which is
21 now Aventis Pasteur performed extensive tumorigenicity
22 and oncogenicity testing on a Vero cell at multiple
23 passage levels. In other words, not just at the cell
24 bank level or end of production passage level, but at
25 further passage levels well beyond the level at which

1 a vaccine would be produced.

2 Next slide. The tumorigenicity test, as
3 I described, is in immunosuppressed rodents. The
4 Institut Merieux found that immunosuppressed newborn
5 rats were the sensitive model for assessing Vero cells
6 and so they performed these tests at 10^6 or 10^7 cells
7 per animal and the readouts for this and I think again
8 it's important to keep this in mind that what we're
9 saying by tumorigenicity is looking at the size of the
10 nodules at the injection site and whether they
11 progress or regress at 21 days. So all these tests
12 are 3-week tests.

13 In addition, they're looking for lung and
14 node metastases. For all of these studies that they
15 published they use positive controls which essentially
16 all these cell lines are Hela cells and in each case,
17 the positive control does form progressing nodules in
18 the animals and often it forms metastases.

19 Next slide. The results that are
20 published are at the working cell bank to end of
21 production passage levels and at least ten passages
22 beyond, so between passages 137 and 159, the Institut
23 Merieux found no nodules were formed, not even
24 regressing nodules and that no metastases were
25 observed in 225 rats when 10^6 per rat were injected.

1 In one study in 10 rats at end of production passage
2 level, in this case, 146, no nodules were formed and
3 no metastases were observed when 10^7 per rat were
4 injected.
5 Next slide. Now this is the study where
6 they actually looked at the passage levels and at
7 varying passage levels. In all cases, these are end
8 of production passage levels and beyond. Basically,
9 what this is is looking for whether there were still
10 a nodule present at Day 21 and then assessing whether
11 it had regressed in size, if there was a nodule
12 earlier, that it's gotten smaller or whether it's
13 getting larger. And then finally looking for
14 metastases. From passage level 169 and below, in all
15 cases the nodules were regressing in size and there
16 were no metastases and this was at various numbers of
17 cells per animal, 10^6 and 10^7. So from 169 and below
18 with the Institut Merieux Cell Bank, they did not form
19 metastases or progressing nodules. From 191 and
20 above, at 191 and 211, at both cell concentrations all
21 the animals formed nodules. All of them were
22 progressing in size at Day 21 and many of the animals
23 had metastases. So this is more comparable to the
24 positive controls. In fact, this is identical to the
25 sorts of things they see with positive controls, but

1 at the passage levels just at the end of vaccine
2 production, and a few passages beyond 10 to 20
3 passages beyond vaccine production level, they did not
4 see these characteristics.
5 Next slide. Other tumorigenicity tests
6 are reported in these literature which include
7 inoculation into the cheek pouch of hamsters and
8 inoculation into nude mice. Both of these tests were
9 negative and what the Institut Merieux found was that
10 even the positive control rarely formed metastases in
11 the nude mice or the cheek pouch of the newborn
12 hamster, excuse me, hamsters, and so that's why they
13 applied the more sensitive rat test. They felt it was
14 more sensitive and they applied that to their banks.
15 Next slide. Now they have also performed
16 what I refer to as oncogenicity tests. These are
17 tests that assess, don't assess the ability of the
18 cell to form a tumor in an animal, but either assess
19 the ability to have some characteristic of
20 transformation in culture or that the cellular
21 components might be able to cause a tumor in an
22 animal. Basically, the tests they performed in human
23 muscle organ culture or the ability to grow and form
24 colonies in soft agar were, and this is a quote from
25 one of their papers, rather in favor of tumorigenicity

1 of Vero cells. In addition, they tested DNA by the in
2 vitro in NIH 3T3 cells looking for transformation and
3 these tests were negative. Many of these data and
4 this was as question that came up earlier, many of
5 these data, both the tumorigenicity and oncogenicity
6 testing have been replicated at FDA and published in
7 this 1987 paper and basically the conclusions that
8 were drawn by the FDA, they weren't just looking at
9 Vero cells, they were looking at other cells was that
10 these in vitro methods are not reflective of the
11 ability to form tumors in vivo and so that's why the
12 recommendation to use in vivo tumorigenicity testing
13 has persisted in the guidance documents. In addition,
14 this paper is in your packet, the packet of
15 information that went to the Committee. In addition,
16 there's another paper in that packet from our sister
17 agency in Canada where they also assessed
18 tumorigenicity of the Vero cells and while their
19 interpretation may be slightly different, I think you
20 need to look at the data for yourself. In fact, the
21 standard interpretation of regressing nodules and no
22 metastases, in their hands, the Vero cells also pass
23 the tumorigenicity test.
24 Next slide. Now what I'm going to do is
25 give you a composite characterization of the Vero

1 cells. Basically, this is a table that lists all the
2 testing that has been done by any sponsor, so this is
3 all the testing that's been submitted to products
4 under IND in the U.S. No one sponsor has performed
5 absolutely every test and many of these tests have
6 been performed by each or most sponsors, so I'm going
7 to present that data now. We're having a multi-media
8 approach here, so I'm going to go to the overhead now.
9 Now I'm probably standing in someone's way
10 and I apologize. Basically, what I'm listing here is
11 again I want to reaffirm that this is testing that's
12 been done by someone, not all tests have been done by
13 every sponsor and I'm not telling you which sponsor
14 did what here, but testing has been performed at
15 multiple stages of Vero cells.
16 The master cell banks, working cell banks,
17 what I'm going to refer to as production cells which
18 is usually control cells that are run in parallel with
19 the production and so they're not actually infected
20 with a viral vaccine, but they're run in parallel so
21 that you can look -- sometimes the vaccine virus can
22 interfere with the testing, so control cells might be
23 used. Or end of production passage levels. That's
24 cells that were not necessarily in production, but
25 were passaged out to a level beyond the end of which

1 cells would be at during production.

2 And testing that's -- all of this is

3 testing that's recommended in the 1993 Points to

4 Consider for Cell Lines, including bacterial and

5 fungal sterility, mycoplasma testing, both

6 cultivatable and noncultivatable. I'll go into the

7 tissue culture testing more in a minute. Suckling

8 mice, adult mice, guinea pigs, rabbits and embryonated

9 hens' eggs have all been exposed to either supernatant

10 fluids or in some cases cells and one test that's

11 recommended, if appropriate, has not been performed

12 and that's testing in monkeys, although tumorigenicity

13 testing has been done in monkeys and I'll show you

14 that in a minute, but specific adventitious agent

15 testing has not been done in monkeys.

16 Transmission electron microscopy has been

17 performed. Retro virus testing has been performed by

18 the RT assay, both conventional and PCR-based RT

19 assays. Retro virus infectivity and again, I'll go

20 more into this, showing you the cell lines that have

21 been used for either co-cultivations or direct

22 supernatant inoculation.

23 In addition, at least one, if not more

24 sponsors has performed MAP testing on the Vero cells

25 looking for the ability of mice to produce antibodies

1 to murine agents. Porcine parvovirus and the bovine
2 agents that are recommended in the 9 CFR testing have
3 been performed. Testing for LCM, the lymphocytic
4 choriomeningitis virus of mice.
5 In addition, human Epstein-Barr virus and
6 human CMV have been tested. The hepatitis viruses
7 have been tested. Human papilloma viruses have been
8 tested. Although recommended, if appropriate, in the
9 Points to Consider, human adenovirus has not been
10 looked for. Both HHV-6 and HHV-7 have been looked for
11 and microbacterium has been looked for by both
12 methods.
13 Now to go into more information about the
14 cell cultures, basically, these cells have either been
15 co-cultivated or supernatant fluids from the cells
16 have been exposed to human diploid cells, to the Vero
17 cells themselves to primary rabbit kidney and primary
18 monkey kidney, primary human amnion or a human amnion
19 cell line and Hela cells, Hep-2 cells are like Hela
20 cells, chick cells, monkey cells of all different
21 kinds, so basically looking for viruses that these
22 cells are susceptible to the viruses of many different
23 species, but they use human, monkey, rabbit and
24 various kinds of monkeys to look for adventitious
25 agents.

