U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

PUBLIC HEALTH SERVICE

FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

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VACCINES AND RELATED BIOLOGICAL PRODUCTS ADVISORY COMMITTEE

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MEETING

OPEN SESSION

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FRIDAY,
MAY 12, 2000

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The meeting took place in the Kennedy Room, Holiday Inn, 8777 Georgia Avenue, Silver Spring, Maryland at 8:00 a.m., Dr. Harry B. Greenberg, Chair, presiding.

Present:

DR. HARRY B. GREENBERG, Chair
MS. NANCY CHERRY, Executive Secretary
DR. ALICE S. HUANG, Member
DR. STEVE KOHL, Member
DR. KWANG SIK KIM, Member
DR. DIXIE E. SNIDER, JR., Member
DR. DIANE E. GRIFFIN, Member
MS. BARBARA LOE FISHER, Member
DR. WALTER L. FAGGETT, Member
DR. DAVID S. STEPHENS, Member
DR. DONALD BLAIR, Temporary Voting Member
DR. PHILIP MINOR, Temporary Voting Member
DR. SIDNEY WOLFE, Temporary Voting Member
DR. REBECCA SHEETS, FDA Representative
DR. KEITH PEDEN, FDA Representative
DR. ANDREW LEWIS, FDA Representative

Present: (cont.)

DR. PHILIP KRAUSE, FDA Representative
DR. PETER PARADISO, Public Comment
MS. JANET ROSE CHRISTENSEN, Public Comment
DR. FIRELLI CAPLEN, Public Comment

Also Present:

DR. BILL EGAN

I-N-D-E-X

P-R-O-C-E-E-D-I-N-G-S

(9:25 a.m.)

CHAIRMAN GREENBERG: I'm a slow learner. Good morning, everybody. I'd like to welcome you to the second day of the VRBPAC meeting. We have a very interesting and important set of discussions today, so I'd like to get it started.

The first thing I will do is turn the meeting over to Nancy Cherry who will read -- no, there are no -- outstanding. So without further ado I'd like to for the record introduce the panel members to the public and introduce our visiting members. So if we could start up there, David?

DR. STEPHENS: Dr. David Stephens, Emory University, Atlanta.

DR. KOHL: Steve Kohl, Oregon Health Sciences, University of Portland.

DR. SNIDER: Dixie Snider, Centers for Disease Control and Prevention, Atlanta.

DR. HUANG: Alice Huang, Cal Tech.

DR. FAGGETT: Walter Faggett, Pediatrics Section, AMA, Washington, D.C.

DR. GRIFFIN: Diane Griffin, Johns Hopkins.

DR. KIM: Kwang Sik Kim, Children's

Hospital, Los Angeles.

CHAIRMAN GREENBERG: Harry Greenberg, Stanford University and the Palo Alto VA Hospital.

DR. BLAIR: Don Blair, NCI, Frederick, Maryland.

DR. WOLFE: Sid Wolfe, Public Citizen Health Research Group.

DR. MINOR: Philip Minor from the National Institute for Biological Standards in the U.K.

DR. KRAUSE: I'm Phil Krause from the Office of Vaccines Research and Review for the Center for Biologics.

DR. SHEETS: I'm Rebecca from the same office.

DR. PEDEN: Keith Peden, Office of Vaccines, CBER.

DR. LEWIS: Andrew Lewis, Office of Vaccines, CBER.

CHAIRMAN GREENBERG: And Ms. Barbara Loe Fisher, the consumer representative is out of the room for a second, I guess, but she is here.

I would like to now have open public hearing and I know of two people who wish to address the Committee. The first will be Dr. Peter Paradiso from Wyeth.

DR. PARADISO: Thank you, Harry. I'd like to just take a couple of minutes this morning. I know that you're going to be talking about cell substrates today and have already begun there. As Harry said, I'm with Wyeth Lederle Vaccines. We've been working on viral vaccine products for a number of years and have been investigating the use of Vero cells in that research and so we were asked to just come and make a couple of comments on the value of the Vero cells from our perspective and in our programs. And so I'll just take a few minutes and do that today.

We have several programs on-going currently using Vero cells as substrates. Those are live RSV vaccine program, the subunit vaccine program and the influenza viral vaccine program. We first started using Vero cells with the subunit vaccine program in the mid-1980s and in fact, I presented first to this Committee in 1987 the use of Vero cells for the production of a purified component vaccine which is listed in the middle there as a subunit RSV vaccine. And more recently have adapted our live programs to the Vero cells.

During the course of those 15 years, there has been a lot of progress made in our ability to do testing on these cell lines and so while those cells

were qualified in 1987 they continue to be updated and I'll show you a little bit of that information as we go here.

The benefits from our perspective of Vero cells, in particular, is that they're quite well characterized. They've been used in vaccines that are marketed in various regions of the world and studied quite extensively.

Now from our perspective, they have the advantage of giving very high yields of virus and that high yield of virus has a couple of advantages that we see, obviously, from a manufacturing perspective that's an advantage, but the other advantage is that it allows you to, in fact, dilute your product fairly significantly and so in that dilution reduce nonantigen specific components so if you can get two or three logs higher of virus at V cells as compared to a different cell, then that two or three log dilution helps you in your purity.

The Vero cells can be grown in serum in mammalian product free media and this is a very important aspect, at least from our perspective because we've moved more and more now towards serum free medias and particularly now mammalian product-free porcine and bovine and other components

that we know may potentially cause problems.

And then we can achieve with these Vero cell lines very low levels of DNA in the final product, in part, through purification and in part, as I said, through fairly large dilutions of product end product.

As I said during the course of the last 15 years, these cells have been tested extensively and I have three slides with lists of tests that I'm not going to go through each one of those tests, but I wanted to just characterize them briefly. There's a series of tests that have actually been long-standing tests and what I would call more general types of tests that include everything from morphology, karyology, sterility, etcetera that are standard testing for any cell line for vaccine production. And then added to that then are more specific tests for adventitious agents that often focus on groups of adventitious agents or viruses, in particular, but aren't necessarily, although in some cases are, but are necessarily specific for any one virus. And so it would include cultivation in cells, co-cultivation, other methods for growing viruses of unknown origin and so are nonspecific in that regard.

