1 So what are factors that could influence
2 adventitious agent risk in a vaccine? Well, if you
3 are thinking about the cell substrate obviously, the
4 species from which the cell substrate comes can have
5 an influence on the kinds of agents you would look
6 for. You would look for different agents in non-human
7 versus human cells, for example.
8 The cell type or tissue of origin
9 obviously makes a difference. Under this I include
10 three points. Various previous exposures while the
11 cell was still in its host could have an influence on
12 the kinds of adventitious agents one might want to
13 look for.
14 For instance, if one were dealing with
15 cells that were derived from fetal tissue, one would
16 look for agents that are known to be able to cross the
17 placenta, for instance, whereas if they are derived
18 from an adult, one would look for appropriate viruses
19 for that, and depending on the type of tissue, one
20 might look for agents that are trophic for those kinds
21 of tissues.
22 Tumor association also could influence
23 one's thinking about adventitious agent risk, and in
24 particular, the knowledge of the transforming event
25 could potentially mitigate that, and in the designer

1 cell substrates that we're talking about today, if one
2 does have definite knowledge of the transforming
3 event, then perhaps one might be less worried about
4 the tumorigenicity of those cells, although if a cell
5 line were derived from an actual tumor, one might be
6 more concerned, especially if one had no knowledge of
7 the transforming event.

8 And then the ability to bank the cells and
9 to characterize them in some detail before they're
10 actually used such that they can be used each time in
11 the same way is also a very useful property in terms
12 of reducing adventitious agent risk.

13 And then whatever is known about the
14 maintenance or passage history of the cell obviously
15 also is an important component of thinking about what
16 kinds of adventitious agents need to be looked for.

17 So if we look at potential vaccine cell
18 substrates and think about ones ability to
19 characterize them for adventitious agent risk, well,
20 whole animals, for instance, embryonated hen's eggs
21 (phonetic) or even the mice which are used to make
22 Japanese encephalitis virus vaccine may be less well
23 characterized than some of the kinds of cells that can
24 be studied in tissue culture before they're
25 inoculated, although in the case of embryonated hen's

1 eggs, one can use a specific pathogen free flocks,
2 which can substantially mitigate one's risk.
3 If one is using primary cells, and of
4 course, the classic example of this or the primary
5 monkey kidney cells were used in oral polio vaccines,
6 one has some period of time prior to inoculation to
7 characterize. One can also maintain uninoculated
8 control cells for longer periods of time as was done
9 in the production of that vaccine.
10 But it is, in general, less easy to
11 characterize these cells than it is either diploid
12 cell strains or neoplastic cell lines. So diploid
13 cell strains are cells like WI38 and MRC-5, which have
14 been used for many years to produce vaccines line
15 rubella and varicella, and because these cells can be
16 banked, one can characterize the bank of cells and go
17 back to it repeatedly and assume that it will be the
18 same each time.
19 Well, what about neoplastic cell lines?
20 They can be banked and, therefore, can be
21 characterized, and they actually also have several
22 other potential advantages which are worth considering
23 today as we think about designer cell substrates.
24 One of them is host range. In many cases,
25 there are viruses that can be grown in these cells

1 that simply can't be grown in other cells.

2 Another one is the ability to bank them.
3 Another one is that very often neoplastic cell
4 substrates can be adapted to serum free growth, which
5 is a property which is not as readily given to the
6 other kinds of cell substrates we talked about.

7 And they can be made, as in the case of
8 designer cell substrates, to express complementing
9 genes.

10 So why then would someone be concerned
11 about neoplastic cell substrates and adventitious
12 agents, and in particular with oncogenic viruses?
13 Well, if there is a neoplastic cell line for which the
14 mechanism of transformation is not completely
15 understood, there is, of course, always the potential
16 that an oncogenic virus was involved in the cell
17 line's neoplastic transformation.

18 And if that were the case, one would want
19 to know that and be certain that that virus did not
20 survive through to the cell substrate.

21 Some of these cell lines, just by virtue
22 of the fact that they grow very well and have been
23 around for a very long time in many different
24 laboratories are more likely to have uncertain
25 histories, and that provides an opportunity for

1 contamination of them.

2 And of course, when one is talking about

3 oncogenic viruses, oncogenic viruses have the

4 potential to lead to very severe consequences that are

5 quite difficult to evaluate in short-term clinical

6 studies.

7 So this slide shows the kinds of testing

8 that are normally done on cell substrates for viral

9 vaccines. So the tests are listed here to the left,

10 and I describe here in these two columns, one of them

11 whether there's an amplification step involved in the

12 test, which gives you some sense of the sensitivity of

13 the test, and to the right the potential to detect the

14 unsuspected, which then gives some sense of the

15 ability of this test to find something that one

16 doesn't know is there and to give some additional

17 assurance on this point of unknown or undetected,

18 unsuspected oncogenic viruses.

19 So tissue culture assays, of course, have

20 been used for many years in qualifying cell

21 substrates. If a virus grows in the specific cells

22 which are being used in the tissue culture assay,

23 there is an amplification step, and it can be fairly

24 substantial.

25 On the other hand, some viruses will not

1 grow in tissue culture, and so although there's an
2 amplification step and although there is the potential
3 to detect the unsuspected, there is a hole in tissue
4 culture testing. We try to make up for this hole by
5 animal studies, which include inoculation of eggs,
6 inoculation of animals with either death or weight
7 loss as an endpoint, and also animal antibody
8 production assays, the so-called mouse antibody
9 production, rat antibody production, and hamster
10 antibody production assays, which also work to the
11 degree that an agent will replicate in the systems
12 which are being dealt with and have amplification
13 steps associated with that, and in general have the
14 potential to detect the unsuspected as well with the
15 exception of the animal antibody production assays,
16 which only detect the agents which the antibodies are
17 shown to be directed at.
18 And then there also are molecular assays,
19 in particular, specific PCR, which is a very sensitive
20 way of finding a given agent, but it doesn't help you
21 at all in detecting the unsuspected, and then newer
22 assays like the PCR based reverse transcriptase assay,
23 which is very sensitive because it has a molecular
24 amplification step. It also has the potential to
25 detect any unsuspected retrovirus.

1 And then there's electron microscopy,
2 which is a very insensitive test because it does not
3 include any amplification step, but of these tests,
4 perhaps there's the greatest potential to detect the
5 unsuspected because it could potentially detect any
6 virus if it were there in adequate quantity.
7 I think it's worth saying a word about
8 comparisons between PCR and biological assays in just
9 thinking about testing of adventitious agents. PCRs
10 tend to be much more sensitive for small samples with
11 low residual DNA, and the reason is because the PCR
12 can detect a very small amount of nucleic acid, but it
13 only can detect it in a fairly small sample. So the
14 amount of a product or of a cell substrate that can be
15 evaluated by PCR is necessarily limited.
16 On the other hand, biological assays are
17 much more sensitive for large samples because you can
18 put many doses either into an animal or onto a tissue
19 culture flask.
20 PCR assays are very specific, whereas
21 biological assays have a greater potential to detect
22 the unknown. PCR assays will work independently of
23 growth characteristics, and so even if a virus does
24 not grow in a specific system that it's being tested
25 in, one can detect it by PCR.

1 Biological assays, on the other hand,
2 require growth in a specific system.
3 On the other hand that's also a flaw in
4 PCR because a positive PCR result doesn't necessarily
5 mean that there's a live virus.
6 Biological assays, on the other hand,
7 provide a more relevant endpoint. For instance, it's
8 only in a biological assay that one can detect
9 oncogenicity.
10 So if one's interested in improving one's
11 ability to detect oncogenic viruses, I guess one way
12 to look at that is to think about the methods which
13 have been used to discover oncogenic viruses or
14 discover viruses in the past, and really four main
15 methods that have been used are listed on this slide.
16 These include animal inoculation and
17 looking for some kind of an endpoint, and for
18 oncogenic viruses, this has traditionally been the
19 appearance of tumors, thereby verifying the
20 oncogenicity of the virus. A tissue culture, electron
21 microscopy, and molecular methods.
22 Now, tissue culture and electron
23 microscopy are fairly well covered by the tests that
24 are generally currently asked for with viral vaccines.
25 So I'm going to go into a little more detail on animal

1 inoculation and molecular methods as potential ways of
2 better understanding or better providing greater
3 assurance that a cell substrate is free of an
4 adventitious virus.

5 Animal inoculation to look for a
6 tumorigenicity endpoint or an oncogenicity endpoint is
7 a method which has been used for many, many years, and
8 really this recapitulates practically the entire
9 history of virology. Rous sarcoma virus, for example,
10 was discovered in 1911 based on the ability of tumor
11 extracts to cause tumors when inoculated into animals,
12 and this method has been used to detect pox viruses,
13 papova viruses, and adenoviruses up through and
14 including the 1960s.

15 And again, I point to SV40 as an example
16 of a virus which was originally detected by this
17 method, and it is the kind of thing that one would
18 like to be able to avoid in dealing with cell
19 substrates.

20 If one looks at the ability of viruses to
21 induce tumors in animal assays, this slide shows some
22 examples of what happens when you inoculate these
23 viruses into either baby hamsters, mice, or rats, and
24 a yellow, which actually is orange on my screen, but
25 it looks sort of yellow from here, means that you do

1 get an oncogenic endpoint. So a tumor of some kind is
2 formed.
3 And what you can see is that if you use a
4 combination of hamster assays and rat assays, one has
5 a pretty good chance of detecting the viruses that are
6 detectable by these means, which include retroviruses,
7 polyoma viruses, and adenoviruses. In general, human
8 Herpes viruses and papilloma viruses though can't be
9 detected by these kinds of methods.
10 So what can we say about animal tests for
11 oncogenic viruses? Well, they could be used in cases
12 where additional confidence that a product is free of
13 adventitious oncogenic viruses is desired. We know
14 that many tumor viruses are cell associated, and so we
15 would argue that inoculating two animal models of
16 either rats and the hamsters with cell free lysates of
17 cell substrates, where lysates would have a better
18 chance of getting cell associated viruses, followed by
19 fairly extensive observation would lead to the maximum
20 sensitivity in these kinds of assays.
21 So what can one say about the kinds of
22 material that ought to be tested? I already alluded
23 to the fact that testing the cell substrate
24 independently of just the final product would have
25 some value, but I wanted to go into that in just a

1 little bit more detail here as well.
2 So testing a cell substrate has value for
3 insuring product consistency because that way you know
4 what the material is that you're dealing with each
5 time you make the product. If you're concerned about
6 the potential for existence of an adventitious agent
7 in a cell substrate, you certainly want to make sure
8 it's not there in the cell substrate rather than just
9 look at the final product because that's the only way
10 you can be sure that there is -- or it's the best way
11 to insure that there aren't going to be interactions
12 between the vaccine strain and whatever adventitious
13 agent is present.
14 You have to look at the cell substrate.
15 You need to know how much is there or isn't there in
16 order to apply the principles of viral clearance that
17 I discussed, and I mentioned that we think that
18 looking at the lysate probably makes a little bit more
19 sense than looking at the supernatant because the
20 lysate will also capture viruses that are cell
21 associated, although one could potentially make an
22 argument in favor of looking at supernatants as well.
23 What about looking at the final product?
24 Well, one concern with looking at final products,
25 especially of vaccines, of adenovirus vaccines is the

1 potential for interference. We heard that at very
2 high titers there is some leakage with these vectors,
3 and so there's the potential that cells that otherwise
4 might show an oncogenic phenotype will simply be
5 killed. If one inoculates enough of this stuff, one
6 will get a generalized inflammatory response, which
7 then also might interfere with an oncogenic endpoint.
8 And we know that E1 has an effect on
9 apoptosis, and E4 also has a little bit of a pro
10 apoptotic effect, which then might also lead to some
11 potential interference of a final product then with
12 whatever one might be trying to rule out in the
13 presence of a cell substrate.
14 On the other hand, testing of final
15 product would give assurance that the vector itself is
16 non-oncogenic, and so would potentially have some
17 value in this kind of assay.
18 It looks to me like a slide was skipped.
19 Can you back me up?
20 So I just want to talk a little bit about
21 where this is going and talk about molecular
22 approaches to virus detection and how one might be
23 able to use a newer, broadly specific approaches in
24 finding adventitious viruses.
25 And one approach which has been used to

1 discover several different Herpes viruses over the
2 last few years has been to use consensus PCR primers,
3 which are directed to the DNA polymerase region to
4 detect related viruses. So generic primers or
5 consensus primers, which detect Herpes virus DNA
6 polyemerases are used, and then samples that are
7 suspected to have new Herpes viruses in them can be
8 evaluated using those primers, and new viruses have
9 been discovered that way.
10 This same general scheme also was used to
11 identify the West Nile virus when it recently came to
12 New York.
13 Molecular subtraction assays also have
14 been used to discover viruses. These include methods
15 like representational difference analysis. Two
16 viruses have been discovered over the last few years
17 using this method. One of them is Human Herpes Virus
18 8 and the other is TTV.
19 And the problem with molecular subtraction
20 assays is that you need something to subtract from
21 your sample. So if you have a cell substrate, unless
22 you have something which is genomically identical,
23 except for the potential adventitious agent, it
24 becomes very difficult to interpret one's result.
25 In our laboratory we've been trying a

1 slightly different approach which we call
2 nonspecifically amplifying viral nucleic acids, and
3 the basic principal here is that instead of doing a
4 molecular substraction, we're doing a physical
5 subtraction in which we take a sample and attempt to
6 get rid of all of the cellular nucleic acids in a way
7 which preserves the viral nucleic acids and then use
8 completely nonspecific PCR methods to amplify what's
9 left over.