1 The retrovirus infectivity, again, these
2 were co-cultivations or direct inoculations. In some
3 cases they were on induced cells, induced Vero cells
4 and here they were looking at all kinds of murine
5 agents, looking for simian foamy virus, simian
6 immunodeficiency virus. They're looking for -- this
7 is a thal erythro leukemic line, so it's susceptible
8 to multiple kinds of retroviruses. They're looking
9 for HIV. They're looking for human retroviruses. So
10 they did a variety of retrovirus testing, co-
11 cultivations or direct inoculation to look for
12 retroviruses of multiple species. This is in addition
13 to doing the TEM and the RT test.
14 Now sponsors have also done additional
15 testing which I should have changed -- it's not that
16 it's not recommended, it's that it's not described in
17 the current testing and we recommend that any
18 additional testing you want to do, please do so. It's
19 very helpful. Sponsors have performed testing for
20 simian immuno deficiency virus, simian STLV, herpes
21 viruses, adeno-associated virus, Mason-Pfizer monkey
22 virus, a retrovirus of monkeys, SB-40, simian CMV,
23 bovine polyoma virus and the HIV-1 and HIV-2. These
24 have all been performed by in vitro techniques such as
25 PCR or Southern Blot, in addition to this one

1 infectivity test I just described earlier. So all of
2 these agents have been looked for by sponsors.
3 In addition, the WHO has done some testing
4 that would add to this list. I know Herpes B virus
5 has been looked for and I think that simian adeno
6 virus has been looked for, although human adeno virus
7 has not. And I don't know whether those primers would
8 be cross reactive, but basically a lot of testing has
9 been done and in all cases, all of this testing has
10 been negative.
11 And then finally here, this is
12 characterization of the other -- the non-adventitious
13 agent characterization. Basically, karyology and
14 isoenzyme analysis have been performed. Some -- one
15 or more sponsors have performed DNA fingerprinting to
16 look at the stability of the cells at the master cell
17 bank and endoproduction level to see that the DNA
18 fingerprint has not changed over time. This was a way
19 of assessing the stability of the Vero cells.
20 In addition, tumorigenicity and
21 oncogenicity have been assessed in newborn rats, in
22 African green monkeys, in nude mice, amolygous monkey
23 and hamsters and all of these tests were with cells at
24 10^6 or 10^7.
25 In addition, oncogenicity testing has been

1 done on cell extracts or vaccine concentrate which is
2 essentially a cell lysate or the cell extract is an
3 uninfected cell extract lysate. And here, 10^6 or 10^7
4 cell equivalents in nude mice or newborn rats. In
5 addition, DNA has been assessed in newborn rats.
6 There's been growth in organ culture looked at and
7 colony formation in soft agar.
8 With the exception of these last two, the
9 conclusions that have been drawn from the testing is
10 that Vero cells are not tumorigenic of oncogenic. The
11 conclusion, as I explained later about these last two,
12 was that it was "rather in favor of tumorigenicity."
13 So those are the data, the composite of all the
14 testing that's been performed by any sponsor.
15 Next. It has to warm up a little bit.
16 Okay, next slide. Investigational vaccines have been
17 proposed to be made in Vero cells and these include
18 live attenuated vaccines, both of the conventional
19 type and recombinant type vaccines, but they're still
20 live; attenuated vaccines. And they've been proposed
21 for use as prophylaxis vaccines. In addition, live
22 vectors where the virus that's being grown is not the
23 disease antigen that's being used for the vaccine, but
24 in fact, they're expressing the vaccine antigen.
25 These are all recombinant and they're being proposed

1 for prophylaxis or therapy.

2 Inactivated vaccines, both of the

3 conventional type and recombinant vaccines, are being

4 proposed for prophylaxis, and purified subunit

5 vaccines, either purified from virus or recombinant

6 type products are proposed for prophylaxis.

7 Next slide. The investigational vaccines

8 are proposed for use either or adults and they're

9 either delivered by mucosal route, intranasal or oral

10 or they're injected. These include vaccines which may

11 be minimally purified or highly purified. So, for

12 instance, the injectable vaccines proposed to be used

13 in infants are highly purified. And the parenteral

14 vaccines proposed to be used in adults are either

15 minimally or highly purified. In addition, and this

16 is not a one to one correlation, even though it may

17 look that way, there are live viral vaccines proposed

18 to be given by the mucosal route in infants and the

19 parenteral injection for infants are all inactivated

20 or subunit vaccines, whereas for adults they're either

21 live or inactivated, injectable vaccines.

22 Next slide. Vero cells are favored by

23 manufacturers as a continuous cell substrate for the

24 production of viral vaccines including minimally

25 purified live viral vaccines. Obviously, we have a

1 licensed product that is a purified inactivated
2 product, so we deem that an acceptable cell substrate
3 for those kinds of products, but the question really
4 is now there are many of these investigational
5 products that are only minimally purified. The reason
6 that Vero cells are favored to produce these kinds of
7 vaccines is that they're susceptible to infection with
8 a wide variety of viruses, so they can be used to make
9 many different kinds of viral vaccines, so for some of
10 the larger manufacturers where they're making many,
11 many different products, this is a good quality. They
12 use the same cell bank that they've characterized to
13 produce vaccine X and vaccine Y and vaccine Z.
14 In addition, they grow well in bioreactors
15 on micro carriers and I think you've heard some in the
16 open public hearing from some of the -- at least from
17 Wyeth, you heard this facilitates growth in serum free
18 media or media that's free of animal products and
19 obviously that introduces an element of primary and a
20 risk of adventitious agents that we'd like to avoid.
21 And finally, they produce a high yield viral titre and
22 this is important for scale up, for being able to meet
23 the demands for a successful vaccine and for -- as
24 Wyeth said the higher the titre, the less cell
25 contaminant per virus that's there so they can dilute

1 it further and have a purer product.

2 In addition, there's a great public health
3 need for the types of vaccines that are proposed to be
4 produced in Vero cells.

5 Next slide. Licensed, purified and/or
6 inactivated vaccines, we have years of experience and
7 millions of doses have been given. There's more
8 limited experience with minimally purified live viral
9 vaccines, but thousands of doses of these kinds of
10 products have been given under investigational new
11 drug applications. And there are no data from
12 clinical trials or clinical experience that are
13 clearly correlated and clearly indicate adverse events
14 that are associated with the usage of Vero cells.
15 Most of the adverse events that are observed have been
16 correlated with, for instance, the live virus that's
17 in the preparation, rather than with the cell
18 substrate. And this is true for all the cell
19 substrates, I think, well, primary cells being aside.

20 Next slide. However, there are unresolved
21 questions about this information because there's no
22 long term active follow-up for recipients of vaccines
23 manufactured in Vero cells. When, for instance, when
24 the IPV was licensed in the 1980s, we did not have as
25 much work going towards requiring long term

1 post-marketing or large scale post-marketing studies.
2 We're trending more towards requiring those for new
3 vaccines now. But the IPV that's licensed and the
4 products that are licensed in France, they haven't
5 really done these kinds of long-term 20, 30, 40
6 follow-ups on large numbers of vaccines.
7 In addition, the testing that's done for
8 any of these investigational products, it hasn't gone
9 into that many people and minor or low frequency
10 adverse events may not have been detected as yet.
11 Next slide. Data supporting the safety of
12 Vero cells includes the tests by sponsors for known
13 agents, have all been negative. These include tissue
14 culture tests, animal tests, retrovirus tests, generic
15 tests, transmissional electron microscopy, PCR for
16 specific agents and the bacterial fungal sterility
17 mycoplasma and microbacterium. Extensive use of the
18 Vero cells of diagnostic and research laboratories has
19 also not revealed any contaminating viruses. While I
20 didn't present any of this information, I think
21 there's an extensive experience with Vero cells
22 outside the realm of vaccines held production, vaccine
23 virus production and I think that shouldn't be
24 ignored.
25 Next slide. In addition, tests to detect

1 unknown agents or tests that are capable of detecting
2 a virus which might not presently be known have been
3 negative. This includes injecting lysates into
4 immunosuppressed newborn rats and nude mice, injecting
5 cells into immunosuppressed animals, including
6 cymalogous and African green monkeys and various
7 rodents. And extensive tissue culture search for
8 adventitious agents has been negative. This would
9 have detected agents that propagate in the cells
10 tested. That's a caveat. There's no evidence of
11 retroviruses by PCR based RT assay and there's no
12 evidence of viral contamination by transmission
13 electron microscopy.
14 I believe that's my last slide. And now
15 I have the questions for the Committee discussion.
16 CHAIRMAN GREENBERG: What I'd like to do
17 first, Becky, if you don't mind, you gave us a lot of
18 data which was very helpful and before we do directly
19 to the questions, I'd just like to ask the Committee
20 members if they have any questions of all this
21 information. I'm sure there might be questions as we
22 go over your questions, but just generic questions
23 about Vero cells that were not answered?
24 Dr. Wolfe?
25 DR. WOLFE: If you had a larger budget at