And then more recently over the last five

to 10 years there's been a much expanded use of very specific tests as they become available for specific viral components or other potential tests for DNA and other methods for characterizing the purity of the product and the safety of the cell line and those are predominantly PCR based tests, whether they be PERT assays or a standard test and you can see they include characterization of any DNA that might be in the product and other very specific testing schemes.

And thus in conclusion, in our hands and in our thinking these are a very well characterized cell line and so therefore have all of the characteristics of a viral vaccine production system. They've passed all of those tests that I have shown you and so as we continue to update those tests and do more tests, obviously, we'll continue to evaluate the characteristics of the cell line.

The high virus yields are very significant for us, obviously, as I said, for production as well for purity of product. They're superior to diploid lines because of their ability to grow in serum-free and product-free media and as I said, very large scale production can produce obviously millions of doses that will be required for these vaccines.

Thank you.

CHAIRMAN GREENBERG: Thank you, Dr. Paradiso. We have time for a few questions.

Dr. Faggett?

DR. FAGGETT: Dr. Paradiso, thank you. By the way, Harry, was the test that you mentioned on that list, he flashed it up there real quick. I didn't know if that was specific --

CHAIRMAN GREENBERG: No, what I mentioned would be an investigational analysis at this point would be transcriptional analysis, using micro-array technology but I was, for your interest, Walter, I was approached by a member of the FDA whose name I've already forgotten who -- the FDA has just gotten some money to do exactly that type of analysis.

DR. FAGGETT: Great. My question for Dr. Paradiso, we've heard reports that in one of your tests there was at a passage of 150, there appeared to be tumor development. Just a general -- okay. You do have tumorigenicity tests that you do, correct?

DR. PARADISO: Correct.

DR. FAGGETT: How do you interpret your results at different passage levels? That's a dumb question.

DR. PARADISO: The tests that we've done so far, there's been no evidence of tumorigenicity.

We have our cell banks started a low passage, passage 126 in our virus production out, quite a few doublings, 50 or 60, not passages, but the doublings of the cells pose that, have that for tumorigenicity and there's never been any evidence of tumorigenicity.

DR. FAGGETT: Thank you.

CHAIRMAN GREENBERG: Excuse me.

DR. BLAIR: Yes, just a question about the serum-free. At what point do the cells go into serum-free, is this after the master bank is prepared or only during production that they are grown in serum-free media?

DR. PARADISO: I will have to ask one of my colleagues for the answer to that question.

CHAIRMAN GREENBERG: Please go to a microphone and identify yourself.

DR. CAPLEN: Fi Caplen from Wyeth. The cells are grown in serum-free media during production.

CHAIRMAN GREENBERG: Okay. If there are no further questions, thank you, Dr. Paradiso and we'll go to our next speaker who is Dr. Janet Christensen, I think, from Targeted Genetics. Did I get the name right?

DR. CHRISTENSEN: Thank you and good morning. I don't have any slides, but I would like to

take a few moments to make a few remarks because we do believe this is a very important issue. It's really a pleasure to have a few moments of your time to address you and the invited guests this morning. This is really a timely topic for Targeted Genetics Corporation, as well as for many other companies and organizations in the industry. As you may or may not know, Targeted Genetics is participating with the International AIDS Vaccine Initiative, better known as IAVI and Children's Hospital of Columbus, Ohio to develop a gene therapy derived vaccine for HIV. And as such, we have an interest in the topic of cell substrates from neoplastic cell lines.

As scientific capabilities continue to grow in the areas of genetics, molecular biology and microbiology, new opportunities have arisen that may result in solutions to some very serious and life threatening diseases. We believe that viral vectors containing individual genes from infectious agents, as well as other new technologies may provide a means to deliver safe and effective vaccines for disease prevention.

That new technology, specifically genetic vaccines, may be derived from a neoplastic cell line and doing so is not necessarily a new concept. As

early as 1954 Hela cells were used to produce to produce an adeno virus vaccine that was administered to a small number of human volunteers. These subjects were followed for 11 years and there were no reported evidence of neoplasm formation. Researchers have spent many years enhancing their understanding of tumor producing capabilities of neoplastic cell lines and as well as production and analytical methodologies. Obviously, much has been learned and changed since the 1950s.

It is very important to point out that there are significant differences between the use of cells to produce a vaccine versus the cells to produce and subsequent incorporation of these cells or cell substrates and fragments into a vaccine. These cases give rise to very different risk assessment issues. In assessing risk, it is imperative that several factors be considered. For example, the number of cells per dose, the quantity of host cell DNA and other impurities per dose, purity of the antigen itself or the immunizing agent and lastly, the control of vaccine components personnel, manufacturing process and the final bulk product. For example, the traditional type of vaccine may contain a determinable number of cells per dose which can be monitored by a

purity limit assay. For these traditional products, it is clear that there would be potentially a significant amount of host cell DNA in each dose. For genetic vaccines, it may be possible to produce a product that has no cells per dose as .2 micron filtered thus enhancing the potential for sterility and potentially minimizing the risk requirement of thimerosal or other preservatives and have less than 100 picagrams of host cell DNA per dose which is in line with that of the recombinant DNA products and as Dr. Paradiso mentioned, are well characterized biologic.

Additionally, the new processes can reduce the size of remaining DNA to small fragments that no longer have the capabilities to encode for a functional oncogene or infectious entity. These steps serve to increase the safety of the vaccine and therefore reduce the potential risk. These factors, including the source and cell substrate derivation must be addressed in a balanced and risk associated fashion.

The draft document entitled "A Defined Risks Approach to the Regulatory Assessment of the Use of Neoplastic Cells as Substrates for Viral Vaccine Manufactured" issued by the Division of Viral

Products, Office of Vaccines Research and Review, was the subject of discussion at a CBER co-sponsored meeting in September of last year and it raises many excellent points and the quantitative risk assessment for vaccines derived from neoplastic cell substrates. We support the development of objective policies and guidance that is consistent with the mission of the FDA and protecting public health. We also believe, however, that it is imperative that current technological developments be more adequately addressed to insure that risk be evaluated with the best available technologies and done so in an objective and scientifically sound fashion.

We agree that the potential benefit that may be derived from a vaccine regardless of cell substrates source must be evaluated in terms of risk to the vaccinated population. We also believe that not only should the risk be assessed by strictly empirical analysis of the bulk or finer product, but also through a use of an integrated approach to minimize any risk opposed by any vaccine. These include the process, the personnel, the facilities and the components which is consistent with the philosophy and mission of CBER.