10 And the kinds of techniques we used for
11 that separation include ultra centrifugation and
12 nuclease digestion. We haven't investigated
13 filtration to a great degree yet, but we will.

14 And this next slide just shows one of our
15 early experiments in which we took fairly small
16 quantities of Varicella Zoster virus, spiked it into
17 a million Vero cells and applied this method to ask
18 whether we could find any VZV, and so we did these
19 nonspecific PCRs on the nucleic acid that we extracted
20 from this and ran them on these gels. We then cut
21 these bands out of the gel and cloned them, and both
22 to our delight and our surprise, all of these bands
23 turned out to have VZV sequences in them.

24 We have since taken this general approach
25 and have expanded it to a large number of other

1 viruses, including RNA viruses by adding a reverse
2 transcriptase step, single stranded DNA viruses,
3 smaller DNA viruses, retroviruses, have also looked at
4 cells that constitutively produce viruses in addition
5 to cells into which viruses are spiked and have been
6 successful in finding viruses under these
7 circumstances.
8 So we think that this kind of method has
9 a lot of promise also for doing a better job at
10 finding adventitious agents when one doesn't know
11 exactly what it is that we might be looking for.
12 Did I skip a slide here? Maybe not.
13 It's important in discussing any of the
14 issues that we're talking about today to not only
15 consider the theoretical issues that we're worried
16 about and some of the risks from viral adventitious
17 agents are theoretical, and these issues all have to
18 be placed in the context of the entire product.
19 And a very important component of that is
20 potential benefit of the product. And so it can be
21 easy to look at these kinds of issues and to become
22 discouraged by them, but these issues are only one
23 side of the equation, and it's important to remember
24 that.
25 So how do we think that this entire way of

1 thinking can be applied to Adenovirus Type 5
2 transformed human designer cell substrates for vaccine
3 production?
4 Well, if we think about TSE testing, as I
5 said earlier, it's important to consider the cell type
6 and potential exposures to BSE. The tests that one
7 can consider are a sequencing of the PRNP gene,
8 Western blot, or the ELISAs that Dr. Priola talked
9 about, and the idea of adding newer, more sensitive
10 tests as they become available.
11 Also we believe it's very important that
12 research subjects and investigators and IRBs be well
13 informed of these issues, and so the informed consent
14 investigator brochure and other documents should
15 reflect that need.
16 One other point I wanted to make about TSE
17 issues that I forgot to mention in my introduction is
18 that this entire issue will be presented in a separate
19 meeting of the TSE Advisory Committee in the near
20 future, and so the reason we're discussing TSE issues
21 so heavily at this meeting is really not for the
22 purpose of coming to a final resolution of them, but
23 instead for the purpose of having the Advisory
24 Committee discuss our general approach and also for
25 providing information to the Advisory Committee and to

1 the public on that general approach.

2 And then as this slide shows a potential

3 approach to virus testing of Adenovirus Type 5

4 transformed human designer cell substrates for vaccine

5 production, and that includes obviously to perform the

6 standard testing as I've show it, including extensive

7 tissue culture and electron microscopy. One would

8 want to insure that the test would detect any agent

9 based on the fetal origins of the cells, the cell type

10 and the cell history.

11 And we would argue that although the

12 mechanism of transformation of these cells is likely

13 Adenovirus Type 5 genes, we would recommend doing

14 extensive testing for potential oncogenic adventitious

15 agents as well, which would include cell lysate

16 oncogenicity testing and other tests as they become

17 available.

18 And as was the case on the other slide, we

19 regard it as being very important that research

20 subjects, investigators, and IRBs be well informed of

21 the issues associated with moving into these new kinds

22 of cell lines.

23 So thank you very much.

24 ACTING CHAIRMAN DAUM: Thank you, Dr.

25 Krause. You touched on many issues that I hope the

1 Committee will come back to during our discussion
2 period this afternoon, but for now we'll see if
3 there's questions specifically about the material that
4 you've covered.

5 Dr. Decker and then Dr. Goldberg.

6 DR. DECKER: It seems clear that the
7 designer cell substrates pose issues with respect to
8 the neoplastic line of tumorigenesis that are
9 difference from those posed by currently used cell
10 substrates, but it's not clear to me -- so if I'm
11 missing it, point it out -- it's not clear to me that
12 the designer cell substrates pose any new or different
13 issues with respect to adventitious agents.

14 DR. KRAUSE: I think that one really has
15 to look at each cell line by itself, and so there
16 certainly is the potential for that to be the case.
17 If, for example, the history of 293 cells as it's gone
18 through different laboratories is not as well known as
19 one would like, then that may be a special
20 adventitious agent related issue for 293 cells that
21 one would want to consider.

22 But I think that what you're really trying
23 to get at though is the question of the designer cell
24 substrates having a known mechanism of transformation,
25 and many of the issues that I discussed with respect

1 to neoplastic cells having to do with the notion that
2 if one doesn't know the mechanism of transformation
3 one might be more worried about certain kinds of
4 adventitious agents.
5 And I guess the argument that we would
6 make is that although we're fairly certain that we
7 understand the mechanism of transformation in these
8 designer cell substrates, in order to provide the
9 greatest possible public confidence as one moves into
10 cell substrates that are tumorigenic, even if we
11 believe them not to be oncogenic, that doing tests
12 that provide additional levels of insurance on that
13 point will bolster public confidence in these new cell
14 substrates.
15 DR. DECKER: Well, I'm trying to separate
16 the issues here, and I still haven't heard that the
17 designer cell substrates with respect to adventitious
18 agents pose any challenge that wouldn't be faced by
19 any new, non-designer cell substrate line.
20 In other words, if I was going to create
21 MRC-5 cells now new, you would ask the same questions
22 about adventitious agents and do you want me to do the
23 same things. The fact that these are transformed or
24 designer really has nothing to do with what you want
25 done for caution with respect to adventitious agents,

1 right?

2 ACTING CHAIRMAN DAUM: If I can help maybe
3 bridge the understanding here, I think he means with
4 respect to adventitious agents.

5 DR. DECKER: Yeah, only with respect to
6 adventitious agents, separating adventitious from
7 oncogenic. Okay? Two separate questions.
8 Contamination with PRP protein is a question, and the
9 ability to replicate that has nothing to do with
10 whether or not they're designed agents. It would be
11 true of any novel cell substrate, wouldn't it, that
12 we'd want to look at that?

13 DR. KRAUSE: One would want to look at it.
14 We might have greater concerns though about a cell
15 that has retinal origin, as was mentioned.

16 So since one of the designer cell
17 substrates we're talk -- I'm not sure how you can. I
18 agree with you that in general if the only difference
19 between a new diploid cell line and a designer cell
20 substrate is the fact that the designer cell substrate
21 has had some manipulation which has caused it to
22 become immortal, and if one also stipulates that
23 everything else about it is very well controlled and
24 that one understands the milieux in which that
25 occurred, and if one is absolutely certain that the

1 intervention that you made to that diploid cell line
2 to turn it into a designer cell substrate is, in fact,
3 what caused it to become immortal, then I think you
4 would be right.
5 I think that one can make arguments in
6 favor of doing the kinds of things that Dr. Hughes
7 spoke about to provide additional assurance on those
8 kinds of points. So if one adds an immortalizing gene
9 to a diploid cell strain and then demonstrates it by
10 turning it off, then the cell line is no longer
11 immortal, and that provides a very high degree of
12 assurance that that immortalizing gene is, in fact,
13 the only thing which caused the immortalization of
14 that cell.
15 ACTING CHAIRMAN DAUM: Thank you very
16 much.
17 Dr. Goldberg, please.
18 DR. GOLDBERG: Yeah. When you talked
19 about the needing a quantitative framework for
20 decision making, which goes to some of the questions
21 that were asked this morning, and the first thing that
22 you said was to estimate the pre-test probability of
23 a problem. I mean, have you any thoughts about what
24 you mean by that or can you --
25 (Laughter.)

1 DR. GOLDBERG: -- indicate to some of us?

2 DR. KRAUSE: Sure.

3 DR. GOLDBERG: To some of us who are

4 living.

5 DR. KRAUSE: I think it's very difficult

6 to do, but --

7 DR. GOLDBERG: I agree.

8 DR. KRAUSE: -- for example, in applying

9 the principles of viral clearance to a therapeutic

10 product, if you know by electron microscopy of a

11 certain sensitivity that there are viral particles

12 present which you believe not to be infectious, but

13 you just want to be sure. Then you start off with

14 some pre-test probability based on a positive electron

15 microscopy result if there was something there.

16 And so if you figure, just to pick round

17 numbers, if you could figure that the sensitivity of

18 the EM test allows you to pick up ten to the sixth

19 particles per cc, if you see something there that

20 implies that there could be as much as ten to sixth,

21 you want to have some safety factor built into that,

22 and so you may then require 12 logs of clearance if

23 you want to have a ten to the sixth safety factor.

24 If, on the other hand, that test is

25 negative, it's not clear to me that you would want to

1 start off with the assumption that it would have been
2 positive or that it would have been positive only at
3 the level of its sensitivity.
4 So if you believe the sensitivity of that
5 test, you'd be ten to the sixth. If that test is
6 negative, it's not clear to me that you would start
7 off with the assumption if there are 9.9 times ten to
8 the fifth particles there.
9 So I think that one has to look at each of
10 these products individually, but I think that the idea
11 of spending at least some time initially thinking
12 about how likely you think a problem is and using that
13 to guide the sensitivity of the assays that need to be
14 done is an important component of thinking about doing
15 this.
16 DR. GOLDBERG: Well, that gets back to the
17 kind of questions that Dr. Moulton raised this
18 morning, which really is what sorts of assumptions can
19 you make and what kind of distributions can you put on
20 the possible sensitivity or probability of detection
21 by any of these assays, and you're really accumulating
22 a battery of tests and so that you can model this and
23 get some ideas.
24 And the question is can you do this with
25 any of the even in vitro data to see what you might be

1 missing under various models and assumptions and has
2 anyone done that sort of work?
3 I'm not saying necessarily you.
4 ACTING CHAIRMAN DAUM: Can I interrupt
5 here for just a second?
6 DR. GOLDBERG: yes.
7 ACTING CHAIRMAN DAUM: I think this is a
8 very, very important point, and I think it's the
9 essence of what I would imagine our discussion is
10 going to be when we finish the presentations, but what
11 I'd like to just do is just make sure there's no
12 comments or questions about these presentations
13 because what you're hitting at, I think, is the
14 essence of where we're going to go with this.
15 You were first and then Dr. Katz and then
16 Dr. Kohl, and then we're going to move on.
17 DR. AGUILAR-CORDOVA: I think I would like
18 to follow up on the previous questions by Dr. Decker.
19 That is, if the designer classification just means
20 that you know one of the events that occurred in
21 immortalizing or transforming that cell line, and I'm
22 a big concerned on this because if one detects any
23 oncogenic transformation in any tumor cell line, then
24 you know that one event as well, and it would just
25 become the same as a designer cell, I would imagine.

1 And then it's only the infectious or
2 adventitious agents that we're talking about that you
3 would be concerned with; is that correct?
4 DR. KRAUSE: I guess I'm not sure I
5 understand.
6 DR. AGUILAR-CORDOVA: So as an example,
7 somebody spoke about A549s and 293 cells earlier on,
8 293 cells, you know, the E1A and E1B section. It's
9 A549s you find out that have a mutation in p53. Now
10 you know one transforming event, and you can take that
11 away or test for that.
12 If you know that, then would they become
13 designer cells?
14 DR. KRAUSE: No, I think the way we --
15 we've defined designer cells fairly narrowly as cells
16 which have been immortalized by defined means where
17 the immortalization is part of the design of the cell.
18 So those may well be cells that can be well
19 characterized and ultimately with enough information
20 one could determine they're safe to use.
21 But I don't think we would say that those
22 cells have the same degree of information about them
23 as a cell where one is starting with just defined
24 information about the mechanism of transformation.
25 ACTING CHAIRMAN DAUM: Dr. Katz, is this

1 about adventitious agent testing?

2 DR. KATZ: Absolutely. Not testing; just

3 an amplification or a clarification of those who are

4 less than 60 years old.

5 It was implicit in your statement, but I'm

6 not sure everyone appreciates that SV40 contamination

7 was not confined to live oral polio vaccine. It was

8 inactivated polio and inactivated adeno. because the

9 formalin step that was sufficient to inactivate those

10 viruses did not inactivate SV40.