1 your disposal, could you just tell us, maybe, the top
2 two or three priorities that you have from your
3 observation or other people in your group could answer
4 with respect to undone research with respect to Vero
5 cells. What would you like to know that you don't
6 know? You alluded to at least one thing, but could
7 you just -- not a long list, the top three priorities.
8 DR. SHEETS: I think the more cutting edge
9 research, looking for unknown agents would be the top
10 priority and I think that Dr. Lewis and Dr. Krause
11 have described a little bit what those kinds of tests
12 might be looking for inducible retroviruses, looking
13 for agents by these more generic tests, micro arrays
14 and that sort of thing. Did you want to add anything
15 to that?
16 I think it's the unknown agents.
17 Obviously, there's been a pretty extensive
18 characterization for known agents by one or more
19 sponsors, but it's the unknown things that are always
20 going to be the problem.
21 DR. WOLFE: You mention in your slide the
22 surveillance which is --
23 DR. SHEETS: Yes, I think long term
24 surveillance, I'm not sure, and I guess I would ask
25 Dr. Patriarca if he'd like to comment. These

1 epidemiological studies, I think, are important either
2 as post-marketing surveillance or as more uniform,
3 like our VAERS system, but I'm not sure the logistics
4 of doing all that. Did you want to comment?
5 So I think that long term surveillance
6 would be very helpful for all of these substrates
7 because emerging topics come up, as you very well
8 know, concerns about thimerosal or about, you know, RT
9 in chicken cells, etcetera. These things emerge and
10 having long-term follow-up to look for adverse
11 outcomes in vaccinees would be very helpful.
12 Dr. Krause?
13 DR. KRAUSE: One of the things that could
14 be done over the long term would be to maintain banks
15 of serum on individuals, just a slide of the
16 population over time to get an idea of whether -- in
17 order to have samples available if later somebody were
18 to allege that an adventitious agent were in a vaccine
19 and we would have pre-vaccine introduction serum
20 specimens readily available to do those kinds of
21 tests. But that, of course, would require someone to
22 establish such a bank and to maintain it.
23 CHAIRMAN GREENBERG: Other questions? By
24 the way, my own addition to that list would be to try
25 to think of other ways to understand what happens

1 between passage 149 and 169 which I like the Vero
2 cells at 142 and I don't like them at 169 and I want
3 to know why.
4 Dr. Huang?
5 DR. HUANG: Another issue I'm not sure if
6 we've really touched on is the fact that when we now
7 think back to the polio virus vaccines that we've been
8 using and the discussions of possible contamination
9 with both SV40 and Hooper's Hypothesis of
10 contamination possibly with HIV, we wish now that we
11 had saved samples and materials and substrates so that
12 we could easily go back and look at all of this and so
13 I would suggest that certainly as we progress in using
14 Vero cells or any of the other cells, that we don't
15 throw things away and that when we have batches of
16 vaccines that we put some of that away and we not use
17 it all up.
18 CHAIRMAN GREENBERG: The beauty, in fact,
19 of Veros is that they're in the ATTC as opposed to
20 those original polio cell substrates which were
21 primary which we don't have. So you are 100 percent
22 right and I think we will be in better shape if we can
23 use cells like Veros for that purpose.
24 DR. SHEETS: May I respond to that? I
25 think it's important for manufacturers to hear that

1 concern because they have the clinical specimens to
2 bank. In addition, certainly what was not necessarily
3 done in the 1960s and 1970s, but is now part of good
4 manufacturing practices is to keep samples from
5 production lots. Now how long they're kept, I think
6 that may depend on the manufacturer's freezer space,
7 etcetera, but they're certainly required to be kept
8 for some period of time, according to good
9 manufacturing practices which was not necessarily in
10 place in the 1960s.

11 CHAIRMAN GREENBERG: Ms. Fisher?

12 MS. FISHER: Vero cells are, under certain
13 conditions, tumor producers, so the biological
14 mechanism is there, correct?

15 DR. SHEETS: At various passage levels it
16 has been shown that Vero cells behave like known human
17 tumors in immunosuppressed animals.

18 MS. FISHER: Right.

19 DR. SHEETS: Whereas at other passage
20 levels they do not seem to have that capacity.

21 MS. FISHER: Do not seem to. But the
22 mechanism is there, so we do not know with any
23 certainty that the Vero cells that we have been using
24 have not contributed to cancer.

25 DR. SHEETS: We don't know that anything

1 that we've ever used is --

2 MS. FISHER: No, but I mean the

3 theoretical possibility is still there.

4 DR. SHEETS: The theoretical possibility

5 exists that -- I mean that's the concern. That's been

6 the long held concern. It was the same concern that

7 was expressed by Sabin and others about use of human

8 diploid cells. And it's true that we haven't done

9 these kinds of long term surveillance studies or we

10 don't have -- when they have been done, they're in

11 small numbers of individuals that have been followed

12 for maybe 20 years or 25 years. So it's very

13 difficult to draw conclusions about that sort of

14 question.

15 MS. FISHER: And I have one other

16 question. Is there an alternative to continuing to

17 rely on these cells? Is there another way to produce

18 these vaccines?

19 DR. SHEETS: It depends on the product.

20 Some products, for instance, let's use HIV as an

21 example. If you were going to make an inactivated or

22 live attenuated HIV vaccine, you would have to use

23 either a transformed or a tumor-derived human T-cell

24 to propagate the HIV. So for some products it's not

25 possible. For other products, they will grow in other

1 cell substrates, but the viruses may grow to such low
2 titer or have such poor propagation that they do not,
3 either you can't get enough clinical trial material to
4 even to do the clinical trials and certainly they
5 wouldn't be commercially feasible vaccines.
6 CHAIRMAN GREENBERG: Do we have other --
7 what I'd like to do is get background clarification
8 done with so then we can address the specific items in
9 the FDA's questions.
10 Dr. Minor?
11 DR. MINOR: Are all Vero cells from
12 sponsors the same? I mean if they put them through
13 the same tumorigenicity kind of studies would you see
14 the same thing you see with Institut Merieux because
15 I would have predicted that you might not because they
16 carry on under different conditions?
17 DR. SHEETS: Not all sponsors have done
18 the same level of extensive characterization that the
19 pioneer group did. They have all -- well, let me back
20 up. All these other products are under IND, so
21 they're not licensed, so they may not have -- by the
22 time of licensure they may have done more extensive
23 testing, but at a minimum what we're seeing is that
24 they do the single test in rodents usually end of
25 production passage level cells. They are all getting

1 these cells either from the ATC -- well, they're all
2 getting them from the ATTC either before they went to
3 the Institut Merieux and the WHO bank or after that.
4 So they're all coming from the same original source,
5 but they're all being handled differently in their --
6 so they're all being characterized in their own labs.
7 They've all done these immunosuppressed animals. Some
8 of them do nude mice. Some of them do rats, etcetera,
9 so they aren't uniformly being handled, but they are
10 all assessing tumorigenicity and they've been
11 negative.
12 CHAIRMAN GREENBERG: I'd like to get one
13 clarification. The ATTC is dishing it out at passage
14 of approximately 120 and none of the manufacturers are
15 going beyond passage 150 in anything they describe to
16 you?
17 DR. SHEETS: That's correct.
18 CHAIRMAN GREENBERG: So while there is
19 somewhere in the range of 30 passages and there's
20 plenty of room for divergence there, it's not back at
21 passage 1. It's relatively demarcated from where the
22 manufacturers are starting and where they're stopping,
23 right?
24 DR. SHEETS: Yes. All the banks are in
25 the passage levels from 130 to 140s and so the end of

1 production passage level is around 150 or earlier.

2 CHAIRMAN GREENBERG: Okay.

3 DR. SHEETS: It depends on where the banks

4 are.

5 CHAIRMAN GREENBERG: Yeah.

6 DR. BLAIR: Yes, just two questions. One

7 is if most, if not all, of the tumorigenicity studies

8 on the three week read?

9 DR. SHEETS: Most are.

10 DR. BLAIR: Most are. And secondly, is

11 there any data on sort of P53 suppressor genes in

12 Vero? Is that data known?

13 DR. SHEETS: There was that one paper that

14 I included in your packet. I think they were looking

15 at oncogenes, not tumor suppressor genes.