These include, for example, the on-going

assessment of raw materials derived from animal sources and other components. We had an example of that by Dr. Paradiso. Multiple downstream purification steps that provide a combination of virus removal and inactivation and demonstration thereof consistent with the existing ICH and FDA guidance documents, removal of impurities such as host cell DNA, manufacturing components such as detergents and solvents and the prevention of subsequent contamination with unintentional impurities such as microbes and viruses.

The removal of host cells from the finished product to enable 0.1 micron filtration of the bulk and final product. Assurance that appropriate facilities controls exist to prevent the contamination of a product with adventitious agents and testing of the master working and extended cell banks to confirm the absence of adventitious agents or other undesirable attributes.

The challenge for all us comprised of industry, academics and the regulators is to provide a safe and efficacious and cost-effective vaccines for the global population.

I've briefly outlined some of the challenges as well as the potential advantages of

these types of vaccines, both the traditionally and genetically derived in neoplastic cell lines.

As the process of developing policies and guidance proceeds, we believe that the following points should be considered:

Number one, insure the understanding of the processes employed to produce, as well as assess the risk associated with the genetic vaccines.

Number two, the development of objective and scientifically sound methods, based on the current state of the art methodologies.

Three, that all data referenced in the development of these policies and guidance be derived from peer-reviewed scientific works and four, ensure fair balance of risk versus benefit.

It would not be in the interest of world-wide public health to allow perceptions, misinformation and the requirement of yet to be infected technologies and methods to restrict the development of genetic vaccines that may be derived from neoplastic cell lines. This would be analogous to telling NASA not to proceed to the moon until they've developed warp drive.

This really is a very exciting time for the vaccine community and as these new technologies

and especially the genetic vaccines emerge I, as a public health microbiologist, am very eager to participate in the emergence of these next wave of vaccines and their promise for the future.

Targeted Genetics Corporation is ready to participate in the enhancement understanding of these issues associated with genetic vaccines and in the development of policies and guidance. These must balance the need to protect and preserve public health, while supporting the emergency of vaccines derived from new technologies that may include the use of neoplastic cell line substrates to provide novel vaccines for disease production.

I thank you for your time.

CHAIRMAN GREENBERG: Thank you. We don't have warp drive yet?

(Laughter.)

DR. CHRISTENSEN: I do on my computer.

CHAIRMAN GREENBERG: We have time for some questions from the panel if there are any.

DR. CHRISTENSEN: I would like to comment that I will be providing my talk to Ms. Cherry for incorporation into the appropriate record.

CHAIRMAN GREENBERG: Okay, if not, thank you very much and that represents all the known public

commentaries that we have. Is there anybody else in the audience that wishes to make a statement at this time?

Okay, let the record show that nobody has stepped forward in which case can I just proceed?

I'd like to make one announcement. We announced it in the closed session, but because we have basically a single topic this morning, we're going to work through lunch so there will not be a lunch break and then coming back, we will try to do the entire program and then adjourn.

So we now are moving into the FDA's presentation and the first speaker will be Dr. Keith Peden, who will be giving us an introduction.

DR. PEDEN: So welcome to the Open Session. My name is Keith Peden. I'm with the Division of Viral Products in the Office of Vaccines, Research and Review of CBER. I'm going to give a brief introduction and on the following first slide is what I'm going to say.

Following my brief remarks, Andrew Lewis is going to present his talk on the draft policy proposals for the use of neoplastic cells, the substrates of vaccine manufacture. Then Dr. Phil Krause is going to present some CBER research related

to neoplastic cells. At this point I think there's going to be some discussion and then Dr. Rebecca Sheets will present specifically the history and characterization of one neoplastic cell line, the Vero cells.

First, to clarify what types of cells we're dealing with, I'd like to describe the types and characteristics of cells that are being used or could be used for cell substrates of vaccine production. What we have is several times the cells and in this slide I've shown primary cells. These are isolated from an animal and established in culture.

The cells were used without passage and examples of these are the African green monkey kidney cells that are used for polio vaccine, IPV. If you pass and expand the primary cells, you get diploid cells and these are cells that are used only after a limited passage and they have a finite lifespan and cells such as these of the chick embryo fibroblasts which are used for measles and mumps vaccines.

Because the cells are used either directly or over limited passage, the opportunity to assess these other types of cells for adventitious agents is by necessity limited. In the approach taken with nonhuman primary or diploid cells is to assay the

donor animals to make sure they don't contain simian agents such as SIV, simian foamy viruses or others. And in the case of the chick embryo fibroblast is to use specific pathogen-free flocks.

The other types of cells are what we call diploid cells strains in the old nomenclature, what people now generally refer to as diploid cells lines. These are cells, diploid cells that are expanded to sufficient numbers and then cryopreserved. Again, these cells have a finite lifespan and examples include WI38, MIC5 and the fetal rhesus lung cell line, diploid cell lines.

If you carry cells, diploid cells out for several passages, what often you arrive at are what we call spontaneously transformed cells and these are cells that have been passaged in vitro to survive crisis. These cells are immortal. The mechanism of transformation is unknown. Cells are generally aneuploid and they have chromosomal rearrangements and they may be or become tumorigenic. And examples of these are the Vero cell lines from African green monkeys, the BSC-1 line and the CV-1 also from African green monkeys and the Chinese hamster ovary cell.

Even cells have generally been unable to be passed to develop spontaneously transformation.

The reason for that is not clear. You can also immortalize cells by virus transformation and so these virus transformed cells are immortal and the mechanism for transformation now is learned because you use a specific agent. The cells are usually aneuploid and often have chromosome rearrangements and the cells are usually tumorigenic and such cells such as the Epstein-Barr virus transformed B cells and herpes virus transformed T cells.

At the present moment we don't really think these cells will be used for vaccine manufacture, but cells immortalized by known oncogenes very well might, so these are immortalized with either specific cellular or viral oncogenes, the cells are immortal, the mechanism of transformation is known since we used specific agents. The cells are usually aneuploid and may have chromosome rearrangements and cells are often tumorigenic, but not always the case. Examples here are human embryonic kidney 293 cells and the PER.C6 cell line transformed by adeno virus E1 region.