11 And an anecdote at least for a long-term

12 observation, I can give you the names of three

13 individuals who were injected repeatedly with SV40

14 inadvertently in trying to prepare polio skin test

15 antigens, and none of us has a brain tumor, and we're

16 all still alive.

17 (Laughter.)

18 DR. KRAUSE: I'm pleased to know that.

19 ACTING CHAIRMAN DAUM: Thank you.

20 As we all are, Dr. Katz.

21 PARTICIPANT: But we're going to keep an

22 eye on you.

23 ACTING CHAIRMAN DAUM: Dr. Kohl, please.

24 DR. KRAUSE: And, in fact, Dr. Katz is

25 right. I did not mean to imply that it was only oral

1 polio vaccines. In fact, if you look at the people who
2 seroconverted to SV40, you had an easier time finding
3 seroconversion among people who received the
4 inactivated vaccines for precisely the reason you say.
5 DR. KATZ: We had very high titers.
6 DR. KRAUSE: Right.
7 ACTING CHAIRMAN DAUM: Dr. Kohl, is this
8 about adventitious agent testing?
9 DR. KOHL: Yes, adventitious agents only.
10 Phil, I enjoyed your talk, and the thing
11 that I'm sure causes you to lose sleep at night, and
12 some of us as well, is the unknown adventitial agent
13 that we, you know, at this moment can't even
14 anticipate.
15 And you mentioned some new molecular
16 studies, the subtraction studies, et cetera. Are
17 those being recommended or suggested? What's the
18 status of those for new products?
19 DR. KRAUSE: I think these are tests which
20 perhaps are not yet in a state where they can be
21 recommended universally in a regulatory sense because
22 they may not be well standardized enough or may not
23 have a good enough sense of what the controls ought to
24 be and so forth, but this is, I think, a direction in
25 which we need to move.

1 Certainly the kinds of primers which can
2 detect families of viruses are becoming better known,
3 and so if there are people in the audience who are
4 involved in putting together these kinds of tests, I
5 think it would be very valuable to do that.
6 ACTING CHAIRMAN DAUM: Thank you very
7 much.
8 I think at this point we're going to move
9 on to our final scheduled presentation. Dr. Golding
10 will review OVRR, CBER issues with the use of
11 adenovirus vector vaccines and their complementing
12 designer cell substrates.
13 Following her presentation, we'll have an
14 open public hearing, a brief break, and then we will
15 begin getting at these issues that the Committee is
16 chafing at the bit to begin discussing, which is a
17 good thing.
18 DR. GOLDING: Okay. So as many of you
19 know, I'm Hana Golding, the Chief of the Laboratory of
20 Retrovirus.
21 And the task that I was given today is to
22 try and put all of this very detailed and informative
23 talks that you have heard today into some sort of
24 perspective and to extract the essence of all of the
25 talks and then translate them into the key issue that

1 made our approach in trying to move forward into new
2 cell substrates, and specifically designer cell
3 substrate, into the arena of new vaccine development.
4 And while thinking of how to do that, I
5 thought it was really the right time to put it all in
6 the wider perspective of risk-benefit and to remind
7 the Committee that one of the driving forces behind
8 these efforts in CBER and specifically in OVRR that
9 started almost two years ago is the new development in
10 the HIV vaccine development field.
11 And I think we all are very aware of the
12 fact that the HIV epidemic is still continuing
13 unabated and with the 16,000 new infections a day,
14 that there is quite an increased disillusion with the
15 ability of antiviral therapies to either curtail the
16 epidemic or to cure infected individuals.
17 And I think there's increased hope and
18 belief that the appropriate vaccines that will be
19 tailored to countries around the world will eventually
20 lead to the scope of these epidemics.
21 And we're very excited. I think we're
22 having a very important period during this development
23 stage because many investigators both in academia and
24 in companies have taken up the challenge of developing
25 new vaccine approaches to HIV.

1 And what I tried to list here is some of
2 the new viral vectors and some other approaches that
3 have been developed and are in various stages of
4 development, and most of them still in preclinical,
5 but some are already in the clinic.
6 And there was a whole lot of viral vectors
7 that are currently under development starting with pox
8 viruses, such as canarypox, MVA and the NYVAC. You've
9 already talked about the Adenovirus 5, and they both
10 can be used as both replication competent and
11 nonreplicating vectors. Adeno-associated virus, the
12 Venezuelan equine encephalitis vectors, Seliki Forest
13 virus, and Herpes virus. This is not an inclusive
14 list, but are all types of nonreplicating vectors that
15 are under development.
16 And activated HIV vaccines are definitely
17 under consideration and new ways to inactive the virus
18 to increase the level of safety are under development
19 in multiple labs, and we shouldn't forget recombinant
20 plasmid DNA vaccines, as well as purified protein,
21 peptides and lipopeptides under development.
22 There are several bacterial vectors that
23 are under development, and in combination with all of
24 them are novel adjuvant, cytokine, and co-stimulatory
25 proteins. Many of the new vaccine approach will

1 include dual or with triple modalities, which have
2 been termed prime boost approach with one type of
3 vaccine that may be used for the priming, and then a
4 second modality will be used for boosting, and the
5 hope is that the appropriate arm of the immune
6 response will be generated to, indeed, protect
7 infection, and if not the initial infection, at least
8 reduce the initial viral load and slow the progression
9 of the disease.

10 So new HIV vaccine may require novel
11 substrate, and you heard a lot about the need for
12 complementing cell lines for nonreplicating viral
13 vector vaccines, and both the 293 and the PER.C6 are
14 good examples of them because they are providing in
15 France the E1A, 1B genes that are missing in the
16 vectors.

17 They may also be used for optimal
18 production of recombinant live, attenuated viruses,
19 and definitely new novel cells are required for
20 production of the activated HIV vaccines.

21 So what could be the advantages of
22 designer cell substrate as we move forward into these
23 new classes of cell substrate? As you heard during
24 the day, designer cell substrate as we define them are
25 derived from either primary cells or from well

1 characterized diploid cell lines.

2 The cellular localization is achieved with
3 nontransformed genes that can be either viral or
4 cellular derived, and therefore, the essence of the
5 immortal line genes and/or their product can be
6 closely monitored during vaccine production and final
7 product characterization.

8 I would like to then focus your attention
9 specifically on Adeno. 5 E1 transformed designer
10 cells, and again summarize the advantages that you've
11 heard during this day.

12 So Adenovirus 5 in cells is non-oncogenic
13 in humans. For the more adenovirus or Adeno. 5 E1A/B
14 transformed cells are not tumorigenic in uni-competent
15 (phonetic) animal models. The reason is that the
16 Adeno. 5 E1 expressing cells are killed quite
17 efficiently by cytotoxic T cells and by natural killer
18 cells.

19 In addition, as you heard from Dr. Cook,
20 Adeno. 5 E1 expressing cells are highly sensitive to
21 mediators of apoptosis, such as TNF alpha.

22 We also know that Adeno. 5 from swollen
23 (phonetic) cells are only weakly tumorigenic and
24 immunodeficient athymic nude mice, as you heard from
25 Dr. Andre Lewis, with a TPD-50 of around 6.5 times ten

1 to the sixth cells required to see tumors in about 50
2 percent of athymic nude mice.
3 We also heard that Adeno. 5 transformed
4 HEK cells, 293, have already been used in the
5 production of adenovirus based vector for gene
6 therapy, and there's quite a large number of Phase 1
7 trials, and there was a considerably amount of safety
8 data.
9 However, you also heard that when we move
10 into this new class of cell substrate, the designer
11 cell substrate, there are still potential safety
12 concerns, and I just want to reiterate them here.
13 Incomplete medical history of the original
14 tissue and incomplete documentation of the tissue
15 culture ingredients used in the propagation of the
16 cell substrate may be of concern, as well as the long
17 history of immortalized cell may result in exposure to
18 adventitious agents and potentially to TSE/BSE agents
19 due to undocumented bovine ingredients in the culture
20 medium.
21 So now we'd like to summarize briefly OVRR
22 approach and reiterate what you've heard early on. Do
23 we really believe that extensive safety testing should
24 be conducted on the new master cell banks
25 independently of the vaccine vector, and the rationale

1 is that the same MCB can be used for production of
2 multiple product.
3 If an adventitious agent is detected in
4 the master cell bank, it is important to document its
5 removal during product processing and purification and
6 its absence in the final product.
7 As you heard from Dr. Krause, some viral
8 vaccine vectors may interfere or reduce the
9 sensitivity of certain safety assays. We would also
10 like to recommend that sponsors should be encouraged
11 to place the results of the new master cell banks in
12 the public domain in order to increase public
13 confidence in the safety of the new cell substrate.
14 And I'd just like to briefly summarize the
15 testing that we have discussed during the day with
16 regards to master cell bank tumorigenic and
17 oncogenicity studies. It is useful to actually test
18 the intact cells and to determine their TPD-50, and in
19 order to do that, one needs to use several cell doses
20 and observe nude mice for five to six months because
21 different cells have different kinetics of developing
22 tumors.
23 With regards to the high molecular weight
24 that's extracted from the cells, it's important to
25 conduct oncogenicity studies to establish the

1 inability of oncogenic sequences, viral or cellular
2 derived to cross-tumors in animal models.
3 With regard to adventitious agent testing,
4 as you heard from Dr. Krause, in addition to the
5 standard assays, it's important to try to incorporate
6 new state-of-the-art assays for detection of agents
7 that can infect human cells as needed and as become
8 available.
9 We think it will be important to test cell
10 lysates in order to detect occult oncogenic viruses
11 and the way to approach probably to inoculate the
12 animal species, for example, the newborn hamster and
13 the rats that was shown in the nice table that Dr.
14 Krause showed you, and to observe them for about five
15 to six months.
16 With regard to the potential risk of TSE
17 and BSE, it may be important for all new cell
18 substrates, including designer cell substrates to
19 sequence the PrP gene of the MCB to exclude the
20 presence of the familial variant that was described by
21 Dr. Priola, and to test for the presence of protease
22 resistant PrP protein by sensitive Western blot.
23 As far as residual DNA is concerned, a
24 concerted effort should be made to reduce the amount
25 of cell substrate derived DNA in the final product to

1 less than ten nanograms per human dose, and as you
2 heard from Dr. Keith Peden earlier, that may be
3 translated into probability of less than one in ten
4 million or 100 million, probability of transmitting an
5 oncogenic sequence per ten million or more human
6 doses.
7 For vaccine administration via the oral
8 route, as was also mentioned earlier, higher level of
9 residual cellular DNA may be allowed, especially if
10 studies demonstrated known tumorigenic potential.
11 So I would like to end by actually
12 presenting the Committee with several points to
13 discuss. Please discuss the adequacy of OVRR approach
14 to the evaluation of designer cell substrates for use
15 in the manufacturing of viral vaccine. You can make
16 specific reference to tumorigenicity and oncogenicity
17 studies, residual cell substrate DNA, potential
18 contamination with adventitious agents, including
19 occult oncogenic viruses and TSE/BSE agent, and feel
20 free to discuss any additional safety concern that you
21 may have.
22 Thanks.
23 ACTING CHAIRMAN DAUM: Dr. Golding, thank
24 you very much.
25 With your permission I wonder if we could

1 leave this last --

2 DR. GOLDING: I have one for an overhead.

3 It may be easier to --

4 ACTING CHAIRMAN DAUM: That would be

5 great. Whatever is audiovisual's pleasure, and we'll

6 use that as soon as we come back from our break and

7 leave it up for the whole Committee discussion.

8 Questions for Dr. Golding's presentation

9 only, please? Ms. Fisher.

10 MS. FISHER: All of these tests that you

11 want to have performed and these assays, is the

12 manufacturer going to be doing this? Is FDA going to

13 then be retesting? I mean, how is it going to be

14 insured once something is come up with, you know,

15 that's actually going to be followed?

16 what kind of oversight will there be on

17 the testing?

18 DR. GOLDING: Well, this is actually part

19 of the normal development through the procedures.

20 When they manufacture and develop a new cell substrate

21 or a new vaccine that is made in a new cell substrate,

22 they will usually ask for a meeting with the agency in

23 the form of a pre-IND.

24 During this time any new safety issues

25 regarding the cell substrate or the vectors are

1 discussed, and we start to get dialogue regarding the
2 type of additional testing that are needed. It's
3 quite understood that this type of test has to be done
4 and presented. The result has to be presented in the
5 IND application, and we are looking very carefully at
6 the results of this test, and if we find that they are
7 inadequate, we have to make a place to stop the
8 beginning of Phase 1 trials.
9 But we are looking at each product and at
10 the target population that it's designed for. We look
11 how much tests were already done and how much tests we
12 still need to be done, and based on very extensive
13 internal discussion and discussion with the sponsors,
14 a decision is then made whether to stop the initial
15 clinical trials before everything is done or whether
16 to allow progress or at least initiation of small
17 Phase 1 trial and try to then -- and require the
18 company of the sponsor to complete additional safety
19 studies before going to much larger scale studies.
20 ACTING CHAIRMAN DAUM: Thank you very
21 much.
22 I think we'll move on then to the open
23 public hearing portion of the meeting. Is there
24 anyone in the audience that would like to address the
25 Committee at this time?