16 DR. BLAIR: Yes.

17 DR. SHEETS: I'm not sure whether there's

18 been specific looking at p53 or RB. Does anyone else

19 know that? I don't think so. I don't know of any

20 data.

21 CHAIRMAN GREENBERG: Other -- okay. I

22 think it's time for you to put up your --

23 DR. SHEETS: I'll go through these and

24 then I'll put up an overhead that has all of them

25 listed.

1 CHAIRMAN GREENBERG: Okay, and for all of
2 you, I think you all have it, but this is I think the
3 single piece of paper in your packets entitled
4 "Discussion Points for the Committee Regarding Vero
5 Cells." Correct?
6 DR. SHEETS: Yes, thank you. What we'd
7 like to have the Committee discuss is, in fact, CBER
8 has received numerous IND and pre-IND proposals to use
9 Vero cells to produce viral vaccines including live
10 viral vaccines that are given intranasal or orally, as
11 well as live, inactivated or recombinant sub-unit
12 vaccines for injection.
13 The target populations for these products
14 include infants and young children. They also include
15 adults and older children.
16 CBER has received numerous IND -- I'm
17 sorry, next slide. Some of these vaccines, including
18 live viral vaccines that will be administered
19 intranasal or orally are minimally purified and the
20 purification is done to clarify the vaccine viral
21 harvest of cells and cellular debris. Others of these
22 products are more highly purified, for example, by
23 chromatography or sucrose gradient centrifugation and
24 they may have sterile filtration or dialysis that
25 would help remove any live cells.

1 Next slide. Considering the information
2 that's available about tumorigenicity and adventitious
3 agent characterization of the Vero cell line, we would
4 like you to please discuss the suitability of the cell
5 line as a vaccine substrate, including its use for the
6 production of live viral vaccines that are minimally
7 purified.
8 Next slide. We'd ask that you please
9 include in your discussion the following topics:
10 residual cellular DNA. I think we've had a lot of
11 discussion about DNA today, so we'd like to discuss
12 that in the context of Vero cells, but it's important
13 for you to recognize that CBER has not previously set
14 a limit on the amount of residual cellular DNA in
15 mucosal vaccines and that's, as you said earlier, we
16 eat DNA every day and the intranasal or oral vaccines
17 end up in the gut and so we haven't required the
18 setting of a limit for the amount of residual cellular
19 DNA in these vaccines given mucosally. Nor have we
20 set a limit for injectable vaccines made in diploid
21 cells such as the ones we've already discussed.
22 We would ask that you discuss your
23 concerns if you have any regarding the amount of
24 residual Vero cell DNA in a human dose of vaccine and
25 talk about it in context of both a delivery by mucosal

1 route or by injection and as a guidepost the WHO
2 recommendation currently is for products to have less
3 than 10 nanograms per human dose of continuous cell
4 DNA.
5 Next slide. Also, some of the vaccines
6 are not filtered. They're minimally purified by
7 centrifugation so there is a theoretical possibility
8 or there's -- it's not theoretical, but there is a
9 possibility that residual Vero cells could be present
10 in these unfiltered vaccines that are given intranasal
11 or orally. We'd ask you to please include in your
12 discussion whether production processes should be
13 required that remove live cells.
14 Next slide. And I think this is the most
15 important. The next two are going to be the most
16 important for you to discuss, whether additional
17 testing including testing for adventitious agents or
18 tumorigenicity should be performed by manufacturers on
19 their Vero cell banks.
20 Next slide. If there are any other
21 concerns that you may have regarding the use of Vero
22 cells to provide viral vaccines, what are your
23 recommendations to sponsors for how to address these
24 concerns.
25 Thank you. I appreciate your attention.

1 CHAIRMAN GREENBERG: Okay. I'm just
2 trying to think how we should address these issues.
3 It seems to me that and maybe I'm wrong, that these
4 all actually might be addressed at least at the end in
5 sort of statements from each of us about what we
6 think. But I guess what I'd like to get, at least
7 when we start is just the feeling from each of you
8 about any points addressing any of these issues. And
9 I'm going to start out saying something categorically,
10 you can all disagree. My feeling is I'll take the
11 easiest one since that's my prerogative for me and
12 that is that there should be a clear cut assay that to
13 the best of our ability demonstrates the elimination
14 of live cells in any vaccine that is delivered and
15 that -- I'm not sure what the right methodology is and
16 the manufacturers can each have their own methodology,
17 but there should be spiking experiments or something
18 like that to demonstrate the elimination of live cells
19 in what is being administered.
20 I can see no reason in my mind to allow
21 the ability for a vaccine to have live cells. Does
22 not anybody disagree with that? Okay, so we're done
23 with that one.
24 I got my points. Now you guys all have to
25 deal with the topics.

1 DR. SHEETS: And that covers both vaccines
2 given mucosally --
3 CHAIRMAN GREENBERG: That covers total.
4 I just don't see it. Of course, for Vero cells these
5 are monkey cells. In an immunocompetent individual it
6 would be hard to imagine how they could do anything
7 since there are xenografts, but it just doesn't seem
8 reasonable to me. It seems quite primitive to have
9 live cells there.
10 I open it up to the rest of you. Don't
11 overwhelm me here.
12 MS. FISHER: Well, we all know the answer
13 to that.
14 CHAIRMAN GREENBERG: I know you all know
15 the answer, but the chair has certain. Let's just
16 take -- let me just take a general feeling, polling.
17 How do we feel about going forward and extending the
18 use of Vero cells so we're already in this country,
19 basically permitting an inactivated polio vaccine that
20 is quite purified to be made in Vero cells. This
21 Committee, before any of us were on it, I assume,
22 agreed with that.
23 Now we're talking about using these cells
24 for live viral vaccines, some of which will be more
25 purified, some of which will be less. I think we've

1 had extensive discussion about what are the pros and
2 of course we know the cons are both known and unknown.
3 Are you convinced that the pros outweigh the cons as
4 a general sort of feeling?
5 And okay, Ms. Fisher?
6 MS. FISHER: Well, we may eat DNA every
7 day, but it is not of African green monkey origin.
8 And I believe that most mothers do not support the
9 idea of having their children exposed, no matter what
10 the route to DNA of African green monkey origin and I
11 stated at the SB40 conference in 1997 and I want to
12 state again that I think we should move away from
13 reliance on simian and other animal -- using those
14 ways to produce vaccines.
15 CHAIRMAN GREENBERG: Thank you.
16 As just a note of humor, if I was in
17 charge, you would eat nothing -- nobody would have any
18 DNA.
19 (Laughter.)
20 DR. SHEETS: And I think it depends on the
21 country you're in, but I think that's true in the U.S.
22 most likely.
23 CHAIRMAN GREENBERG: It is clearly true in
24 the United States. Any other -- simply addressing
25 whether we think as a generality we should move

1 forward with this? First Phil and then --

2 DR. MINOR: I think the issue is the Vero

3 cell grown vaccines that you have at the moment is

4 slightly different insofar as they are -- yes, the

5 polios are clearly highly purified. It seems to me

6 that if you have a highly purified product where you

7 have no cell substrate contamination at all, assuming

8 it was possible, that the cell substrate would not be

9 an issue in that particular context.

10 Where you're talking about minimally

11 purified materials, then I think maybe the cell

12 substrate does become more of an issue and I think

13 there are questions which occur to me, even if there

14 is no scientific basis for them for which I apologize

15 about the nature of what would happen if you had Vero

16 cell DNA encapsulated in a particular live viral

17 vaccine which you couldn't get rid of which then went

18 into your patient.

19 Now it may well be and I think from what

20 evidence there is that it would do absolutely nothing,

21 but it does seem to me that the evidence is not very

22 strong to say --

23 CHAIRMAN GREENBERG: That possibility

24 already exists with currently licensed vaccines, that

25 is, that the polio virus could encapsulate Vero DNA.

1 DR. MINOR: I think --

2 CHAIRMAN GREENBERG: Nobody has proven
3 that that is not so. So that issue, I think will
4 exist no matter what happens.

5 DR. MINOR: All right, polio is probably
6 not the best example. I like measles vaccines would
7 be a better example, I suspect.

8 CHAIRMAN GREENBERG: Then it would be very
9 hard to purify a vaccine.

10 DR. MINOR: But again, you're dealing with
11 a substrate which is nontumorigenic, whatever that
12 means. So the issue is does the tumorigenicity of the
13 substrate actually affect your concern about the DNA
14 that you may be introducing. And if you are concerned
15 about introducing DNA by this route, it seems to me it
16 doesn't necessarily matter whether it's going in
17 mucosally or parenterally because the virus will take
18 it in and protect it. Right?