Another type of cell line which could be used is the tumor derived cells. These are established from tumor cells and in many cases the human tumor cells. These cells are immortal. The

mechanism of transformation is usually unknown although hints can be gleaned from the type of cell that was used, the type of tumor that we used which was for example Hela cells with human papilloma virus. Cells are usually aneuploid. They have chromosome rearrangements and they're usually tumorigenic.

The advantage of these cells, we've just heard about -- sorry, let's just define what we mean by neoplastic cells, include these four classes, the spontaneously transformed cells, the virus transformed cells, oncogene transformed cell lines and the tumor-derived cell lines. This is what we're generally calling neoplastic cells and Dr. Andrew Lewis in the following presentation will give his reasons, give our reasons why we think this is an appropriate term.

Now the advantages of using the neoplastic cells you've heard some about. They're immortal and can be expanded and tested indefinitely. That's certainly an advantage for adventitious agent testing. They usually grow well in culture. They can be adapted to growth in serum-free medium and that's clearly an important issue to remove certain animal products. Immortalization allows the growth of certain fastidious cell types, for example, certain

cells, primary cells say kertinocytes require fetal layers, but if you immortalize them, transform them, then they can grow alone.

They can be single cell cloned which is impossible for most human cells, diploid cells. Cells in the cell banks can be characterized and tested repetitively. In addition, cells themselves cna be engineered genetically to either express the protein products which can be used as a vaccine or act as a helper cell substrate, for example, the packaging cell line or even a complementing line such as 293 and PER.C6. The viruses often grow to much higher titres and therefore as we heard the purification level is higher and they're much less expensive and finicky to propagate.

So the last slide, what's driving our consideration of these cells now? Well, as you also heard and again new vaccines coming down the road for development of HIV vaccines, many of these are to be produced in neoplastic cells. In addition, there's rapid development of vaccines to emerging diseases such as the H5N1 and H9N2 influenza viruses. These are probably going to be grown in neoplastic cells.

Progress in understanding carcinogenesis, the cell substrate meeting last September, the panel

discussed that and it was clearly, carcinogenesis is a multi-step component process, therefore, if we are worried about such things as DNA, provoking an oncogene it's unlikely that a single introduction of even if you did introduce a single oncogene that would result in a cancer. It's a multi-step process.

Progress in detecting adventitious agents has made leaps and bounds since the introduction of PCR and all ceratin reverse transcriptase assays such as the PCR base reversed transcriptase such as the PERT assay about which this Committee, I'm sure, has heard quite enough over the last few years. The ability to detect adventitious agents gives us a much higher level of competence that the cell substrates are lacking such agents.

Experience we have now with biological, purified biologicals produced in tumor cells. There has not been any adverse events associated with the cell substrates and products produced in neoplastic cells may not be adopted to alternative cell substrates and finally the practical considerations and advantages mentioned in the previous slide.

So that's one reason why we now are starting to deal with that and I don't think there's any need for questions, so if I can go call on Andrew

Lewis to present his presentation.

CHAIRMAN GREENBERG: I'd just like to see if there's anybody, any Committee -- Dr. Minor?

DR. MINOR: You described oncogene transformed cells and you said that's okay because what -- in that example, you know where they're transformed. If you take something like a PER.C6 cell and you take the E1 out of it, somehow, does that mean that they're no longer transformed, even though they aneuploid?

DR. PEDEN: No one has done that specific experiment. What we've heard is that people have looked at say, for example, Hela cells and expressed antisense to human papilloma virus E6 and E7 and now they appear not to be transformed. That's not published, but that's what we've heard. So those kind of experiments could and perhaps should be done on PER.C6 cells.

CHAIRMAN GREENBERG: Any other questions? Okay, thank you very much, Dr. Peden. And now we'll move on to Dr. Lewis who is going to give us a draft interim policy or is it an interim draft policy?

DR. LEWIS: I seem to be missing the first slide.

(Pause.)

The first slide must have dropped out when I was running through these things last night. It simply gives my name and the title of the talk which I think is listed on the program. Just to repeat that I'm Andrew Lewis with the Division of Viral Products in the Office of Vaccines at CBER.

I'd like to begin my talk by pointing out what I'm going to say today has evolved over the past several years from discussions within the Office of Vaccine Cell Substrate and Adventitious Agent interest group. The work of this group on the use of neoplastic cell substrates has not been completed, but we think enough has been accomplished at this point in time to develop as an exercise several draft policy proposals to serve as a focal point for the discussion of the use of neoplastic cells in the manufacture of viral vaccines.

As outlined in this slide, the presentation of the events and discussions that provided the basis for these draft policy proposals and the draft policy proposals themselves will be the focal point of my talk today. I'm going to conclude by reviewing our plan to continue our deliberations until it's possible to develop a more comprehensive draft policy statement.

The members of the cell substrate group who were participating in these discussions are listed in the next slide.

Now before I begin to review the events and the discussions that provided the basis for our draft policy proposals, I'd like to go over two items for you to keep in mind. The first of these items which Dr. Peden has already mentioned is the definition of neoplastic cell lines which is shown in the next slide.

From my talk I'm using the term neoplastic cell in its broadest sense to include all types of immortalized and continuous cell lines. These cell lines are derived by the process of neoplastic transformation in contrast to the other uses of the word transformation. They include cells transformed spontaneously by unknown mechanisms, transformed by clone, viral cellular oncogenes or transformed by oncogenic viruses.

We immortalize our continuous cell lines in the use categories, can either be tumorigenic or nontumorigenic, as I think most of you are aware of that.

As you'll see later on in the talk the nature of the transformation event itself, that is,

whether the mechanism of transformation is known or unknown is an important feature in the development of our draft policy proposals.

The next slide deals with the products that we're covering in these proposals. The products that we're covering in the draft policy proposals is limited to parenteral viral vaccines that are developed in neoplastic cells. Proposals regarding the use of neoplastic cells to develop oral or mucosal vaccines will be covered in future drafts.

As I hope the Committee is aware, first presentation of the possible use of neoplastic cells as vaccine substrates was at the advisory committee meeting in November of 1998. At that time several of us reviewed the issues that we believed to be associated with the use of neoplastic cell substrates and we propose the development of what we termed a defined risk approach as a way to think about managing these issues. The basic aspects of this approach are shown on the next slide.

The defined risk approach that we proposed then consisted of first assessing quantitatively the risk posed by issues associated with the use of neoplastic cells for vaccine manufacture and second to evaluate these risks individually and cumulatively.