1 (No response.)

2 ACTING CHAIRMAN DAUM: In the absence of
3 recognizing a rush to the microphone, I would like to
4 declare us in recess for 15 minutes. It's 3:25. We
5 will reassemble at 3:40.

6 Thank you.

7 (Whereupon, the foregoing matter went off
8 the record at 3:27 a.m. and went back on
9 the record at 3:44 a.m.)

10 ACTING CHAIRMAN DAUM: One of the nice
11 things about having our information presented to us in
12 discrete bits this morning is that we had an
13 opportunity to discuss a lot of issues related to
14 those that the FDA wishes to hear our advice about.

15 And so now comes a time rather than
16 perhaps needing to start from square one to be sort of
17 well immersed and understanding what some of the
18 issues are and concerns.

19 There are no actual votes today, but there
20 are issues for discussion, and they're nicely
21 summarized on this slide, which I asked Dr. Golding to
22 put up for us again, and I'd like to just focus a
23 little bit on how we proceed here.

24 Please discuss the adequacy of OVRR's
25 approach to the evaluation of designer cell substrates

1 for use in manufacturing of viral vaccines.

2 Now, this morning's presentations were
3 divided into three discrete kinds of concerns, one,
4 the tumorigenicity and the oncogenicity studies;
5 secondly, the residual cell substrate DNA concerns;
6 and, third, of course, the adventitious issue
7 concerns.

8 So I would like to have or invite
9 Committee members and consultants to begin this
10 discussion by lumping those three things and talking
11 about whichever of those issues you would like to
12 bring up and discuss.

13 We have most of our, perhaps even all of
14 our speakers from this morning available as resources,
15 and I think the issues have a lot of commonality to
16 them.

17 If we don't get good discussion on all of
18 the issues, then I might take the Chairman's
19 prerogative of refocusing the discussion and doing
20 them sort of one by one, but let's sort of see what we
21 get.

22 For consultants that are new to this
23 process, I really would like to hear at some point
24 during the discussion from everyone at the table, and
25 we will help people remember to shut off their cell

1 phones and beepers, and also to participate in the
2 discussion at some point.
3 So with that, Dr. Decker cannot wait for
4 a comment. So I'll begin with him first.
5 DR. DECKER: Well, Bob, as you know, I've
6 got to meet a prior commitment. I have to slide out
7 early. So I'll say my piece now and then be gone.
8 I'll try to take --
9 ACTING CHAIRMAN DAUM: We're grateful.
10 (Laughter.)
11 ACTING CHAIRMAN DAUM: I mean for the
12 first part.
13 (Laughter.)
14 DR. DECKER: You know I have your home
15 number.
16 (Laughter.)
17 DR. DECKER: The first question or the
18 first issue laid in front of us was the reminder of
19 the nearly half century old bias against using
20 immortalized or neoplastic cell lines for production
21 of vaccines or biologicals, and one implicit if not
22 explicit question was whether the time has come to
23 overturn that prejudice and to exploit these based on
24 the new developments and the marked dramatically
25 improved capacity for understanding and auditing them,

1 and I think the answer to clearly is yes.
2 If we're going to move forward, it's time
3 to take advantage of these things, but then, of
4 course, it has to be done with I would love as a
5 writer to say absolute, but as a scientist I know
6 that's ridiculous; so as near to absolute assurance of
7 safety as we can humanly attain at our present level
8 of scientific development.
9 With respect to the three issues raise, I
10 think the one that's of probably the broadest general
11 concern is the adventitious agent one, but I think
12 that's one that is by no means particular to the
13 designer cell substrates, and I think that we would
14 approach them or we would want to see FDA approach
15 them with the same level of caution and thoughtfulness
16 as they do with respect to any biological in the
17 developmental substrates coming forward.
18 And so I in my own mind, I pretty much set
19 that one aside with respect to the specific issue,
20 which is the designer cell, and for them we've got
21 more particularly the residual DNA and the
22 oncogenicity questions, which are legitimate, but it
23 seems to me that we have more than adequate tools and
24 techniques to address them, and it seems to me that
25 the OVRR's approach to this is perfectly adequate.

1 So I'm quite comfortable with the way
2 these things are progressing now.
3 ACTING CHAIRMAN DAUM: I take it these
4 comments are from your perspective as an industry
5 representative here, and some people have raise issues
6 this morning about how the agency and industry are
7 interacting about these issues, and so are you
8 comfortable with that from a point of view of -- I
9 mean some of these bars are pretty high here. It
10 sounds like people are going to be asked to jump
11 through who are making designer cell vaccines.
12 And from an industry perspective, does
13 that sound like something you think people who are
14 making them will comply with, can comply with, must
15 comply with?
16 DR. DECKER: Well, that's an interesting
17 question, and let me back up on that because although
18 I'm here as the industry rep., and I try to be
19 conscious of that all the time, obviously you get the
20 whole person, and I've been in industry for six months
21 and in academia for 20 years. So I guess you get that
22 balance of it.
23 Before I came to this meeting, as before
24 every meeting, I E-mailed my colleagues at all of the
25 major vaccine companies, reminded them that the

1 meeting was coming, and asked them to instruct me on
2 anything they thought was industry relevant.
3 I received zero replies. So, therefore,
4 I come uninstructed.
5 (Laughter.)
6 DR. DECKER: And largely uninformed until
7 I heard this morning's meeting, other than with my
8 background in vaccinology.
9 And because of that, you're not getting
10 the response of the head of our production or the head
11 of our research labs. You're getting the head of our
12 Medical Affairs Department responding, and as such, I
13 see nothing here that doesn't strike me as within the
14 bounds of reason.
15 That doesn't mean at some later meeting I
16 won't have some information or instructions that I can
17 share with you concerning a technical problem that's
18 arisen that it's hard for industry to respond to, but
19 right now you're getting my response mostly as a
20 vaccinologist, and it seems reasonable.
21 ACTING CHAIRMAN DAUM: Okay. Thank you
22 for that.
23 But anybody else in the Committee can now
24 feel free to chime in or our consultants on any one of
25 these points that you wish. Yes.

1 DR. WOLFE: These are general comments on
2 the points that at on the board. About a year and a
3 half ago at one of these meetings I commended CBER
4 generally, this group particularly on the remarkably
5 good research they had done, and there was at that
6 point some question about adequate funding to keep
7 doing what they're doing, and it sounds like that's at
8 least somewhat better. It's never as good as it
9 should be.

10 But given this whole -- as most, if not
11 all of you know, this was originally part of NIH,
12 still physically there, was not even a regulatory
13 agency until 1971 or two, whatever the year was. It
14 can be looked on as a research.

15 The first question is adequacy of OVRR
16 approach. From a research perspective, I think it's
17 excellent. Some cutting edge studies are being done,
18 things that Phil talked about, nonspecifically amplify
19 nucleic acid when you're sort of hunting for
20 adventitious agents that haven't been identified
21 before.

22 But to reflect the questions that came up
23 23 several times this morning, what's the match between
24 that and the first product or the first products that
25 are coming through the hoop?

1 And this morning it was is it just a
2 recommendation; is it would it be nice to do or what?
3 And I think that although we will and I will certainly
4 direct these questions tomorrow at the sponsor when
5 there's a presentation, it would seem to me that there
6 are certain at least state-of-the-art diagnostic, if
7 you want to call them, techniques for either
8 tumorigenicity or adventitious agents or for residual
9 cell substrate DNA which are far enough along that
10 they should be just automatically requested of the
11 company as part of their package.
12 There are other ones that are in
13 development, and even though we don't have the final
14 word on how sensitive and specific they are and so
15 forth, there should be some consideration given to
16 having the company at least do a certain amount of
17 those.
18 And I think that's really the dilemma. It
19 isn't so much, particularly after what we've heard
20 today, as to whether OVRR has done a good job. I
21 think we would all agree they've done a tremendous
22 job, but how does this translate into the regulatory
23 aspect of the agency?
24 I think that's a difficult question, and
25 I certainly would like us to move in terms of our

1 recommendations in the direction of as safe as
2 possible, never, you know, 100 percent safe, but as
3 safe as possible, and that means sort of pushing the
4 companies to take advantage and be required for their
5 specific products to do all that has already been
6 done.

7 ACTING CHAIRMAN DAUM: Thank you very
8 much, Dr. Wolfe.

9 Dr. Aguilar-Cordova.

10 DR. AGUILAR-CORDOVA: I would echo that.
11 Some tremendous research was presented here, but
12 addressing the issues directly posed there about
13 tumorigenicity, oncogenicity studies, and designer
14 cells, I would caution that we might get a false sense
15 of security based on this designer classification of
16 these cells. The only thing they have that's really
17 designer is that they are designed to be able to
18 propagate a virus. They weren't transformed or
19 immortalized on purpose by that gene.

20 And in fact, from the data that we saw
21 today, it seems like it requires multiple events to
22 occur to become a tumorigenic agent. E1A and E1B was
23 presented to be a very poor transforming, you know,
24 tumorigenic agent. So therefore, this cell might
25 probably have multiple event.

1 And I think it would be dangerous to have
2 the false sense of security that if we cannot detect
3 E1A and E1B as a contaminant, that that would be any
4 better than not detecting anything else from an
5 unknown transforming event, and thus, I think that
6 designer cell classification should be looked at
7 carefully.

8 On the other hand, we were also shown that
9 it is a very unlikely event that this transforming or
10 oncogenic activity would come through from the minute
11 amounts of contaminating DNA that are currently
12 allowed on a per dose basis, and given that very
13 unlikely event that I believe was ten to the minus
14 eight, ten to the minus ninth range, then the
15 additional events that might have occurred in those
16 cells really probably don't change the probability of
17 or the risk of that contaminating DNA since one in ten
18 to the ninth is probably no different than five in
19 ten to the ninth.

20 But lastly I would then follow Dr.
21 Decker's, that the adventitious agent portion of this
22 discussion would probably become the most critical of
23 all of those since that was apparently logs of
24 difference in risk.

25 ACTING CHAIRMAN DAUM: Thank you very

1 kindly.

2 We have Dr. Kohl, then Dr. Hughes.

3 DR. KOHL: I'd like to go back to
4 something Dr. Decker said, and if we could achieve a
5 consensus on that, it would make it easier for me and
6 maybe for the Committee.

7 I'm having trouble with at least three
8 issues that we're being asked to discuss, and Mike
9 suggested, and I think I concur, that the adventitial
10 agent issue regarding designer cells, other than the
11 fact that they're around longer and maybe have a
12 slightly or some undefined increased risk of being
13 infected with something, but other than that, I don't
14 think we have been presented with anything suggesting
15 that the adventitial agent issue in designer cells is
16 any different than the adventitial issue in any new
17 cell substrate.

18 So the question is: should we be
19 discussing that pertinent to this or can we leave that
20 issue alone and let CBER go through their routine
21 adventitial issue discussion, which they have to do
22 for every cell line, and move to the two things that
23 seem to be pertinent to designer cells in particular?

24 ACTING CHAIRMAN DAUM: I would respond by
25 saying that I hear a consensus building for the

1 concept that the adventitious agent issue is not
2 unique to these designer -- I hate that term --
3 designer cell lines. Nevertheless, we have been asked
4 to comment on whether the new cell lines we've heard
5 about today in a generic kind of way -- how to address
6 these issues, and more importantly, is the approach
7 that's being taken adequate to the concept?
8 So while I agree with your point, I think
9 the agency still needs our opinion. So I'd like to
10 continue to leave it on the table as an issue,
11 although I think you're right personally that it's no
12 different than any new cell substrate.
13 I have Dr. Hughes, Ms. Fisher, then Dr.
14 Katz.
15 DR. HUGHES: I'd like to respond a little
16 bit to the point that was raised a few moments ago
17 about whether or not designer cell substrates are, in
18 fact different than transformed cell lines derived by
19 other means.
20 And I think the first thing that's worth
21 discussing is that the cell line that's transformed in
22 vitro by whatever means is not necessarily equivalent
23 to a tumor cell, and one of the things that's
24 particularly important if one is talking about the
25 collaboration of a number of genetic changes necessary

1 to create a frank tumor cell for the kinds of things
2 we're discussing here, the development of cells that
3 are permanent in culture, it may not be necessary to
4 have as many change, and that I think automatically
5 distinguishes the kinds of things we're discussing
6 here in terms of creating designer cells certainly
7 from cell lines derived from tumors from animals or
8 humans, and I think that's an important distinction.
9 And the other thing, I think, that matters
10 in that regard is that particularly if in the creation
11 of the cell in vitro, the designer cell, if you like,
12 if one does set the system up in such a fashion that
13 the controlling gene -- and this is the point I tried
14 to make this morning -- can be shut on or off, one can
15 ask then by shutting it off whether the cell returns
16 to what one might call a normal phenotype or not, and
17 there are some relatively simple assays which are
18 probably beyond the scope of the discussions we're
19 having today to ask whether or not those cells are
20 reasonably normal in terms of their behavior.
21 And I think you can get at by doing things
22 of that sort whether or not there are additional
23 changes that are associated with the establishment of
24 those cell lines, and I think that's one of the
25 reasons that that type of experimentation is worth

1 doing.