19 Now having said that, I'm not clear that
20 there is a risk, but nonetheless, it does seem to me
21 it's a question which should be asked.

22 CHAIRMAN GREENBERG: Just so I have it
23 right, Dr. Minor has postulated that if some virus is
24 grown in Vero cells that virus, no matter how purified
25 it was, could pseudotype the Vero cell nucleic acid so

1 to speak, at least and hence, if your virus is going
2 to immunize the person, nucleic acid from the cell
3 line would be introduced into the host. Isn't that
4 what your hypothesizing?
5 DR. MINOR: That's right. It also seems
6 to me that it's testable. You could actually maybe
7 assess the amount --
8 CHAIRMAN GREENBERG: Right, it is
9 absolutely testable and of course, it is also below a
10 level of -- you run into the same detection since I
11 would actually argue another way and test Vero cell
12 nucleic acid in some sort of read out assay as opposed
13 to seeing how much got pseudotyped by a virus, but in
14 any case that's a theoretical risk, 100 percent. You
15 begged the question that I asked, however, which is
16 you added yet another con which is good. But what I'm
17 trying to get at now and what this Committee is
18 supposed to be trying to get at is just are you con or
19 are you pro, as a general feeling?
20 DR. MINOR: I think it's necessary to
21 proceed with caution.
22 CHAIRMAN GREENBERG: Okay. Dr. Kohl?
23 DR. MINOR: So that's a clear answer,
24 isn't it?
25 DR. SHEETS: No, we want clear answers

1 from you, Dr. Minor.

2 (Laughter.)

3 DR. KOHL: I'm going to give you another

4 clear answer. I think like everything we do on this

5 Committee, things aren't absolute and in the best of

6 all possible worlds, it would be great if we had the

7 world's safest cell line for every vaccine and that's

8 what we're striving for. And for some vaccines we

9 think we have safer cell lines.

10 I think the Vero cell or some cell like it

11 will have a role in certain vaccines and the one that

12 comes to mind that you mention in particular is HIV

13 and if there were an HIV tomorrow that looked good in

14 Vero cell line, I think we will probably all strongly

15 urge moving forward quickly with that. So what I'm

16 saying is I think there is probably going to be a role

17 for Vero cells or something like Vero cells.

18 I would hope we would proceed with that,

19 but with caution and where it's gradated for the total

20 necessity to use it and the risks involved versus the

21 disease we're trying to protect. So for instance, I

22 would not be in favor of an injectable unpurified Vero

23 cell based vaccine to prevent rotavirus, injectable,

24 unpurified.

25 CHAIRMAN GREENBERG: Right. Just to

1 remind everybody, you've got a spectrum, I think, from
2 the FDA, of INDs for a variety of vaccines that are up
3 there and my own impression was that respiratory
4 syncytial virus, parainfluenza virus, rotavirus, were
5 in fact, in some ways maybe even further along and
6 closer than HIV, so if the feeling is that only --
7 that's an important thing that Dr. Kohl raised. What
8 level are you going to be drawing this line?
9 Dr. Wolfe?
10 DR. WOLFE: I think that the point is well
11 taken that within the 53 INDs that we looked at during
12 the closed session, I think there were 28 of them that
13 were live viral vaccines so that if you look at the
14 data that are available or the data that are provided
15 thus far for these 53, you've seen an enormous
16 difference in terms of how many have had DNA assays,
17 as to how much is there, how many are live versus how
18 many are you killed, how many are mucosal versus
19 parenteral and so forth.
20 So I think that part of the answer to the
21 question should we go ahead with Vero can be answered
22 in seeing what I think is a disturbing spectrum within
23 the 53 that we've looked at as to (a) the diseases for
24 which they are being used as vaccines; (b) the extent
25 to which they are parenteral or not; (c) the extent to

1 which they are live or not and I think that within the
2 53, if one needed to make a regulatory decision about
3 green light or red light, there might be some green
4 lights, but there would be at this point a lot of red
5 lights which could possibly be filled with more data
6 between now and the time that the FDA is going to
7 consider approval, but I also noted that the FDA is
8 expecting another rush of pre-IND things coming into
9 IND.
10 I just think that there isn't any simple
11 answer and it's going to be case by case and
12 particularly when these are guidance up to a point and
13 not regulations that places an enormous burden on the
14 FDA to sort of say yes, no, whatever. So my answer
15 would be case by case, huge difference between the 53.
16 CHAIRMAN GREENBERG: I think that's good
17 advice and of course, remember, that these are
18 guidelines. They're here to help manufacture. I
19 think the FDA wants to get a feeling. You certainly
20 don't want manufacturers to move along for the next
21 five years thinking that they're going somewhere and
22 then get a no, but each vaccine will have to be
23 approved on the merits and that will be a weighing and
24 that's a heavy burden for the FDA and for this
25 Committee.

1 Diane?

2 DR. GRIFFIN: Well, as I'm sure everybody

3 in this room knows, viruses have to grow in cells and

4 therefore all virus vaccines are going to be

5 contaminated, quotes, with cellular DNA and so what

6 we're really talking about is what kind of cellular

7 DNA is going to be there and so that therefore to

8 address the concerns of Phil or whoever about that

9 you're going to package some DNA that then might

10 transform human cells or be in some way tumorigenic

11 which I guess is the primary concern, then I do think

12 that characterizing the cells, even though you're

13 going to remove, I mean given as a prerequisite that

14 all cells be removed, but still characterizing the

15 cells as extensively as we can by as whatever the most

16 modern methods are so that we at least understand

17 either why they're oncogenic or whether they are and

18 in what circumstances and have a better

19 characterization of those cells will be an important

20 part of our eventually being able to accept this with

21 comfort.

22 CHAIRMAN GREENBERG: Other issues that

23 people want to raise?

24 So let me -- I'm sorry, Dixie.

25 DR. SNIDER: Well, I just wanted to ask

1 Becky or others at FDA about this issue of delivery
2 systems because we've been talking about the INDs that
3 have come in of oral, intranasal or parenteral, but
4 there are other delivery systems that are being
5 thought about and developed and it seems to me that
6 some consideration needs to be given to moving away
7 from parenteral administration whenever that's
8 possible.

9 From what you said, it also suggests to me
10 that just because we move away from parenteral, we
11 shouldn't be satisfied with a messier product and take
12 some shortcuts and I just wonder what folks think
13 about moving more toward nonparenteral delivery
14 systems and purity of products that are not
15 administered parenterally --

16 CHAIRMAN GREENBERG: Dixie, I think that
17 may help us give the FDA some more specific advice, so
18 if I could reformulate what you said, I'd like the
19 Committee to pipe in now on whether they have any
20 differences in how they would evaluate Vero cell grown
21 vaccines that were administered parenterally first,
22 and by parenterally I mean either by injection or by
23 some vehicle that makes it go directly through the
24 skin in some other way versus mucosally, that is
25 orally, rectally, intranasal or some other process

1 where we're using traditional absorptive mechanisms of
2 the body to transport things that have been there for
3 the last million years.
4 Do we feel those are the same, the risks
5 are the same or not? And if so, can you sort of
6 quantitate your differences in feeling, specifically
7 about Vero cells and that really gets to one of these
8 things here, right, did we talk about oral? Any
9 feeling about that?
10 I'll pipe in. I personally feel that oral
11 or intranasal administration, I have -- and I don't
12 have tremendous -- well, I would assume that
13 contaminating nucleic acid can be shown to be less,
14 that there's a gradient and that less of the
15 contaminating nucleic acid in a vaccine is
16 systemically administered when you deliver the vaccine
17 orally versus parenterally and that number can be
18 quantified.
19 DR. KOHL: Harry, that's assuming that the
20 target for a downstream transmissional event is
21 somewhere systemic. What if it's in the nasopharynx
22 and you're delivering something nasopharyngeally? I'm
23 not sure we can make that assumption, that it's safe
24 for giving it nasopharyngeally.
25 CHAIRMAN GREENBERG: I think each member

1 of the Committee needs to address their points of view
2 here.
3 No?
4 DR. WOLFE: Again, it would be different
5 if it were a live versus a killed vaccine because then
6 even though you were delivering it via a nonparenteral
7 route you'd have more possibility.
8 CHAIRMAN GREENBERG: I think, yeah, I
9 think kill inactivated vaccines, okay, let's address
10 inactivated. From my own feeling, inactivation, viral
11 inactivation in some way is going to almost certainly
12 create a margin of safety. It depends what
13 inactivating mechanism you use. Were you to use
14 psoralins or some other nucleic acid inactivating I
15 would assume that you would -- although they
16 themselves have some problems, might lower yet even
17 more tumorigenicity problems.
18 I thought most of us here are concerned
19 about live viral vaccines as the biggest worry, is
20 that not the case?
21 No? Okay. And I think those are going to
22 be the biggest questions for us as they come up with
23 individual vaccines.
24 So I'm looking for input from committee
25 members about -- excuse me, Phil.