From this type of assessment, potential benefits and risks posed by the use of substrate, various substrate vaccine combinations could then be compared with the risk presented by the disease that requires immunoprophylaxis.

Now in addition to the defined risk approach, we presented a plan to move forward with an evaluation of the issues associated with the use of the neoplastic cells as vaccine substrates. And this plan is presented on the next slide.

Again, and I hope the Committee will recall that they agreed to the plan that we put in motion and that plan was to prepare a draft proposal of our defined risk approach within six months and to use this proposal as a focal point for an international workshop on neoplastic cell substrates. And finally, we were going to take the comments from the expert panels that convened during this workshop to develop draft policy proposals.

Now in implementing this plan, the proposal, the draft of our defined risk approach was completed in May and June of 1999 and as you can see on this slide the International Workshop on Cell Substrates was held in Rockville, Maryland, September 7th through the 10th last year.

I think as the Committee will remember I summarized the outcome of the September meeting at the Advisory Committee. It was held three days later on September 14th. Time doesn't permit a detailed review of the September cell substrate workshop, so in this slide I've listed six of the consensus agreements that were reached by the expert panels that played an important role in developing the draft policy proposals that I'm going to be presenting today.

These agreements are as follows: first, vaccines should not contain adventitious agents. Second, adventitious agents pose significant challenges to the use of neoplastic cells as vaccine substrates. Third, primary cells pose a greater risk for adventitious agents than neoplastic cells. Fourth, neoplastic cells pose risk for the presence of unrecognized oncogenic agents. Fifth, although residual neoplastic cell substrate DNA should pose little risks available data are insufficient to dismiss it as a concern and finally, although the defined risk approach is a useful way of organizing regulatory thinking, conclusions that are drawn from this type of data analysis should be made with caution.

And since the September 1999 meeting, the

cell substrate group has met almost weekly to review data, notes and the transcripts of the meeting and to discuss ways to address the concerns associated with the use of neoplastic cell substrates. During review of the group's work in early April it was apparent that a development of detailed guidance to insure the safe use of neoplastic cell substrates would require more information than was currently available.

As the pace of proposals for the use of neoplastic cell substrate for vaccine development is picking up and as we in CBER have no choice but to respond to these proposals, the decision was made at this time to summarize the work of the groups since last September by developing draft policy proposals to serve as a focal point for further discussion.

As recognized by the expert panels at the September meeting, the most difficult issues posed by the use of neoplastic cell substrates is the issue of their possible contamination with the adventitious viruses, especially unknown viruses. Thus, the draft policy proposals were designed from the adventitious agents' perspectives that I've outlined in the next slide.

These perspectives include the need to recognize that adventitious agent contamination

represents a major concern of the use of neoplastic cells as vaccine substrates. The set levels of risk with the possible presence of adventitious agents in neoplastic cell substrates to establish features of neoplastic substrates that can contribute to adventitious agent evaluation. And finally, to possibly identify neoplastic cell lines that may not require the application of a defined risk approach.

To develop the draft policy proposals, these perspectives were expanded by the cell substrate group as shown in the next series of slides.

Perspective 1 and 2 is developed by the cell substrate group deals with the adventitious agent as a major concern and with setting levels of adventitious agent burden. Perspective 1 states that neoplastic cell substrates should be documented not to contain adventitious agents.

Now in general, viral vaccines prepared in neoplastic substrates should pose no greater risk of containing adventitious agents than purified biotech products such as monoclonal antibodies which are prepared in neoplastic cells as well. The limits of adventitious agent burden that we are suggesting for vaccines is less than one infectious unit per million doses and we should point out that the limit set for

viral clearance of monoclonal antibody products should exceed by a thousand fold the level of any initial contaminant.

Now everyone agrees that vaccines should not contain adventitious agents, but the problem of defining "not contained" means establishing limits for adventitious agent burden. As I've noted on the last slide, we're suggesting that less than one infectious unit per million doses to be an appropriately safe level of adventitious agent burden for vaccines. The question of how to go about establishing this level of adventitious agent burden for vaccine manufacturing in neoplastic cells is stressed in the next slide.

There are three obvious approaches to establishing limits for adventitious agent burden and they are to apply the process of viral clearance and inactivation as useful biotechnology products, to borrow vaccines that can be highly purified. The second method is to determine if there are any neoplastic cell substrate vaccine combinations that due to the safe use of the substrate itself may not require establishing quantitative limits on adventitious agent burdens. Third, is to develop assays of defined sensitivity to detect or establish the absence of adventitious agents at the desired

levels.

On this slide I've outlined the concept of viral clearance and inactivation. And this concept is based on the fact that the presence of adventitious agents in neoplastic cell substrates can be reduced substantially during the manufacture of highly purified or inactivated vaccines. And by measuring the reduction of model viruses that are added to the substrates it is possible to determine the levels of virus removal or inactivation that occurs during the manufacturing process.

And from these viral clearance and inactivation of these model viruses can be used to establish the level of potential adventitious agent burden in neoplastic substrates that do not contain adventitious agents.

And perspective 3 I think is presented in this slide. Perspective 3 deals with features of neoplastic cells that contribute to adventitious agent evaluation. These basic features include the origin in terms of the species, the donor tissue and the passage history of the cells and whether the mechanism of transformation is known or unknown.

To give you several specific examples, concerns with testing cells from rodents will

certainly differ from concerns with testing cells from primates. Concerns with testing cells from adult will differ from concerns with testing cells from a fetus and concerns with testing cells from a kidney will obviously differ from concerns with testing cells from the lung or the brain. If the cell substrate is derived from cells transformed by clone, viral, assay or oncogenes they might be less suspect than if they were derived from a tumor that develops spontaneously.

Based on these perspectives, the cell substrate group developed five draft policy proposals that I'm going to be presenting in the next series of slides. There was actually a handout that summarizes these proposals and it might be easier to follow, for the Committee to follow if they refer to that handout because I think we tried to summarize all that information in that, in the information on these slides in that table.

CHAIRMAN GREENBERG: Andy, is that the single --

DR. LEWIS: It's a table, it's a single table, yes.

CHAIRMAN GREENBERG: I assume this --

DR. LEWIS: It's that table, yes.