2 And finally, to just comment very briefly
3 on what's on the list and to respond to some of the
4 other points that were made earlier, I think in a
5 sense the question is not even so much whether we
6 should proceed in this direction, but more of a
7 question of how we should proceed. In fact, not
8 whether or not we should move in this direction, but
9 how, and what are the tests that are necessary to
10 provide the responsible margins of safety that we hope
11 always, whether we're involved in creation of
12 receiving of vaccines, that we think we deserve.

13 And I think it's a question -- the real
14 question is to think very carefully and to try as best
15 we can to determine the best ways to be safe, and I
16 think for some of these issues and for some of the
17 things that we need to test for that the answers are
18 reasonably straightforward.

19 Not all of the work is necessarily done
20 yet, but I think we know more or less how to do it.
21 I think some of the other questions are more
22 difficult, and probably in a sense one of our tasks
23 ought to be to try and think carefully about not
24 whether to do it, but how to do it.

25 ACTING CHAIRMAN DAUM: Thank you very

1 much.

2 I have Ms. Fisher, then Drs. Katz,

3 Griffin, Goldberg, and Coffin.

4 MS. FISHER: Well, it appears that the

5 risk assessment of residual DNA infectivity and

6 tumorigenicity and how much should be allowed is

7 almost entirely dependent upon the assumption that the

8 population to be injected with this DNA is

9 immunologically competent or what is being termed as,

10 quote, normal.

11 But hundreds of millions of humans have

12 genetic predisposition to cancer and autoimmunity or

13 are suffering from active cancer and autoimmune

14 disorders, and it doesn't appear to me that there's

15 been enough consideration given to biodiversity that

16 narrows the definition of what is normal, and that

17 this has an impact on the validity of the animal

18 studies conducted so far and affects the premise that

19 what counts most is how much DNA rather than who is

20 being exposed to it.

21 And I think that before we walk down this

22 road, a lot more has to be known about the differences

23 between people and their response to this DNA.

24 ACTING CHAIRMAN DAUM: I want to make sure

25 I understand you clearly. Are you speaking about

1 expanding the scope of the animal research?

2 MS. FISHER: Yes, because to truly give

3 informed consent, you're going to have to know more.

4 Individuals are going to have to know if they have a

5 higher risk because of their, you know, predisposition

6 potentially.

7 ACTING CHAIRMAN DAUM: Thank you very

8 much.

9 Dr. Katz.

10 DR. KATZ: I was going to debate with

11 Michael Decker, and I'm sorry he left. I would

12 turn --

13 ACTING CHAIRMAN DAUM: As I am.

14 (Laughter.)

15 DR. KATZ: I would turn his neutral

16 position into an even more positive one. I think

17 there's less concern with these cells than with

18 primary derived cells. The problems we had with SV40,

19 with avian leukosis virus. We didn't use

20 Sudimangabese (phonetic) or chimpanzees. So we didn't

21 run into HIV, but all of the potential adventitious

22 agents have been from cells from a natural source,

23 from other primates or other non-human sources.

24 Whereas I think these are much better

25 defined and much better tested. I would give them a

1 more positive approach as what' being done and what's
2 potentially available is much more reassuring than
3 were we to use another primary cell line.
4 ACTING CHAIRMAN DAUM: Thank you.
5 I have Drs. Griffin, Goldberg, Coffin,
6 Blair, and Diaz.
7 Dr. Griffin, please.
8 DR. GRIFFIN: Well, I think that with
9 respect to oncogenicity, first of all, we know at
10 least the 293 cells can cause tumors in mice. So by
11 definition the cells themselves can cause tumors at
12 least in some animal models. So, therefore, the
13 issues become whether there's something else besides
14 which are really the issue that Dr. Hughes has just
15 made, whether there's something else besides the
16 adenovirus transforming genes that are in the cell
17 lines that we should know about, and the only way
18 we're probably going to be able to figure that out is
19 if we can knock out in some way the function of those
20 genes and see if those cells still can cause tumors in
21 mice.
22 So something else that we should be
23 worrying about is there, but then the issue become
24 really then the DNA issues because you aren't going to
25 be injecting cells as a part of what you give as a

1 part of a vaccine. Presumably you can be sure of
2 that.
3 But then the quantities of DNA and having
4 very good data on how many nanograms or the real
5 quantitation in mice of different varieties that have
6 both increased susceptibility to tumors because of
7 oncogene mutations and also increased susceptibility
8 because they're young or immunodeficient in one
9 respect or another, including immunodeficiency in NK
10 cells, not just T cells.
11 So skid mice or something that perhaps is
12 even more susceptible than a nude mouse. So I think
13 that it's really moving toward just a clear
14 understanding on characterization of these cells and
15 this quantitation issue, which is what it's all going
16 to come down to at the end in looking at the final
17 product basically.
18 And the other point is that, which really
19 hasn't been brought up because it's in many ways the
20 easiest to deal with, is a real risk obviously is
21 recombination in these cell lines, and that the
22 product that you can actually get viable virus out,
23 and the engineering that's going on right now will
24 probably deal with that issue. So that makes it very
25 unlikely, but that is in the other tumor models at

1 least where even though adenovirus is probably not
2 that oncogenic in humans, but in other situations
3 where you really have the increased risk is if you get
4 recombination.
5 ACTING CHAIRMAN DAUM: Thank you very
6 much.
7 Dr. Goldberg.
8 DR. GOLDBERG: First of all, I'd like to
9 commend the agency for the excellent presentations.
10 It's really clear and basically are allowing me to ask
11 questions, which I couldn't do from just the reading.
12 I think the idea of having a framework for
13 the testing of adventitious agents is absolutely right
14 on. It's very important, but I think even now it's
15 time to start looking at the assumptions and looking
16 at the results that you've observed under various
17 modeling assumptions, whether it's <u>in vitro</u> or in the
18 early animal work.
19 I mean, it's very hard to say "never," and
20 statistically you never can. there's always a finite
21 probability of something occurring, and therefore, I
22 think it's very important that you start to think in
23 terms of the different underlying truth that could
24 produce data, such as the data that are observed.
25 You're also using batteries of tests as I

1 see it, and you can come up with rules for combining
2 the batteries that would give you more assurance and
3 reduce the probabilities that you're missing
4 something, and I think that some of that needs to be
5 done.

6 It can be done crudely in terms of worst
7 case analysis. Everything is under the worst possible
8 scenarios that you could think of. What would your
9 numbers look like, and then you accumulate them, but
10 there are more sophisticated ways to do that, but
11 that's a way to start.

12 You can also design sequences of the
13 testing that may be able to make it more efficiency
14 for manufacturers and for you to be able to work your
15 way through based on some of these results, and those
16 things all need to be worked out. We can't do it
17 here, but they are things that I think need to be
18 thought about.

19 The other issue is the carcinogenicity
20 studies. I think that that needs to be done, but the
21 system obviously would need to be stressed to the
22 limit because these are very low -- the likelihood of
23 any of this occurring is very low.

24 ACTING CHAIRMAN DAUM: You're breaking up,
25 Dr. Goldberg. Can you speak right into the microphone

1 for us?

2 DR. GOLDBERG: Okay. I think you have to

3 stress the CARCI studies, I mean, and do them in the

4 way that you do standard carcinogenicity for any drug,

5 which can be much longer than five or six months. You

6 need to push the thresholding doses that you're using

7 to see whether or not anything does occur at some

8 dose, and then you work your way back to what might be

9 a safe threshold.

10 ACTING CHAIRMAN DAUM: I think that the

11 idea of trying to mathematically model risks of worse

12 case scenarios is one that it does really need to be

13 developed in a more systematic way than we've heard

14 today, and then I think once those models are

15 developed, it's a separate issue of whether we -- and

16 by "we" I mean the most global "we" I've ever said in

17 my life -- we, the public and the people sitting at

18 this table and the FDA and the manufacturers and

19 mostly the people who are going to receive these

20 vaccines then need to decide whether or not they

21 accept those risks, theoretical or not.

22 But I think modeling them with all of the

23 information we do have, incomplete as it may be, is a

24 wonderful concept that I'd like to hear more about.

25 DR. GOLDBERG: I just have one more

1 comment.

2 ACTING CHAIRMAN DAUM: Okay.

3 DR. GOLDBERG: Which is I think that then

4 you evaluate the risks we're going to have to make

5 some distinctions about a preventive vaccine that's

6 given to children versus a therapeutic vaccine for

7 serious illness, and there will be some gradations of

8 acceptable risk which will also play into the

9 evaluation.

10 ACTING CHAIRMAN DAUM: I have Drs. Coffin,

11 Blair, Dias, Faggett, and Kohl, and now Myers and van

12 der Eb.

13 Dr. Coffin, please.

14 DR. COFFIN: Okay. First I wanted to

15 comment to Ms. Fisher's comment on the experiments

16 that are being done regarding the oncogenicity of

17 oncogene DNA in experimental animals. These are --

18 and as to how they would play into the issue of

19 variation of immune confidence in the public at large.

20 These experiments are being deliberately

21 designed to minimize the immune competence of the

22 animals that are being injected. They're going into

23 either newborns, weanlings, or into nude mice and, if

24 necessary, other animals of this type will be being

25 brought in. So they're being set up to be to a first

1 approximation the worst case scenario in terms of what
2 the immune competence of any potential human recipient
3 would be, at least as close as we can come in
4 straightforward animal models and animal models that
5 are reasonable to deal with and don't have a very high
6 background tumor incidence, for example.

7 Regarding the issues on the board, the
8 tumorigenicity and oncogenicity studies of the cell
9 substrate have to be done, but I have a lot of trouble
10 seeing how you use the information you get from them
11 because if you have a cell line that does not give
12 tumors in animals, but yet it's a cell line, do you
13 come to the conclusion that that cell line is
14 necessarily safer and you can relax the stringency
15 with what you have to do other testing because of
16 that?

17 And on the other hand, I'm not convinced
18 that we can come to the conclusion that a vaccine
19 prepared from a cell line which is much more
20 tumorigenic necessarily carries with it, just because
21 of that fact, carries with it a greater potential to
22 contain DNA which will cause bad oncogenic
23 consequences in the recipients of the vaccines.

24 So we have to know this information, but
25 I just don't see how it's used or what its relevance

1 will actually be to what the final decisions and the
2 final way we think about a product are.
3 One thing we haven't considered very much
4 with these that is special to the designer cell
5 substrates in the system that's been particularly
6 under discussion and to others regarding retrovirus
7 helper lines and so on is the issue of recombination.
8 There has been a lot of bad experience
9 with that in retrovirology. I'm not sure that the
10 adenovirus people have gotten anywhere near the levels
11 of the sort of bad things that have been discovered
12 with people generating helper cell line after helper
13 cell line that couldn't possibly give replication
14 competent recombinants and finding that sure enough
15 they do, and it wasn't until things really have to be
16 subdivided in a much more firm way in retrovirus
17 systems than they are in these systems to actually
18 reasonably insure that there are very low levels of
19 recombinants.
20 this will, of course, need to be
21 discussed in much more specific terms, but I think
22 it's an issue that we have to keep in mind.
23 And another issue that is specific for
24 vaccines, viral vaccines and live viral vaccines, is
25 the possibility that the virus itself can bring in --

1 can over and above the DNA fragments that are present,
2 can actually encapsidate or recombine in cellular DNA
3 and enhance the introduction of that DNA into cells.
4 If these were propova virus vaccines,
5 propova virus is very well known to encapsidate genome
6 size fragments of cell DNA and bring them in.
7 Adenovirus, I don't know where they tend to do that as
8 much, but I suspect there's some of that, and I think
9 that's an issue that may have to be looked at
10 separately that hasn't really arisen in our
11 discussions.
12 As far as the issue of DNA contamination
13 in these products and the possible oncogenes, I don't
14 see how we can treat these cell lines any differently
15 than we would any other cell line as far as the
16 standards that have to be applied, which we don't
17 completely know what they are yet, I think, because we
18 don't really have -- I don't think we have the data
19 yet that we need to really judge the risk of this.
20 I think the risk is extremely low, but I
21 don't think we have the data to really put any
22 quantitation on it, but I don't see why there should
23 be a difference between DNA, between viruses grown on
24 any kind of cell line, one to another, as to the way
25 this is treated.