1 DR. MINOR: Is there data on the
2 tumorigenicity of polyoma DNA by mouth as opposed to
3 parenterally in mice? And is it not less?
4 DR. KRAUSE: There are data on the
5 infectivity and tumorigenicity of polyoma DNA given
6 orally and basically they gave a lot of polyoma DNA to
7 a lot of mice, could not get any tumors by having them
8 swallow it. That being said, there are experiments in
9 which polyoma virus DNA was fed to mice by a feeding
10 tube and in that case there was some evidence for
11 infectivity in a small number of animals, giving I
12 think 500 nanograms, is that right Andy?
13 DR. LEWIS: It was 500 yeah, 500
14 nanograms. The incidence was 1 in 18. I think at
15 1,000 nanograms it was 18 or 20 out of 20.
16 DR. MINOR: And how does that compare to
17 parenteral?
18 DR. KRAUSE: Parenteral was 2 nanograms.
19 So it's at least 100 fold and I guess the question is
20 if you put a tube in are you actually creating some
21 kind of a disruption in the mucosal wall that makes
22 the DNA behave sort of like a hybrid between an oral
23 and a parenteral administration. But if you actually
24 just feed the DNA to the mice, it's essentially, in
25 the experiments that were done, you do not get

1 infections.

2 CHAIRMAN GREENBERG: And those mice didn't

3 develop oral tumors?

4 Just simply -- in that one model.

5 DR. LEWIS: Since the material is

6 deposited directly in the stomach, no, they did not

7 develop oral tumors.

8 CHAIRMAN GREENBERG: He said feeding, so

9 that was not --

10 DR. KRAUSE: Right, but when fed they got

11 no tumors of any kind.

12 DR. LEWIS: They got no tumors and no

13 infections.

14 DR. KRAUSE: Right.

15 CHAIRMAN GREENBERG: Dr. Kohl?

16 DR. KOHL: Diane, correct me if I'm wrong,

17 I think there are models where herpes simplex, for

18 instance, given nasopharyngeally in infant mice will

19 cause an encephalitis as it infects some of the

20 anubation in that area whereas if you give it orally

21 or even systemically it doesn't cause that type of

22 illness, so some viruses have particular tropisms that

23 certain routes are just more devastating.

24 CHAIRMAN GREENBERG: So I think there are

25 two issues here, Steve. There's two issues. One is

1 adventitious agents and the other is oncogenicity.

2 DR. KOHL: And I don't see why there may

3 not be oncogenic scenarios where that may be the case.

4 DR. GRIFFIN: I guess I would just say --

5 that's whole infectious virus, so that is an important

6 distinction.

7 CHAIRMAN GREENBERG: This is a very hard

8 topic. I'm actually in my two years here I've never

9 seen this Committee at such a loss for words.

10 Dr. Huang?

11 DR. HUANG: I mean just to focus on your

12 worry of oncogenicity transformation of intranasal

13 epithelial cells, as with other epithelial cells we

14 have the turnover rates are so great that even though

15 we know that infectious virus will attach and go into

16 the central nervous system, transformation of surface

17 type cells, if that does, indeed, happen to any great

18 extent, the ability of -- their ability to survive and

19 propagate is much less than somatic cells.

20 CHAIRMAN GREENBERG: Dixie?

21 DR. SNIDER: Well, I think one of the

22 problems -- well, there are at least two problems

23 here. One -- this time of day it's hard to remember

24 what we've said in open session and what we said in

25 closed session that maybe needs to be repeated.

1 And the other thing is that we're talking
2 at a very generic level and I agree with Sid's point
3 earlier that I mean these are case by case decisions
4 and it's hard to talk about this in the abstract, but
5 having said that, I mean I would agree that I think
6 that oral administration or intranasal administration
7 should present a lesser risk to the recipient to
8 parenteral administration. Nevertheless, we shouldn't
9 be too laid back about intranasal or oral
10 administration or any other mucosal route that may
11 come about.
12 And we also have to be cognizant of th
13 economic issues here, not for the sake of the
14 companies' bottom line although I want the companies
15 to continue to be able to produce current vaccines and
16 develop new vaccines, but also we're talking about
17 vaccines for the developing world, so economic issues
18 are something important to keep in mind for the
19 world's population as well as for the companies'
20 welfare.
21 So I think I would want to agree with the
22 point you made earlier about the cells, but I think
23 it's very difficult to know without the context of a
24 specific vaccine how far to insist they go in terms of
25 purity and how far to go in terms of testing or what

1 specifically to test for, although to -- overall to
2 get to your first question, because I never weighed in
3 on it I think because we have studied Vero cells
4 extensively that I'm comfortable saying, in essence,
5 what Dr. Minor says, that yes, let's go ahead with
6 development of vaccines in Vero cells, let's make sure
7 that we do that with the proper precautions, being
8 cognizant of the things we don't know and try to learn
9 things such as why do they change in these subsequent
10 passages and so forth.
11 DR. BLAIR: I mean given the fact that
12 this is -- the whole advantage of this is it's a cell
13 line we have. We can test it at various stages. I
14 mean it seems like some of the questions about nasal
15 effects or others or potential hazard of different,
16 worse case scenarios can be tested on these cells
17 and/or required to be tested on these cells and that
18 there should be a way to at least eliminate the known
19 or possible known risks to using the cells and the
20 contaminations that would come from the cells. And
21 that that's -- that would lend itself to these cells
22 or this kind of an approach.
23 DR. SHEETS: What sort of readouts would
24 you expect if looking at intranasal application of
25 Vero cell DNA? I mean that's what you were just

1 proposing.

2 DR. BLAIR: I confess I don't know much

3 about intranasal injection of anything.

4 CHAIRMAN GREENBERG: Well, what readout

5 would you use for parenteral administration? It can

6 be the same for Vero cell. I mean what readout would

7 you use to better assess non-whole cell -- Vero cells

8 that are dead, because we've already said there's

9 going to be no live Vero cells in a vaccine, so what

10 more information do we want to get from --

11 DR. BLAIR: I guess the worse case

12 scenario if you inject a very large amount of Vero DNA

13 and do you see a response, a disease, an illness in

14 some susceptible system, whether it's nude mice or

15 hamsters or something else.

16 CHAIRMAN GREENBERG: Has that ever been

17 done, Phil?

18 Has Vero cell nucleic acid been

19 administered to test animals in large amounts?

20 DR. SHEETS: Well, the Institut Merieux

21 injected 10^8 -- oh, I'm sorry. I thought you said

22 DNA.

23 CHAIRMAN GREENBERG: I said nucleic acid,

24 yes.

25 DR. KRAUSE: Cell lysates have been

1 administered --

2 CHAIRMAN GREENBERG: Yes.

3 DR. KRAUSE: Which would include nucleic
4 acids, but I don't think anybody has actually purified
5 nucleic acids as nucleic acids and done that
6 experiment.

7 DR. SHEETS: They've done it parenterally,
8 not intranasal, to my knowledge.

9 CHAIRMAN GREENBERG: And there's no tumors
10 associated with it, cell lysates.

11 DR. SHEETS: I think that was some of the
12 information I presented in closed session this
13 morning.

14 CHAIRMAN GREENBERG: Okay, excuse me. Any
15 other comments?

16 Well, so the Committee seems to be -- I'm
17 not getting a lot more thoughts, so what I thought,
18 what I guess I'm going to do now is simply move
19 through each one of these bullets that the FDA has
20 provided us and ask each of you to give any of your
21 thoughts. If you have any other ways of helping me go
22 through this, let me know.

23 These are not again votes, these are just
24 thoughts. The first bullet, Becky, as best I can tell
25 is -- it's not much of a question here.