CHAIRMAN GREENBERG: It's titled "Summary

of Draft Policy Proposals for Use of Neoplastic Cell Substrates for Manufacture of Viral Vaccines" and it lists draft proposal numbers 1 through 5 on it.

Everybody has it?

DR. LEWIS: Draft policy proposal 1 affects inactivated viral vaccines, purified viral vectored vaccines, a purified viral subunit vaccines. This policy proposal reads as follows: "When produced by manufacturing processes that meet the criteria for viral clearance and inactivation required for purified biotech products, these types of viral vaccines can be developed in neoplastic cell substrates provided that the passage history of the substrate is appropriately documented and the cell substrate does not contain adventitious agents. If these vaccines are manufactured in Vero cells, then conditions required for Vero cells should apply." And the next proposal will be dealing with Vero cells.

"Residual cell substrate DNA in these products should not exceed 100 picagrams per dose."

Draft policy proposal 2 affects minimally purified live attenuated viral vaccines and minimally purified virus vectored vaccines. This proposal reads as follows: "These types of vaccines can be developed or manufactured in nontumorigenic Vero cells based on

current recommendations for cell substrate evaluation. Residual Vero cell VNA in the final product should not exceed 10 nanograms per dose, the limit that was set by the World Health Organization."

Dr. Sheets is going to have more to say about testing and evaluation of Vero cells later on this morning.

Draft policy proposal 3 also affects minimally purified live attenuated viral vaccines and minimally purified virus vectored vaccines. This draft policy proposals reads as follows: "These types of vaccines can be developed and manufactured in neoplastic human and mammalian cells that have been transformed by defined viral or cellular oncogenes and that do not contain adventitious agents provided that (1) current recommendation for cell substrates are met; and (2) any additional recommendations that are deemed appropriate for cells originating from a specific source in tissue are follows. Residual cell substrate DNA in these products should not exceed the 10 nanogram per dose limit."

Draft policy proposal 4, likewise deals with minimally purified live attenuated viral vaccines and minimally purified virus vectored vaccines. This draft policy proposal reads as follows: "These types

of vaccines can be developed and manufactured in neoplastic human and mammalian cells, cells of defined origin that do not contain adventitious agents, that have been transformed in tissue culture by oncogenic viruses that first it can be documented that the vaccine does not contain the transforming virus at the limit of detection of this virus. And this limit will need to be defined. Second, current recommendation for cell substrates are met. And third, any additional recommendations that are deemed appropriate for cells originating from a specific source in tissue are followed. Residual cell substrate DNA in these products should not exceed the 10 nanogram per dose limit.

Like proposals 2 and 4, 2 through 4, policy proposal 5 also deals with minimally purified live attenuated viral vaccines and minimally purified virus vectored vaccines. This draft proposal reads as follows: "The development of these types of vaccines in neoplastic cells derived from naturally occurring tumors from humans and other mammals or from human cells and mammalian cells that have been transformed from unknown mechanisms is discouraged at this time."

Now during its deliberations, the cell substrate group identified a number of items that need

more attention. These items include developing adventitious agent testing assays of sufficient sensitivity to establish acceptably low levels of possible adventitious agent burden; developing methods to assess the risk posed by residual cell substrate DNA; addressing the relevance of the PrP mutations in the genomes and neoplastic human and relevant mammalian cells; evaluating the problems of expressing the levels of risk quantitatively that are associated with implementing a defined risk type of approach; and finally continuing the development of regulatory management plans for the use of neoplastic cell substrates.

The plan to move forward on these issues is presented in the last slide. Protocol study residual DNA are being developed as we speak with scientists in the National Cancer Institute. We plan to hold further discussions on adventitious agent testing issues, on tumorigenicity assays and developing and evaluating quantitative data of the type needed for the defined risk approach as well as issues raised with the possibility of mutations into the gene that produces prion proteins in neoplastic cells from humans and other species.

In the future, we hope that the June 1999

draft of the defined risk approach will be converted into a draft guidance document. When the revised proposal will be ready will depend on our ability to resolve the issues that remain.

Finally, I think we certainly plan to continue our discussions with the Committee in the detail necessary to get over this hump.

I think myself and any members of the self substrate group that are here would be pleased to help with any questions that you may have.

CHAIRMAN GREENBERG: Thank you, Andy for making an effort to try to put some structure to a very complicated question and I think the only way to attack such a complicated question is to pull it apart and look at each piece and you're trying to do that. It's still pretty complicated, so Dr. Kim?

DR. KIM: I have some generic questions. I guess based on your proposal numbers Vero cells would belong to number 5, right?

DR. LEWIS: No. No. 2.

DR. KIM: No. 2, okay. And then second question is that any potential agents of either biologic significance, or nonsignificance, insignificance that can be detected by techniques like the PCR, like again, it doesn't apply here, but

chicken cells were found to have some RT activity by PCR, if that happened let's say in this case, then it would not be acceptable based on your proposal, I guess.

DR. LEWIS: Yes, I think if a PCR assay turned up evidence for the presence of an infectious gene, a viral DNA, for example, or if it was a PERT assay and it turned up evidence for retrotranscriptase activity, certainly that would have to be a very extensive set of testing going on to show what that data actually meant.

You can certainly conceive of situations, for example, if you took Hela cells and that was some residual Hela cell DNA in there. Hela cells can contain anywhere between 20 and 50 copies of a defective part of a papilloma virus type 18 genome and certainly if you applied a PCR assay and you had significant amounts of Hela cell DNA you could pick that up. But to argue for the Hela cell case, no one has ever found an infectious papilloma virus type 18 genome in Hela cells. It's almost certainly defective in at least the data I'm aware of. There have been a number of attempts to document that.

The business of a PERT positive data indicating that there's reverse transcriptase activity

in a human cell might be more difficult for the reason that there are endogenous elements retrotranspose on elements that can make RT activity in human cells. And the question is is there any possibility that that activity represents the expression of an endogenous infectious retrovirus genome and in humans, there's never been that I'm aware of the detection, there's never been any evidence that there are infectious endogenous retrovirus genomes in normal human cells.

However, if you, for example, have a T cell lymphoma that was produced by a person who was infected with human T cell lymphotrope virus Type 1 or 2, then you would certainly have to worry that there was endogenous retrovirus or DNA in that genome and that would certainly be an issue. So you'd have to search very extensively for retrovirus activity in any cell that was already positive and as you went down the species, certainly below humans the chances of that being evidence for an active endogenous virus would increase almost to 100 percent in the case of rodents, 200 percent in the case of rodents.