1 ACTING CHAIRMAN DAUM: Thank you very
2 much, Dr. Coffin. You raised a lot of new points that
3 we haven't really addressed in detail yet.
4 Dr. Blair, then Dr. Diaz, Dr. Faggett,
5 Kohl, Myers, van der Eb, Minor, and Aguilar-Cordova.
6 Dr. Blair, please.
7 DR. BLAIR: I think to follow up, I think
8 I'm probably a little more comfortable that they don't
9 form tumors than John. Maybe I would agree that we
10 may not know what that means, and certainly you can't
11 be less careful with the material that comes from a
12 cell like that, but I think the closer the cell is to
13 a normal what we seem comfortable with, which is a
14 normal diploid cell, the closer it is to that, the
15 more comfortable I would feel, and I think the more
16 comfortable the public will probably feel as a whole.
17 In terms of the biodiversity issue, I
18 think there is an attempt in some of these experiments
19 to test tumorigenicity of things that we believe to be
20 tumorigenic to try and establish some sort of baseline
21 that we are trying to do this in a variety of
22 different backgrounds, as many as to stress the system
23 in such a way as to get a positive response from which
24 we can then determine some sort of sensitivity and
25 hopefully then some level of confidence in negative

1 responses.

2 But I guess it is very difficult to use

3 the rodent or anything else as a real model for all of

4 the varieties of human genetic background that these

5 vaccines will go in, and ultimately we have, I guess,

6 to be as safe as we can be in the model systems and

7 develop as good a model system and as testable a

8 system as we can to test the risk.

9 But there will probably always be a, you

10 know, some risk of some situation, and people will

11 have to be aware of that as these things go out.

12 ACTING CHAIRMAN DAUM: Thank you very

13 much.

14 Let's move on to Dr. Diaz, please.

15 DR. DIAZ: Well, firstly, I'd like to

16 comment that I think that the approach to the

17 evaluation of these newer cell substrates is very

18 thoughtful and certainly based on what data is

19 available currently.

20 And with that in mind, we always worry

21 about the unknown, and I think it's very important

22 that the FDA makes recommendations on what types of

23 testing ought to be done to at least give us as good

24 a feel for the safety of a particular product as is

25 capable at that point in time.

1 And yet I also feel that it merits perhaps
2 even making some perhaps even more specific
3 recommendations about what types of tests ought to be
4 done at what time point and how perhaps often they
5 might need to be repeated or at what time frame they
6 might be repeated in dealing with some of these cell
7 lines.
8 Because of the length of time involved in
9 some of the tests, I would hate to get to a point in
10 time where we're down the road and recommendations
11 were somewhat open to interpretation, and then we come
12 down the road and suddenly the question arises, and
13 we're at a point in time where we have to deal with
14 less information about that product than we could
15 potentially have had.
16 So I recognize the issues about requiring
17 versus recommending and yet perhaps putting some time
18 or at least some recommendations based on certain
19 intervals would be meritorious.
20 Switching gears a little bit, the usage of
21 some of these cells and particularly the usage of
22 vaccine constructs in viruses like adenovirus or
23 perhaps there may be other viruses to which this might
24 even be more applicable, but viruses that are somewhat
25 ubiquitous in the general population.

1 I think despite the fact that issues about
2 recombination can probably be hopefully dealt with and
3 ruled out. I think the question is still going to
4 come up at some point in time with these products when
5 they're used in humans, and suddenly there's an
6 infection perhaps in an immunocompromised host or
7 viral products, viral genes or viral gene products
8 found in tumors in humans, and the question will come
9 up in a vaccinated person: is this related to vaccine
10 or is it related to a wild type virus occurring.
11 And what I haven't heard is any
12 discussion, and perhaps it's not possible from a
13 molecular standpoint, but any discussion about having
14 some kind of marker or the need to have some kind of
15 marker in these constructs that would be able to when
16 that situation does arise to answer that question,
17 whether those products or that infection is a result
18 of recombination with the vaccine virus and/or whether
19 it's wild occurring.
20 ACTING CHAIRMAN DAUM: Thank you very
21 much.
22 Dr. Faggett.
23 DR. FAGGETT: I agree with my colleagues
24 that there's been a high quality of the presentations
25 today, and I really appreciate it, and I especially

1 appreciate the historical perspective from my
2 colleague Dr. Sam Katz, a survivor of potentially an
3 SV40 infection.
4 Stay well, Sam.
5 But I think this has really been an open
6 and honest discussion of very technically challenging
7 concepts. There's been a lot of good science present,
8 and to include admission of limitations of that
9 science.
10 I think we as a panel are now better able
11 to make evidence based decisions with a better
12 understanding of the evolving nature of this science.
13 I think OVRR has been very adequate in its approach to
14 the evaluation of designer cell substrates, to include
15 all of the topics listed.
16 I agree with Dr. Hughes that this is an
17 opportunity to really bring on line the state of our
18 testing to minimize risk contamination by adventitious
19 and other agents yet unknown.
20 I agree with Ms. Fisher that we do need to
21 keep in mind that as we take the animal study results
22 and try to apply them to a diverse target population,
23 that there are a lot of other ethical considerations
24 as well as scientific that we have to consider.
25 But I think as a primary care provider I'm

1 very comfortable that we're in the process of really
2 looking at all available data, and in so doing, we'll
3 be a lot more comfortable to recommend to our patients
4 that, indeed, no stone was left unturned by us.
5 And I'm really impressed that safety has
6 remained a top priority in this discussion. So often
7 we have the science separated from the safety, and
8 it's an afterthought. In this instance I'm seeing
9 safety as a primary concern, and I think with that
10 approach we'll be better able to really anticipate any
11 questions in the future.
12 I think the results of our recommendations
13 will be better accepted because of this in depth
14 examination, and I truly thank you for the
15 presentations today.
16 ACTING CHAIRMAN DAUM: Thank you.
17 Dr. Kohl, I think you're up.
18 DR. KOHL: We've heard a little bit about
19 refining the risk assessment, I think, and trying to
20 get finite kinds of numbers, and it's a little bit
21 deja vu.
22 I was looking at the September '99 panel
23 discussion, which several of the members here were on,
24 and I was struck by Dr. Sedivy's statement. Hopefully
25 I'm not killing his name. "It is worth trying to do

1 calculations so long as you do not believe the final
2 numbers."
3 (Laughter.)
4 DR. KOHL: And I guess I wanted to know
5 from the people who were there and maybe anybody who's
6 wiser than I am. Have we moved further from that
7 statement or are we still pretty much there?
8 It's apparently just an exponentially
9 multiplication system, and as we get further along in
10 multiplication, the numbers get fuzzier and fuzzier.
11 ACTING CHAIRMAN DAUM: I would like to
12 actually begin the -- I think you raise an interesting
13 point, and I'd like to begin and ask Dr. Hughes we'll
14 stay focused on this subject for a moment, but I think
15 the numbers are one more approach to understanding
16 what kinds of situations in terms of safety we're
17 dealing with here.
18 I don't think the numbers are going to
19 solve the problem or provide security that makes us
20 forget about all the other things we've heard about
21 and talked about today, but I think if you're talking
22 about ten to the minus 39th versus ten to the fourth,
23 I think that gives you a certain difference in how you
24 think about what we're talking about here.
25 So I pulled those two numbers out of a hat

1 as a potential illustration, but I think what you
2 quoted as being said a couple of years ago was right
3 on the money. As long as we don't take them too
4 seriously and believe that we've defined something
5 that we really have no idea what we're talking about,b
6 ut it still is useful to get us in the range and give
7 us a little more piece of the puzzle, a little more
8 frame.
9 Dr. Hughes, you probably have something
10 much more erudite to say.
11 DR. HUGHES: I very much doubt that, but
12 I'll be happy to give you my prejudices. I was one of
13 the people, and I'm sure you know if you've looked at
14 the text, who expressed some skepticism of some of the
15 calculations, not in the sense that I don't think they
16 have some use, but as was mentioned a little while
17 ago, because there are uncertainties in each of the
18 numbers that goes into the calculation, you tend to
19 expand the uncertainty issue as you multiply out.
20 And I think that was exactly what
21 motivated us to try and get together with the FDA to
22 try and derive in the animal models which have their
23 limitations, and I think we would be the first to
24 admit that, but to at least for the animal models get
25 some numbers that have much less uncertainty.

1 And I suppose I would apologize in some
2 sense in the name of the federal government that it
3 hasn't been a faster process, but as I think everyone
4 who does science understands, the first thing you have
5 to get is some money, and that part of the problem is
6 solved.

7 And I think if -- and I'm sorry to say
8 that I think it will probably be another year, but I
9 think in another year we will at least be able to give
10 in rodent models some clear preliminary data, and I
11 don't think that's going to solve everything, but I
12 think it will give us more confidence that we know
13 what we're trying to deal with, and I think that will
14 make us feel even more comfortable or less comfortable
15 with what we're doing.

16 But I will always feel more comfortable if
17 I have data that I believe has a firm numerical basis,
18 and I think that's the goal of the experimentation
19 that's being done with the NCI and the FDA.

20 ACTING CHAIRMAN DAUM: Thank you.

21 I think we've spoken to Dr. Kohl's point
22 a little bit, which is well taken, and Dr. Myers is
23 next, then Dr. van der Eb, Minor, Aguilar-Cordova, and
24 Kim, and there's actually a couple more. That will
25 get you.

1 DR. MYERS: I really like the risk
2 modeling approach because even if the numbers are
3 soft, at least put them into some sort of perspective.
4 As you say, ten to the four is a lot different than
5 ten to the 39.
6 It seems to me the two things that are
7 different about designer cells, a lot of people have
8 commented that the cell substrate or the rules for
9 cell substrate, whether we're talking about a new
10 diploid cell line or others are pretty much the same,
11 and one is Dr. Daum's point about what is different
12 about the designer cell is what we put into it, and if
13 we could ablate that function and see that the cells
14 revert to what we expect, I think we learn a great
15 deal about the stability of the cell substrate.
16 But I'd like to say something about the
17 adventitious agents before we completely leave those.
18 I agree with Sam that diploid cells and now continuous
19 cell lines would be, I would think, much safer from an
20 adventitious perspective.
21 We don't want to forget that these cells
22 are somewhat different in that they are derived from
23 fetal neural cells, and so as we're developing the
24 assays to look specifically for adventitious agents,
25 we maybe should be targeting them specifically for

1 vertically transmitted agents.

2 And so, Phil, as you were giving your

3 model of the spiked VZV assay that you did, I was

4 think about if, in fact, you were dealing with

5 trigeminal ganglia and looking for VZV, the

6 sensitivity of the assay and the difficulty of trying

7 to find a latent virus in that type of setting.

8 So while I think the likelihood of

9 adventitious agents -- the risk would be much reduced

10 as we get more experience with these types of cells.

11 I do think as different cell substrates come along, we

12 should think about their origin and target our seeking

13 assays towards in this case vertically transmitted and

14 neurotropic agents.

15 ACTING CHAIRMAN DAUM: Thank you, Marty.

16 Dr. van der Eb.

17 DR. VAN DER EB: I would like to add a

18 comment on the opportuniticity (phonetic) issue. Two,

19 nine, three cells are oncogenic in nude mice. They

20 are weakly oncogenic, and so are the PER.C6 cells.

21 Both cell types of weakly oncogenic in immunodeficient

22 nude mice.

23 The BRK cells, baby rat kidney cells,

24 formed by Adenovirus 5 in our hands are just as weakly

25 oncogenic as the human cells, and in fact, if I

1 remember correctly, but I am not absolutely sure if I
2 am correct, also the BRK cells -- so this is the baby
3 rat kidney cells -- transformed by oncogenic Adeno. 12
4 are also not that much more oncogenic than the Adeno.
5 5 cells.
6 The fact that Adeno. 12 transformed cells
7 are oncogenic in immunocompetent animals and are,
8 therefore, called oncogenic viruses is due at least in
9 part to the fact that E1A of Adeno. 12 can switch off
10 the Class 1 C antigens, the transplantation antigens
11 in the transformed cell, but basically they do not
12 seem to be very much more oncogenic in nude mice.
13 I remember many years ago there has been
14 an experiment where in SV40 transformed human diploid
15 skin fibroblasts, the SV40 large T antigen gene was
16 switched off, and that was due to a temperature
17 sensitive mutation in the large T antigen, and after
18 several packages these cells, when you switch them to
19 the nonpermissive temperature so that the SV40 large
20 T is no longer functional, the cells return to a more
21 or less normal phenotype and, in effect, stop the
22 fighting, so indicating that in SV40 at least
23 transformed cells after a number of passages, but not
24 hundreds of passages, but maybe 20, not much more has
25 occurred.