1 DR. SHEETS: It's to express your
2 concerns.
3 CHAIRMAN GREENBERG: Suitability of the
4 cell line as a vaccine substrate. I think we've done
5 that. So does anybody else want to express their
6 opinion on the suitability of this cell line as a
7 vaccine substrate?
8 Okay. Well, so then please include in
9 your discussions the following: residual cellular
10 DNA. So I'd like people to give me their feeling,
11 whether they have any strong feeling about how the FDA
12 should move forward with this issue, if Vero cells are
13 going to be used as a substrate and can I start
14 somewhere.
15 Dr. Minor?
16 DR. MINOR: I feel it should be measured
17 at least. I don't know what you do with the result.
18 CHAIRMAN GREENBERG: That's the second
19 safest thing. One hundred percent correct. I'm in
20 total agreement that it should be measured.
21 Can I push you a little bit further and
22 say how one is going to use that number?
23 Let's just break this down a little bit.
24 We already have rules about parenteral -- the amount
25 of DNA in parenteral vaccination, correct? Isn't that

1 where the less than 10 nanograms per dose comes from?

2 DR. KRAUSE: Actually, some vaccines that

3 are made in MRC5 WI18, for example, have quite a bit

4 more DNA in it than that and those are given

5 parenterally.

6 CHAIRMAN GREENBERG: It's a

7 recommendation. Do you want to say that for

8 parenteral administration of live viral vaccines from

9 Vero cells that number that is out there should exist?

10 I'm just --

11 DR. MINOR: You mean 10 nanograms?

12 CHAIRMAN GREENBERG: Yes, 10 nanograms.

13 DR. MINOR: I personally at this stage of

14 the game, I would draw a distinction between Vero and

15 MRC5s.

16 CHAIRMAN GREENBERG: Uh-huh.

17 DR. MINOR: I think we have to look at a

18 discussion on the effect of passage on tumorigenicity

19 in Vero cells and I think the burden of the discussion

20 has tended to imply that we think that that matters,

21 although it's not clear to me why, actually, but that

22 has been the discussion and if that's the case then I

23 think you need less from your Vero than you do if you

24 run MRC5 to my mind. If you're really going to say

25 it's a concern which it sort of is to me.

1 CHAIRMAN GREENBERG: Okay, I know I'm
2 being a little pushy here, but I just feel the FDA
3 needs our best guess.
4 Dr. Wolfe, how do you feel about --
5 specifically about nucleic acid in the Vero cell grown
6 vaccines?
7 DR. WOLFE: I assume there is some basis,
8 it's not as scientifically grounded and rational as
9 one would like for the recommended of less than 10
10 nanogram per dose so that at the very least I don't
11 know why that shouldn't be made more formal so that
12 people either don't measure at all which is what we
13 saw in some of those INDs or they have amounts that
14 might be over 10 nanograms. Unless someone disputes
15 the basis for the WHO recommendation, so I would favor
16 that.
17 CHAIRMAN GREENBERG: I'm blocking you, Dr.
18 Blair.
19 DR. BLAIR: I mean I think there is an
20 attempt being made to try and get a quantitative
21 number on as I think was said last fall the subject
22 that's been discussed for 10 years and everyone asks
23 how do you measure it and nobody ever does it. So
24 there is an attempt to at least try to get a
25 quantitative hazard of one measure of the potential

1 measure of hazard of DNA of -- that is inducing
2 tumors. I mean I would think as low as you can
3 achieve the DNA is probably the best level to have,
4 but I don't know what that is and I don't know whether
5 in a live vaccine, the way that it was described as
6 being prepared how low you can go or what you can do
7 to eliminate the DNA.
8 CHAIRMAN GREENBERG: Ms. Fisher?
9 MS. FISHER: Well, I don't think that this
10 Committee can state with any certainty that the
11 introduction of nucleic acids, in essence, the
12 introduction of foreign DNA and RNA of African green
13 monkey origin into the human body does not cause
14 chromosomal change and I just think it's extremely --
15 I think we need to know more before we go forward
16 using this cell line for other vaccines.
17 CHAIRMAN GREENBERG: Okay, Diane.
18 DR. GRIFFIN: I agree that it needs to be
19 measured. I agree to have at least a base of
20 information. If there are subsequent problems or
21 whatever, that we have an idea of what vaccines
22 contain. I can't imagine that these minimally
23 purified live virus vaccines are not going to have a
24 lot, I mean, way more than 10 nanograms, but whether
25 that matters or not is a totally separate issue, but

1 at least if we know what we're dealing with we have
2 our first piece of data.
3 DR. SHEETS: When it is measured, it's in
4 microgram quantities.
5 CHAIRMAN GREENBERG: Yes, Dr. Huang?
6 DR. HUANG: I think I've already
7 previously stated how I felt about this which is that
8 certainly when you're doing mucosal inoculations that
9 you can stand more DNA and if it's parenteral a lot
10 less.
11 Obviously, if it were cheap and easy such
12 as filtering out cells, to filter out DNA, then we
13 would say yes, we should go for the highest possible
14 capability of eliminating all DNA, but in the real
15 world we do have to make these choices and I think the
16 cost and the amount of vaccine that you can make when
17 you have to go through more and more processes, that's
18 going to have to balance out. But certainly to
19 measure and to know what you have is an important
20 start.
21 CHAIRMAN GREENBERG: Dr. Snider?
22 DR. SNIDER: I agree with Alice.
23 CHAIRMAN GREENBERG: Dr. Kohl?
24 DR. KOHL: I think how much DNA we
25 tolerate will, should depend upon how important or how

1 unique the vaccine is and how serious disease is that
2 we're trying to prevent and in that context I don't
3 think we -- I can give a blanket answer to the
4 question. I think it has to be individualized.
5 CHAIRMAN GREENBERG: And again, I'll
6 remind all of you that these are our guidelines to the
7 FDA who is thinking of putting out guidelines, so in
8 no case are we here sort of making hard and fast
9 rules.
10 And for the record, I actually will agree,
11 I think Alice said it best and I may say it even a
12 little stronger, I think very much the amount of
13 nucleic acid in a vaccine that is given parenterally
14 is of more concern to me than that that is given
15 orally, substantially more concerned, despite the fact
16 that I can really imagine given orally that something
17 bad would happen.
18 As a general rule I would go with that as
19 a minimum current WHO recommendations that I would
20 want- parenteral immunization from Vero derived
21 vaccines to be less than 10 nanograms. Now I could
22 change that if somebody had a great HIV vaccine that
23 was going to save all of Africa I might change how I'm
24 thinking about it, but in the abstract, I would feel
25 strongly and given -- I'll even push it a little

1 further and this is just my own opinion that I would
2 say maybe I'll give you ten fold for oral, so if it's
3 10 nanograms for parenteral, then maybe I'm up to 100,
4 it's going to be somewhere between 100 nanograms and
5 a microgram. It's going to be in that range that I
6 think -- I will bet that we come out when we look at
7 individuals. I wouldn't legislate that or write that
8 down, but there's got to be some play, I am sure, as
9 you talk about individual vaccines and you're going to
10 get somewhere between, I'll bet, a log and a 2 log
11 differential in thinking about it.

12 We're getting towards the end here, but I
13 want to keep focused because again this is very, very
14 important. It is very hard for all of us to think in
15 the abstract and we're all worried that in the
16 abstract we're going to make a mistake and that's --
17 I understand that.

18 The next bullet is whether additional
19 testing including adventitious agents and
20 tumorigenicity testing should be performed by the
21 manufacturer on their Vero cell banks. Well, we won't
22 know what testing they've done so additional testing
23 is hard to say, but -- so I'm going to start off here
24 and say that I sure as heck want what we've seen in
25 open session, I think, is extensive testing that the

1 Aventis Pasteur has done to characterize what they
2 have done and I would hope, my own feeling is that
3 each manufacturer, as they come forward, has at least
4 a comparable armamentarium of data of their
5 tumorigenicity and adventitious agents testing for
6 their product. That's my -- I'm starting off and I'll
7 let other people comment.

8 DR. SHEETS: And by that you mean
9 tumorigenicity of multiple passage levels and in
10 multiple species?

11 CHAIRMAN GREENBERG: I mean -- I would say
12 I would want to know, yeah, one of the things I feel
13 good about with the data you said is that there is
14 buffer of they're at around 143 and it's up at 169, at
15 least maybe it's up at 169. They didn't do 152,
16 unfortunately, but it looks like the next point is
17 169. I would not feel good if 142 caused no tumors
18 and 143 caused 10 of 10. That would not make me and
19 the vaccine came in at 142, that would give me
20 anxiety.

21 So I think yes, I would like to see -- I
22 don't know whether I need a lot of data before the
23 level of the vaccine is made, but at the level of
24 vaccine in some number of passages after it, some
25 buffer zone.