CHAIRMAN GREENBERG: Dr. Minor and then Dr. Wolfe and then Dr. Huang.

DR. MINOR: I've got two questions here. The first thing is that I assume the reason that Vero

are put into a different category from Category 5 is because you know a lot about them and vaccines have been made already, is that right?

DR. LEWIS: Yes. I think from our perspective Vero cells represent a special case.

DR. MINOR: Right.

DR. LEWIS: They've been out there in the population as vaccine, as been used for manufacturing vaccines for 15 years and safely as we understand it. So we do not think they fit in the same categories as the other cell lines and I think that our concept is that this represents the example of a substrate that has demonstrated safety of the only neoplastic cell substrate that's been used from what appears to be safely as a substrate for vaccine manufacturer and for that reason in addition to all extensive tests and it's gone on, we segregated that into a class by itself.

DR. MINOR: Okay, the second question is your Category 4 is cells transformed by oncogenic viruses.

DR. LEWIS: Yes.

DR. MINOR: So would you accept an SV40 transformed human diploid cell for vaccine production?

DR. LEWIS: Would I accept?

(Laughter.)

You're getting personal. Probably not. I think that the reason that we put that in there is that there are -- it seems to be very unlikely at this particular stage in technology that anyone would go out and try to produce a cell substrate by transforming it with an oncogenic, an infectious oncogenic virus because it's so easy to do it with cloned viral acellular oncogenes. But there may be situations in which this might want -- people may want to use cells from the past.

Now if those cells, especially SV40 is an excellent example because in most SV40 transformed cells, you can recover SV40 quite easily and so I do not think that we would want to have a substrate in which we had an infectious viral genome that was capable of being liberated with the ease in which SV40 can come out as a substrate for vaccine manufacture. Now that's my personal opinion.

DR. MINOR: I was maybe just proposing that Number 4 needs a bit of looking at perhaps.

DR. LEWIS: Yes.

CHAIRMAN GREENBERG: Dr. Wolfe?

DR. WOLFE: Recognizing that Vero cells are a special case, but recognizing that even when

this was discussed this morning there were some questions about how much follow-up there's been, there's been in all the people who've gotten vaccines from them and some questions about the number of passages. If we look at the Categories 3 and 4, to me they come much closer to Category 5 in terms of the concerns I would have.

I'll mention just two reasons why. Although in the column, in the block diagram chart for adventitious agents, none detected, we know that the whole process of detection of adventitious agents is going through a transformation of its own. We still are wanting better methods, so the fact that none have been detected doesn't mean really -- I would almost put it more safely in the category of difficult to determine, even though none have been detected, the methodology is changing so rapidly even since the meeting I attended a year and six months ago.

Then the other issue is the whole, the residual DNA kind of issue. I'm not sure why one would be less concerned about this issue or significantly less concerned about this issue in Categories 3 and 4, transformed by defined viral or cellular oncogenes or transformed by oncogenic viruses, then in Class 4. I think operationally my

concerns translate into the question why are we or you discouraging the manufacture of vaccines made out of Class 5, but not 3 and 4, given the limitations that I've just mentioned.

DR. LEWIS: Yes. I think that the idea behind that differentiation was that cells that arrive spontaneous or tumors that arrive from cells that arrive from tumors that occur spontaneously in nature, carry with them not only the weight of the problem that they are tumors and they're induced by something, but they also carry with them, I think, the weight of the fact that they generally would probably come from an adult individual or an individual that's been exposed, environmentally exposed, say for many years.

And that would increase the chance that they would have something that we wouldn't know about or we would be unable to detect, whereas if you had cell line, for example, if you transformed the WI38 cell or the MRC6 cell which has been in culture and been used for vaccine manufacture now for many years, if you transform that with a defined oncogene, the chances of that having an adventitious agent inherently would be quite, quite small.

So any adventitious agent that those types of cells would have would have to be either introduced

during the transformation process or induced by the transformation process and this again is speculation, but I'm not aware of any evidence that says you can induce the expression of any kind of viral gene in a human cell as a result of this because if the cell is clean to begin with, there are no endogenous viruses in there that we're aware of. So I think it's this reason that we use it to discriminate between those categories.

DR. WOLFE: What about the residual DNA issue?

DR. LEWIS: The residual DNA is a problem and I think that in making these draft proposals we have stuck with limits that have been accepted based on debate that has been going on mainly on the Vero cell issue now for 20 years. And the WHO's 10 nanogram limit was the outcome of a debate in the latest debate in 1997 in which they established that limit for a meeting in Europe.

The 100 picagram limit was the limit that was acceptable both by the FDA and I think the WHO before 1997, so we stuck with those limits.

DNA is an issue and we want to -- that's why we have this protocol in the works to try to figure out a way to get at that issue, to define it

better than we can define it right now.

DR. WOLFE: Just a 10 second follow up question which would be given the special case nature of Vero cells, I guess I would wonder why you're encouraging the manufacture of vaccines from 3 and 4 in circumstances where I'm not sure a burden can be born that those people wanting to do that can't do them in Vero cells. I just think that they're so much more uncertainty about these two categories than there is about Vero cells. That's really -- that's --

DR. LEWIS: Phil wanted to make another comment.

CHAIRMAN GREENBERG: I have to go in order here.

DR. KRAUSE: Just to briefly comment on the DNA issues, the original 100 picagram limit that had been around for a long time was based on an early assessment of potential oncogenicity of DNA, based on various animal experiments. Additional experiments, more recently, led to the sense that in terms of oncogenicity, even if oncogenes are present and so forth, 10 nanograms would be safe.

The reason under some circumstances we would be worried about lower quantities than 10 nanograms, at least from my perspective comes from the

concern that there might be infectious genomes in that DNA as well. And if you're in Category 3 and 4 the presumption is that you're starting off with cells that don't have known viruses in them and so there should not be infectious genomes in them either. And so that's the reason for that difference.

CHAIRMAN GREENBERG: Thank you, Phil, and now we have Alice and Ms. Fisher and then we're going to break for coffee and we'll catch the rest of the questions as discussion continues.