1 ACTING CHAIRMAN DAUM: Thank you very
2 kindly.
3 Dr. Minor, please.
4 DR. MINOR: Firstly, I think that the
5 studies on the tumorigenicity/oncogenicity of the DNA
6 are very, very welcome, I think. I think the existing
7 data are a bit anecdotal, and I'm quite impressed that
8 they seem to agree with each other as Keith Peden
9 demonstrated, and I think it would be nice to have
10 some real data on these things that you can actually
11 put your hand on your heart and believe.
12 (Laughter.)
13 DR. MINOR: With respect to
14 tumorigenicity, in general, it seems to me that while
15 you have designer cell lines where you put in a
16 particular gene and that results in transformation,
17 nonetheless, you don't really know the full story.
18 I mean, I think there's a difference
19 between the retinal transformed cells and the kidney
20 transformed cells, for example, and it's not clear to
21 me why one goes relatively easily or straightforwardly
22 and the other one doesn't.
23 So it does seem to me that you don't
24 really know the fully story about why the PER.C6 is
25 the way it is and why the HEK cells are not perhaps

1 the way it is.

2 And then I think going on with what John

3 Coffin just said, I think the question of degree of

4 tumorigenicity is something that really needs to be

5 carefully thought through. I'm not sure how

6 tumorigenic a cell line would have to be before you

7 decided it wasn't acceptable or if it could ever be

8 unacceptable depending on how tumorigenic it is.

9 And that's why I think the DNA issue is

10 really a very important one to get to grips with.

11 There are clearly DNA delivery systems like cells, for

12 example, which you wouldn't want to have stuffed into

13 your vaccinees. Oncogenic viruses you wouldn't want.

14 Viral nucleic acid I think you wouldn't want

15 particularly.

16 I'm not sure what happens when you get

17 down to oncogenes and other bits of DNA as well. It

18 seems to me there's a bit of a data gap there in terms

19 of do you really worry about them or not.

20 So I think I very much welcome the DNA

21 studies which are being done by CBER. I think they're

22 all to the good.

23 One thing I wouldn't like to slip through

24 the net actually is the adventitious agent question.

25 It seems to me that considering the viral testing, I

1 don't think there is anything special about these
2 cells in terms of what you would actually do to look
3 at them.
4 The TSE, however, I was a bit horrified by
5 this. My country, as you may be aware, has become
6 increasingly obsessed with BSE over the last 20 years,
7 and the approach which has always been taken, adequate
8 or not, is really to look at the nature of the
9 materials which are going into your culture, system,
10 and so on, this being regarded as the best place to
11 put your resources and the most effective way, if you
12 like, of resolving any issues that arise.
13 And the impression I had from what was
14 said here is that it's now considered at least
15 relatively straightforward perhaps to get a cell in
16 culture infected with a TSE agent or to get a cell in
17 culture spontaneously producing the TSE agent, and for
18 me this is a basic shift in the paradigm.
19 I think it's going to be a very difficult
20 thing to actually do that, which doesn't mean that it
21 shouldn't be done, of course, right? But I would like
22 to not let that one just go by on the nod because I
23 think that's actually a matter of some concern, I
24 think, and it would be a need to balance, if you like,
25 the good against the possible consequences of doing

1 that.

2 For example, if you wanted to go and test

3 calf serum in cows, it would take you seven years and

4 ten million pounds, which is a lot of dollars, as

5 well.

6 ACTING CHAIRMAN DAUM: Thank you very

7 kindly.

8 Next is Dr. Aguilar-Cordova, please.

9 DR. AGUILAR-CORDOVA: So I just have three

10 quick responses or comments to what's been said.

11 ACTING CHAIRMAN DAUM: Please.

12 DR. AGUILAR-CORDOVA: One is with regard

13 to what Ms. Fisher has spoke to, and that is the

14 target population, and obviously I think that there

15 needs to be some consideration of that, and I would

16 hope that at the early stages of this one would not

17 use very sensitive or very skid population, for

18 example, or leap frog mainly (phonetic) patients or

19 such, and I'm more concerned with that.

20 However, there are still two different

21 concerns there. The product and the contaminants, and

22 even within the product one must be a tad bit

23 concerned, but we must keep in mind that the potential

24 for RCA or replication competent adenovirus in the

25 product, that may come in through the product versus

1 what may be in the environment and at what levels
2 might be tolerated or acceptable since we're all
3 exposed to some RCA most of the time.
4 The second was with respect to the testing
5 that was proposed by Dr. Krause and the technology
6 that's being used, and it's fabulous actually. I
7 really like that use of random primers probably to
8 just detect little pieces of DNA in the supernatant.
9 Now, and another thing that may be
10 considered, and I don't know if your group is doing
11 anything, but just to throw it out there, it would be
12 new technology like micro chip analysis, and in fact,
13 that might even be something that would be considered
14 in the TSE or BSE type of environment as to whether
15 the presence of such infectious agents may lead to
16 different genomic expression profiles that might be
17 detectable that way.
18 And lastly, I hate to beat a dead horse
19 into the ground, but I guess often they're the easiest
20 ones to beat.
21 (Laughter.)
22 DR. AGUILAR-CORDOVA: And this has to do
23 again with the designer cell state, and I was looking
24 at the glass here in front of me, and it sort of
25 prompted the analogy.

1 I think when we're talking about
2 tumorigenic cell lines, defined as cells that can form
3 tumors in nude mice, as Dr. Hughes was mentioning,
4 it's perhaps a series of events that were captured <u>in</u>
5 <u>vitro</u>, and that they may not reflect what events have
6 happened <u>in vivo</u>, but they still lead to tumor
7 formation in the nude mouse.
8 And if one has a glassful of candies like
9 this and one more candy puts it over the edge, just
10 because you know what that one candy that put it over
11 the edge is doesn't preclude the fact that there's
12 still a whole bunch of other candies in there, and if
13 you take that one candy out, it won't go over the edge
14 anymore. So it won't be tumorigenic anymore, but all
15 of the other candies are still there, and the only
16 difference in the designer cells, again, is that you
17 know that one candy.
18 ACTING CHAIRMAN DAUM: Thank you very
19 much.
20 Dr. Kim, it's your turn.
21 DR. KIM: Well, I guess looking to the
22 question about whether approaches are adequate, I
23 think that this question certainly is a moving target.
24 As we heard today, there are many new assays, and
25 animal models are being developed to look at the older

1 issues related to oncogenicity and particularly
2 adventitious agent contamination issues.
3 So I think, again, I'm sure that these
4 issues will come back on another basis as we gain more
5 data and experiences. So certainly I think we can
6 address what we have today, and based on that I think
7 I'd like to support the idea of, you know, having some
8 sort of mathematical model, which we talked about
9 earlier, because, again, there are data being
10 generated in our models and questions have been raised
11 whether that is relevant to humans or not, and again,
12 there are some biologic and theoretical concerns with
13 information coming out from in vitro, as well as
14 animal models.
15 I think the best we can do is to try to
16 come up with some sort of worst case scenario, and
17 again, as many people have said, that shared that
18 information with the public and that have the public
19 aware of all of these exercises have gone, you know,
20 before the material has been presented to them. So
21 they certainly will be up to date on these issues.
22 And then lastly, I would also like to see
23 some utilization of current advances made in the
24 genomics, and I think it will be fun to look at some
25 of these issues that were raised and so on and see

1 what are, you know -- if something is entirely benign,
2 then I would hope to see that there are no changes,
3 but if something is coming up, then up and down, you
4 know, that might, you know, imply some potential of
5 some biologic concerns.
6 ACTING CHAIRMAN DAUM: Thank you, Kwang
7 Sik.
8 I put my name down on the list, and it
9 comes up now. So I would like to ask a question. Dr.
10 Peden, I think it was -- is he still here? There he
11 is. Good.
12 Dr. Peden -- my eyesight is bad, too --
13 Dr. Peden, I think you raised the issue of SV40
14 follow-up or someone. Dr. Krause. Excuse me. I was
15 curious as to -- I was taking note of your comments
16 about SV40 now having been found in several patients
17 with human cancer, and I was aware that that was true
18 and wondered how much of a surveillance that finding
19 has prompted.
20 In other words, is there an ongoing
21 screening of human tumors for SV40 among recipients of
22 vaccines?
23 We've heard the issue several times today
24 about the need for not short-term observation with
25 vaccines that may have oncogenic potential. Here's an

1 example of something that might need a very long-term
2 follow-up, and what's being done about that?
3 DR. KRAUSE: Well, Dr. Lewis can probably
4 add to my comments on this because he's been working
5 in this field longer than I, but there have been --
6 the fact that people were exposed to SV40 was noted
7 fairly early on. So it was possible to follow people
8 over the long term and do different kinds of studies
9 to determine whether different kinds of tumors
10 appeared to be more likely in people who were exposed
11 to one of the vaccines versus not, and also compare
12 people from different regions where different vaccines
13 which had different likelihoods of being contaminated
14 were administered.
15 And so the epidemiological data exists,
16 but such as they are, these are not studies that, I
17 guess, are designed to pick up very low risks, and a
18 confusing aspect of the SV40 in tumor issue is that
19 some of the SV40 has also been detected in tumors of
20 people who are too young to have received these
21 contaminated vaccines.
22 So I think that's complicated. There have
23 been very many published studies. I don't know. Is
24 it over 50 at this point? How many published studies
25 have found SV40 in one tumor or another?

1 DR. LEWIS: It's over 50, less than 60.

2 (Laughter.)

3 DR. KRAUSE: Okay. Of course, these
4 studies, in general, have used PCR, which is a method
5 which is certainly subject to contamination, but
6 there's a very large number of laboratories, many of
7 them independent laboratories that have found this.

8 There also are a few negative studies as
9 well, and so it's difficult to know what to make of
10 it, except one thing is clear, that it's a potential
11 problem.

12 I think Robin Weiss wrote a very nice
13 review -- I think it was in Nature -- of the book The
14 River, which basically said regardless of whether or
15 not any of this stuff is right, the fact is if it
16 isn't right, it's just because we were very lucky, and
17 so I think that that may well apply here as well.

18 ACTING CHAIRMAN DAUM: Thank you very
19 kindly.

20 I have Dr. Stephens, Coffin, Priola, Ms.
21 Fisher. So we'll go next to Dr. Stephens.

22 DR. STEPHENS: It's getting late, and most
23 of my comments have already been made. I do have one
24 issue, again, regarding a number, and that's the ten
25 nanograms issue that repeatedly comes up.

1 Dr. Faggett this morning raised the
2 question in terms of the WHO standard change from .1
3 to ten, the '88 to '90 recommendation, and I just
4 wanted to hear some further discussion. I think Dr.
5 Lewis had his hand up at one point and could comment.
6 It seems to me that less is better, and
7 for all of the issues that we've already discussed
8 today, and again, while did we change that standard
9 from .1 to ten nanograms?
10 ACTING CHAIRMAN DAUM: Dr. Lewis, would
11 you comment?
12 DR. LEWIS: Yes. I think one of the
13 driving forces behind the change in that
14 recommendation was the fact that concepts of
15 carcinogenesis evolved from the late '80s through the
16 middle '90s. Generally it was felt that one oncogene
17 was one transformed cell based on the virus models,
18 but I think the work that was done on carcinoma of the
19 colon by Burt Vogelstein and carcinoma of the breast
20 perhaps by several other people whose names I can't
21 recall right at this moment, but the concept evolved
22 during this period of time over about eight years from
23 one gene being involved in neoplastic development to
24 multiple genes being involved in neoplastic
25 development.

1 Now, when the data that was used to
2 compute the ten picogram limit was based on a one-hit
3 model; when you change that to a two-hit model, you
4 basically increased or reduced the risk factor by the
5 square of that risk factor, and the risk factors that
6 I recall were around, I believe, one -- based on the
7 one-hit model, about one in ten to the 11th or ten to
8 the 12th, and so you double that, and now you're up
9 around one in ten to the 20th.
10 And I think based on that, I think that
11 change in concept was one of the driving forces behind
12 the increase in the level of DNA that was proposed.
13 DR. STEPHENS: Can I pursue this just a
14 minute?
15 ACTING CHAIRMAN DAUM: Yes.
16 DR. STEPHENS: Because we're dealing in
17 this particular instance, I think, with a one-hit
18 model, EA-1G, in essence, which is being used to
19 transform cells. Other events likely occur as we've
20 heard today after that one event. So why not limit
21 the issue to a one-event model rather than going to a
22 more liberal standard, if you will.
23 ACTING CHAIRMAN DAUM: Anyone at FDA like
24 to comment on Dr. Stephens' point?
25 DR. LEWIS: Well, I think in terms of the

1 adenovirus E1A, that gene, while it transforms cells,
2 when you have it in its maximal capacity to infect
3 humans and to spread in the population as a virion,
4 the viruses are not oncogenic. So in an artificial
5 system you do get transformation, but when you put it
6 in a situation where it spreads through the
7 population, and in fact, most of us sitting around
8 this table are probably carrying Adenovirus 2 or
9 Adenovirus 5 in our peripheral blood monocytes.

10 So there's no -- and people who have
11 searched very diligently for the presence of
12 adenovirus DNA in various kinds of human tumors, and
13 there's no evidence that it's there.

14 So I think for these reasons, we're not as
15 concerned about the adenovirus E1A gene, especially if
16 it's isolated, as we are about some other things.