1 Sid?

2 DR. WOLFE: I'd just like to put in the
3 form of a recommendation at least what I was observing
4 and I can't remember if it was the open or the closed
5 session, but it doesn't make any difference.

6 CHAIRMAN GREENBERG: They'll jump on you
7 --

8 DR. WOLFE: It doesn't refer to any
9 product. I am very uncomfortable with the fact that
10 the data upon which the observation is made that it's
11 okay at 140 and it's not okay at higher is made with
12 underpowered studies that have (a) only 10 animals;
13 (b) the observational period is 2 weeks or 3 weeks
14 rather and one of them at 22 weeks there was as
15 positive finding; (c) the dose is either 10^6 or 10^7,
16 it may be worth, at least occasionally, trying a
17 higher dose; and finally, that in none of the studies
18 were primates used, I mean they did not use a doubling
19 or previously called passage of cells that was high
20 enough to cause problem in the primates. That does
21 not mean the primates are more resistant because I
22 think 137 or 140 was the highest. I think there are
23 some data that need to be clarified, otherwise, we are
24 magically having faith in this number of doublings in
25 a number of experiments that are really under power to

1 see things that might be occurring at lower doublings,
2 that's all.
3 CHAIRMAN GREENBERG: Thank you, Sid.
4 Other -- Diane?
5 DR. GRIFFIN: Well, and it's also to use
6 perhaps what we know of as better model systems now,
7 I think the only immunosuppressed -- genetically,
8 immunosuppressed mouse was the nude mouse which has
9 tons of NK cell activity and we know can reject tumors
10 and so Scid mice which may also have some of that, but
11 there are other, there are other kinds of immuno
12 compromised rodents that could be tested and followed
13 for a substantial period of time. I certainly
14 wouldn't use a 3 week magic cutoff. As I said, this
15 is not my area of expertise.
16 CHAIRMAN GREENBERG: Harry?
17 DR. LEWIS: Just a thought on the
18 tumorigenicity assays in monkeys. The first problem
19 you have in a situation like that is monkeys are not
20 syngeneic, they're allogeneic. So you would have to
21 overcome the allograft response which is basic to all
22 primates. In order to do that you have to
23 immunosuppress the animal, not once, but you have to
24 keep them immunosuppressed for a fairly long period of
25 time and I don't know that anybody has ever tried an

1 assay like that.

2 What you really need is a positive control

3 and I don't know that such a positive control exists.

4 So I have very much sympathy with worrying about this

5 problem, but I'm not sure how practical it is to try

6 to assess it the way Dr. Wolfe has in mind.

7 CHAIRMAN GREENBERG: You're just talking

8 with respect to primates, not the other variables,

9 right?

10 DR. LEWIS: Yes, exactly.

11 CHAIRMAN GREENBERG: Okay. Thank you,

12 Andy. The other -- I think we're getting -- Dr. Egan?

13 DR. EGAN: I'd just like one clarification

14 that everyone considers that considerable amount of

15 additional tumorigenicity test needs to be done even

16 if the additional passages, given the constraint that

17 we have that it will need to be validated, that there

18 are no Vero cells in the product. So these will be

19 filtered through .2 micron filters and etcetera.

20 We've already established that there will be no live

21 Vero cells in the product. So we accept that advice.

22 CHAIRMAN GREENBERG: Just so that you are

23 all on Bill's wave length there, the vaccine will have

24 no live Vero cells, the tumorigenicity studies that

25 we're talking about are with live Vero cells. That,

1 of course, gives us a lot of margin because if there's
2 no tumorigenicity with live Vero cells and we have no
3 live Vero cells in the product, we feel very good and
4 feeling that we've done a lot.
5 I don't have a big worry about that. I
6 don't see personally that this is an overwhelming
7 burden to put on the manufacturers. We're talking,
8 especially if we're not talking about primate
9 experiments in rodents. These are not killer
10 experiments.
11 DR. WOLFE: The other point that's been
12 made since we don't know the mechanism whereby
13 whenever or at whatever dose the transformation
14 occurs, it is possible that it does have some
15 interaction with the nucleic acid of the virus that's
16 growing there. So even though we are in a cell-free
17 future world, thanks to the recommendations here,
18 they're is still a concern.
19 CHAIRMAN GREENBERG: And -- Ms. Fisher?
20 MS. FISHER: I still think that you have
21 to go and you have to look at the nucleic acids and
22 whether or not the residual DNA and RNA, whether or
23 not it's causing chromosomal change that would damage
24 the immune system or cause tumor production. You have
25 to go down to that level and look at chromosomal

1 change.

2 CHAIRMAN GREENBERG: Thank you. Any other
3 points? Okay, I think we have a sense of the
4 Committee. Does the FDA, did they hear that? You
5 guys heard that? Does it make sense to you? I mean
6 does it make sense that you understand what the
7 Committee said?

8 DR. SHEETS: Yes, it makes sense. I think
9 the one factor that we haven't even tried to talk
10 about today that we also struggle with is at what
11 stage of product development do you require a plethora
12 of testing.

13 CHAIRMAN GREENBERG: I don't want to go
14 there right now.

15 DR. SHEETS: So I guess what I'm saying is
16 that because only one product is licensed and that
17 product had extensive testing, obviously, there is
18 still the open opportunity for products in the
19 pipeline to be tested --

20 CHAIRMAN GREENBERG: What Dr. Huang said
21 that if we were dealing with a more homogeneous cell
22 line, we might be able to not have to worry about each
23 manufacturer of cells as much.

24 I'm going to get to the very last bullet
25 now. Any other concerns? This is a grab bag to catch

1 everything that hasn't been caught already. Are there
2 any last thoughts that any of you have about this
3 issue?
4 Dr. Huang?
5 DR. HUANG: I'll just stress something
6 that was said earlier and that was we're so concerned
7 about cancer and tumorigenicity and all this that we
8 tend to forget some of the other things that are just
9 as important. I believe that Dixie mentioned
10 immunosuppression and I would add neural toxicity as
11 things that one needs to look at either with a product
12 or with a cell line and some of these tests are
13 relatively easy to do and I think we shouldn't just so
14 be concentrated on cancers that we forget about these
15 other things.
16 CHAIRMAN GREENBERG: I totally agree. I
17 think the focus on cancer was because of the fact was
18 that we're now dealing with cells that resemble
19 cancer, but the problems of neural toxicity or other
20 problems are with us, in fact, with all forms of
21 vaccination and as we heard yesterday or potentially
22 they are from additives in a vaccination.
23 Dixie?
24 DR. SNIDER: If I understand correctly I
25 think what we're saying to the FDA and the

1 manufacturers is that we would like to see a
2 standardized set of tests for tumorigenicity and for
3 adventitious agents.
4 In addition, depending upon the particular
5 vaccines and routes of administration, there may be
6 additional tests that would need to be done and one we
7 discussed earlier would be -- and wouldn't necessarily
8 have to be done perhaps with every vaccine, but the
9 whole question about intranasal administration of Vero
10 cell DNA, if you're going to have a product that winds
11 up having a substantial amount of that DNA still
12 there, even though we're not going to have whole
13 cells, there still might be a substantial amount. And
14 so there are going to be, there's going to be this
15 core set of tests and then ceratin additional tests
16 that would be done depending upon particular
17 circumstances.
18 CHAIRMAN GREENBERG: So the FDA needs to
19 use some sense as each individual vaccine comes up to
20 model the safety constraints for that vaccine.
21 If there are no other issues, okay. I'd
22 like to thank all of you. This is a highly --
23 DR. EGAN: Don't worry, Harry, it's not
24 another issue. I just wanted to thank everybody, you
25 know, for their thoughts, deliberation and very

1 crystal clear advice about having any residual cells
2 in the vaccine, whether it's parenteral or oral. I
3 thank you for that.
4 I think it's also very clear, we've got a
5 lot of work to do and also I think it's very clear
6 that I think we were going to be coming back to this
7 Committee on many occasions with very specific
8 vaccines with regard to these continuous cell lines
9 and I promise to do that.
10 CHAIRMAN GREENBERG: I'd like thank
11 everybody also. I think the Committee said they're
12 willing to hear and in some ways the individual
13 vaccines will be somewhat simpler to deal with because
14 you'll be able to sink your teeth into a specific
15 issue, so I'd like to thank all of you. This was the
16 hardest one to run that I've had and thanks and have
17 a good weekend.
18 (Whereupon, at 2:03 p.m., the meeting was
19 concluded.)
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