17 Jim might have something to say about
18 that.

19 ACTING CHAIRMAN DAUM: Does Dr. Cook or
20 Dr. Golding have their hands up? If it's about this
21 point, we'll go now. If it's not, we'll go in line.
22 This point?

23 DR. COOK: Yeah, I'd have to go now
24 because if I wanted until the end of the line, I'd
25 totally forget what I was going to say.

1 ACTING CHAIRMAN DAUM: It's happening to
2 all of us, sir.
3 (Laughter.)
4 DR. COOK: So I would like to respond to
5 this specific point, and that is it isn't fair to
6 think about E1A as a one-hit model. The only way that
7 E1A can do anything in terms of a measurable
8 immortalizing event is what we all use in the
9 laboratory, which is to put E1A into 3T3 cells or
10 something like that, and you can get colony formation
11 by using E1A with a neomarker or something like that
12 alone.
13 If you try to do an E1A alone primary cell
14 transformation, and Dr. van der Eb can comment about
15 this, it's virtually impossible. The only person I
16 know who's -- I mean, you can find a rare, a very rare
17 cell line, but it's going to be several orders of
18 magnitude, many orders of magnitude less efficient
19 than E1A plus X, and X can either be a previously
20 altered cell line that's been immortalized so that now
21 you can get a colony formation or it's a complementing
22 oncogene like E1B or <u>ras</u> or polyoma middle T, but
23 thinking of E1A as a single hit is probably
24 inappropriate.
25 DR. STEPHENS: Yeah, I guess part of my

1 concern had to do with the polyoma virus data where
2 low levels, nanogram or picogram amounts, can be an
3 issue in terms of infectivity, not necessarily -- but
4 the combination is, I assume, what I was concerned
5 about.
6 DR. BLAIR: Right, but I think you have to
7 envision polyoma or any of the polyoma viruses like
8 SV40 or polyoma as the equivalent of E1A plus E1B,
9 considering what they can do. SV40T or polyoma T can
10 do a couple of things, and E1A has to have E1B to do
11 those two things.
12 So E1A is probably only half as good.
13 It's probably only part of a transforming gene. Dr.
14 van der Eb really is the world's expert on this, but
15 that's my take.
16 ACTING CHAIRMAN DAUM: Dr. Golding, did
17 you want to speak to this issue?
18 DR. GOLDING: Yeah, I think that it's
19 important to remember that part of our approach is not
20 to look -- I think we would recommend that the
21 Committee does not look at one aspect in isolation.
22 So I think to get sort of hooked up on the ten
23 nanogram versus .1 nanogram is really not -- it's only
24 one thing that we recommend. That, I think, was
25 derived from some of the worst case scenarios that Dr.

1 Peden described. If you are to translate it into what
2 the probability of transmitting an oncogenic gene,
3 such as activated res, you're talking about something
4 in ten to a million, in 100 million human doses.
5 But in addition, because we're dealing
6 with designer cell substance, because we know what was
7 put into them to immortalize them, you have the
8 additional safety of knowing that that particular gene
9 is not in your final product.
10 So you'll have a way of following the
11 product through the purification, as well as looking
12 at the final product to make sure that it's not there.
13 So to your best ability you're saying whatever we put
14 in these cells to start with is not in the product.
15 It's not in the vaccines that go into people.
16 And in addition, we would like to see a
17 reduction on total amount of DNA to a dose that's
18 really reduced the risk of any unknown oncogenic
19 sequence to very, very low probability.
20 ACTING CHAIRMAN DAUM: You know, I think
21 the committee is not hooked up on this one issue
22 because I think we recognize that you can't just say
23 as long as it's below ten we don't care what's in it,
24 but I think the issue that Dr. Stephens is trying to
25 explore is what prompted a change, and a change was

1 made, and it's intriguing to us because the issues
2 that prompted it may help us in our deliberations.
3 So I think that's why we're sort of
4 exploring it here.
5 Dr. Krause, do you want to speak to this
6 issue?
7 DR. KRAUSE: Yeah, just two very brief
8 points. One of them is in addition to the data that
9 Dr. Lewis mentioned, there also is a lot of data that
10 wasn't available about the amount of DNA that's
11 present in blood, and so if you consider the amount of
12 blood in the form of transfusions that people are
13 exposed to without adverse effect, I think that also
14 had an influence on the WHO and changing their limit.
15 The other caution that I would add is not
16 to over interpret the polyoma virus infectivity data,
17 and the reason is because mice are very susceptible to
18 infection with polyoma virus such that it does not
19 take very much polyoma to infect a mouse.
20 And if there were a virus that infectious
21 for humans, it would be very, very unlikely that such
22 a virus would not yet have been discovered. So the
23 kind of as yet unknown agents that one would be
24 worried about would be very unlikely to parallel that
25 situation.

1 So I think the polyoma virus example is
2 very useful as a worst case type scenario, but I don't
3 think that you can take that number and apply it
4 directly to the human situation.
5 ACTING CHAIRMAN DAUM: Thank you very
6 much.
7 Dr. van der Eb, did you want to speak to
8 this issue or has everything been said?
9 DR. VAN DER EB: I think so. I just would
10 like to add that E1A, indeed, it can practically not
11 transform cells, and the reason is that E1A is a very
12 strong inducer of apoptosis in cells. So you need E1B
13 in order to neutralize that effect.
14 And as to the ten nanograms of DNA, this
15 is ten nanograms of turtle chromosomal DNA, of course.
16 It would be a very different issue if the ten
17 nanograms were only activated ras oncogene or
18 something like that.
19 ACTING CHAIRMAN DAUM: Thank you.
20 I'm going to return to my general list
21 now, and did you want to speak to this very issue?
22 DR. KETNER: Yes.
23 ACTING CHAIRMAN DAUM: I'm sorry. You go
24 first.
25 DR. KETNER: It hasn't been mentioned I

1 don't think that as far as DNA is concerned and the
2 transfer of oncogene size matters, and so it's of
3 interest, I think, to know what the nature of the DNA
4 in these perhaps is likely to be. If it's small
5 pieces of DNA, a couple hundred base pairs, then it's
6 much less likely to pose a possibility of transferred
7 an activated oncogene or E1A or even ras E1A plus E1B.
8 So if the preparation of the product
9 involves, for example, DNA of the lysates, the hazard
10 is very much reduced.
11 ACTING CHAIRMAN DAUM: Okay. I have Dr.
12 Coffin, Dr. Priola, Ms. Fisher, and then I have a
13 question, and that's all the people I've recognized so
14 far.
15 Dr. Coffin.
16 DR. COFFIN: As it turns out, I also
17 wanted to speak to the issue on the floor.
18 ACTING CHAIRMAN DAUM: A double header
19 here.
20 DR. COFFIN: The question I had in a sense
21 relates to that. It goes in a slightly different
22 direction.
23 First, my memory of the change in the
24 standard was that there are also some practical issues
25 involved, such as the ability of the technology at the

1 time to measure small amounts, I think, if I remember
2 correctly played -- and you can correct me on this.
3 There may have been some other issues as well as what
4 was, in fact, in practice achievable in any given real
5 life prep.
6 In my memory of the discussions around
7 this, of which I was present at one or two of them,
8 not everything, was that there are also some issues
9 like that in addition to the theoretical issues that
10 were important. This goes actually to the point also
11 about the size of the DNA, is if it wouldn't for the
12 future be worth considering a more directed standard
13 since we have the ability to do PCR assays and, you
14 know, quantitative PCR assays now that are within the
15 range of anybody that's making vaccines, whether it
16 wouldn't be worth down the road considering developing
17 an assay that's based on numbers of copies of some
18 standard sequence that's present in all cell
19 substrates that might be present as measured by some
20 standard assay, a sort of true copy number standard
21 which would then factor in the fact that most of the
22 DNA is probably degraded and very, very unlikely, you
23 know, a few hundred base pair pieces, and extremely
24 unlikely to be possible to be reassembled into an
25 intact gene in the course of this.

1 That's just sort of an aside.

2 It was pointed out earlier, and this is
3 something that was remarked on, I think, during Dr.
4 Peden's talk about sort of the remarkable concordance
5 of the numbers of copies of DNA that came out of all
6 of these different studies.

7 I was struck by that, too, but then on a
8 little reflection, I realized that that's actually an
9 artifact of the numbers because almost everybody uses
10 somewhere around a couple of micrograms of DNA in
11 these studies, and all of the samples on the studies
12 that were reported gave positive results, and since in
13 all of the molecules the genomes were about the same
14 size, so all of them -- considering that lot together,
15 I think an expected result that once you go through
16 the same multiplication with numbers that are about
17 the same to start with, you end up with the same
18 result at the end, whereas, in fact, the underlying
19 biological differences could have been extremely large
20 while all of the numbers that are on those charts or
21 all of the final conclusions that are on those charts
22 should have had less than or equal to in front of the
23 numbers.

24 Maybe in some cases it was really ten to
25 the 13th molecules, but less than or equal to ten to

1 the 13th molecules could be ten to the fifth molecules
2 or ten to the fourth molecules.
3 So I think the concordance that appears
4 there is actually a little bit of slight of hand,
5 inadvertent slight of hand on the part of the analysis
6 that was done.
7 ACTING CHAIRMAN DAUM: Thank you, Dr.
8 Coffin.
9 Dr. Priola.
10 DR. PRIOLA: Yeah, I'd like to break
11 entirely with this train of discussion and return very
12 briefly to the comment Dr. Minor made about
13 contamination with adventitious agents, and
14 particularly with the TSE, and that is that it has
15 become -- it's apparently much easier to infect cells
16 with TSE agents than we originally thought, and I know
17 that he's aware of this.
18 Experimentally we've learned a bit better
19 how to do that. It's still quite unpredictable and
20 quite difficult to do, and under the circumstances
21 that are being discussed here with these PER.C6 cells
22 and exposure to fetal bovine products that might be
23 potentially contaminated with BSE, we're talking about
24 logs of difference in terms of exposure to
25 infectivity.

1 So when we do these things experimentally,
2 we expose these cells to enough infection, infectivity
3 to kill ten million mice, and if you look at the very
4 few studies that have been done looking at TSE
5 infectivity in blood, there's enough in there to kill
6 a handful of mice.

7 So we're talking about very, very
8 different situations. So under the conditions being
9 described here, I would certainly say that it's
10 unbelievably difficult to passage TSE infectivity in
11 that regard, even though we are getting better at it.

12 I wish it were as easy as it might be, and
13 I'd also like -- yeah, but I'd also like to very
14 briefly address Dr. Aguilar-Cordova's comment about
15 using microray analysis to perhaps pick up other
16 markers of TSE infection that might be useful in a
17 situation like this, and it's an excellent idea.

18 And I know that recently there have been
19 reports, for example, that there is an erythroid
20 differentiation marker whose expression level has
21 changed in TSE infected animals, and those are the
22 sorts of novel approaches that would be extremely
23 useful in these situations, but may take several years
24 to develop.

25 ACTING CHAIRMAN DAUM: Thank you very

1 kindly.

2 Ms. Fisher.

3 MS. FISHER: Well, I would like to return

4 to the SV40 issue. SV40 has been cultured out of

5 brain, bone, and lung cancers of children born to

6 parents presumably who were exposed to polio vaccines

7 contaminated with SV40, and there is a suggestion by

8 some researchers that there was horizontal

9 transmission perhaps involving exposure to SV40 DNA,

10 and my question is: are the animal studies looking at

11 successive generations of mice exposed to residual DNA

12 for tumors?

13 ACTING CHAIRMAN DAUM: Thank you.

14 Dr. Krause or someone from FDA want to

15 take that one on?

16 DR. PEDEN: No, I don't think we know

17 that, and we certainly hadn't factored it in, but

18 maybe we could consider it at some stage.

19 The trouble with the mouse experiments, of

20 course, is you normally are going to sacrifice the

21 mouse, but we could in the future design experiments

22 to address that.

23 And while I'm on the microphone, I just

24 wanted to say in defense of those numbers there were

25 a limited number of studies, John, that did go down in

1 the dose response. Now, admittedly there weren't that
2 many, but there were some.
3 So we do in some cases have a lower limit,
4 albeit in very small studies. So it may not be quite
5 such a slight of hand as you imply.
6 ACTING CHAIRMAN DAUM: Okay. We're
7 getting to the point where I would sort of ask people
8 to really as they contemplate comment, whether it's
9 been said before already or whether it's something
10 new, and also direct your attention to the last line
11 of the slide, which is to discuss any additional
12 safety concerns besides the ones that are raised.
13 Ms. Fisher raised the one of passage,
14 placental passage across to subsequent generations.
15 Does anyone have others?
16 We're trying to sort of sum up. So we're
17 looking for new points for the top issue and comments
18 about additional safety concerns, and then we can come
19 to closure on this, I hope, fairly soon.
20 Dr. Coffin, please, and then Dr. Moulton.
21 DR. COFFIN: Just to make sure it gets on
22 the list. I said this before, but I just want to put
23 it on the list. One is the recombination issue, which
24 I think really was additional to that, and the other
25 is packaging of host cell DNA into otherwise empty