DR. HUANG: I think it's rightfully so that you should focus on DNA and viral particles and infectious units, however, the question of adventitious agents also would include the PrP protein which you've already discussed and the possibility of infectious self-replicating RNA and even the outside chance of stable products such as small double stranded RNA pieces as well as DNA, RNA hybrids that would cause mutagenic chance.

Have those been discussed and what is some of the thinking about that and the measurements for them? I'm just surprised that there's almost no mention or worry about RNA at all.

DR. LEWIS: I have to confess we've not considered the possibility that RNA itself could be

infectious if it were not representative of some type of viral genome. That's not been on our radar screen.

Does anybody else in the group want to say anything about that?

DR. KRAUSE: Of course, most of these products the way they're produced have large amounts of ribonuclease in the cellular milieu and so our assumption has been that DNA is much more likely than RNA to give rise to these kinds of complications, but certainly it is something to think about, especially under circumstances where there could be an entire RNA virus genome present in a product. I agree with you.

CHAIRMAN GREENBERG: Ms. Fisher?

MS. FISHER: Just to clarify, the FDA's attempting to make a standard here with the use of neoplastic cells that would -- the test would be required to guarantee the elimination or the absence of adventitious agents, however, it looks as if there is -- the thinking is that residual cell substrate DNA would be allowed to be in there at a certain threshold.

DR. LEWIS: Yes, well, I think if you're dealing with products that are purified, highly purified, then the level of DNA is usually controlled and it's quite low, usually much less than 100

picagrams. When you're dealing with live viral vaccines, it's sometimes very difficult to remove the level of DNA. So that's why these various limits have been proposed over the years.

Now the business of adventitious agent burden, the point I was trying to make when I was trying to explain when you have to say something does not contain, there is no such thing as absolute freedom when you're measuring. You always have a limit that the assays will be sensitive to and a limit below which they cannot go, so you can never say it's free. There's no absolute here. It can only be measured numerically and a number defined to it. So that's why we've suggested the concept of less than one infectious unit per million doses as a starting point to think about, how to define sometime that's not there.

MS. FISHER: So you are -- there is a threshold for adventitious agent contamination?

DR. LEWIS: There always is a threshold for adventitious agent contamination. In other words, it's impossible to say that something could not be there below your ability to detect it. This is with any product. There's just no way to --

MS. FISHER: I'm not sure the public

understands that. I'm not sure they do.

DR. LEWIS: I think this very well may be a problem that we have to sell.

DR. SHEETS: Dr. Greenberg --

CHAIRMAN GREENBERG: I think Ms. Fisher has made an important observation. In all products, there's no way ever to insure to absence, the absolute absence of anything. That's a major problem in science and it is, in fact, sometimes a difficult concept to convey and I think Dr. Lewis is working hard at doing it and what he said is that what, as a draft idea, is that if you give a million doses, you are sure there is no infectious adventitious agent in those million doses.

But he would not be sure if he gave 10 million doses, that there was no infectious agent in that 10 million because he couldn't do the experiment to figure that out. And most of us, as scientists around the table, understand that problem, but it is not necessarily a simple one to understand and as the consumer rep. that would be something that I think we need help in conveying because it's virtually an impossibility to get to that point. If you had a billion doses, you couldn't prove -- or a hundred billion, that there's no infectious agent in there.

MS. FISHER: But the variation of production methods, in the testing that's used is going to be very important after the thresholds are set.

CHAIRMAN GREENBERG: Well, the threshold is a threshold and it darn well better be correct. I mean you can't have a threshold and then say it's not real. That's a second point. If you have a threshold it has to be there. You're 100 percent right and we're talking before that, you have to set a threshold.

I am going to, because I know that all of you might have various needs and we had a coffee break scheduled for this time, but I'm going to cut the coffee break a little short. It's now 10:37. I'd like you all back here at 10:45 when we will start again.

(Off the record.)

CHAIRMAN GREENBERG: I'd like people to take their seats. Before we start I would just like to add, Dr. Minor asked an interesting question and I'm not sure I have a ton of data on it, but he asked basically the question for cells that were transformed with a specific oncogene, for example, if that oncogene then was removed what happens to the

transformed phenotype. I think you asked that.

And that's an interesting question because you can imagine either that something else happens in addition. And I do think that there's data recently presented using certain oncogenes with inducible promoters like the tetracycline promoter in in vivo tumorigenesis models that show in fact, much to my surprise, that you can sort of turn on and turn off tumors just by the expression of the oncogene and I think that's work by a scientist named Dean Felscher who was then at UCSF which you might look up. It was a surprise to me, but gave me some margin of feeling that, in fact, this very complicated process of tumorigenesis, at least in some cases could be totally controlled by a single gene function.

With that little editorial, we'll now go to Dr. Krause who will talk to us about CBER research related to neoplastic cell lines.

DR. KRAUSE: Thank you. This is an inauspicious start.

Anyway, to get started, at the November 1998 advisory committee meeting, we discussed the issues associated with the introduction of new types of cell substrates. In particular, those that are neoplastic. One of the advisory committees' major

concern was that CBER might not have adequate resources to perform the research necessary to obtain a clear understanding of the underlying scientific issues. The purpose of my talk is to summarize current CBER research projects in this area and to give the Committee an idea of what kinds of information they can expect to be available in the near future.

Current CBER research is focused on three major areas, including the risk of residual DNA, potential risks associated with protease resistant protein instability in neoplastic cells and the detection of adventitious agents.

In thinking about residual DNA, it may be useful to consider that residual DNA could theoretically carry a tumor producing phenotype from a cell substrate or could encode the genome of an infectious virus which could also theoretically be oncogenic. An additional complexity is that some viruses may package cell DNA and could theoretically deliver cell DNA to a vaccine recipient.

Studies of residual DNA are currently focused on two issues. Together with John Coffin and Steve Hughes of the National Cancer Institute, we're developing a protocol to study the potential risk of

injecting large amounts of oncogenes into normal immuno compromise in p53 deficient mice. It is hoped that these experiments will provide a basis for further assessing the tumorigenicity risk of injected cell substrate DNA.

We also are interested in understanding how various parameters influence the infectivity of virus genomic DNA. I will discuss this issue in a bit more detail in the next few slides.

According to the recently revised WHO requirements for the use of animal cells is in vitro substrates for the production of biologicals, the amount of cellular DNA and biological products should be limited to 10 nanograms per dose, an increase from a previous limit of 100 picagrams per dose.

This limit is meant to apply to continuous cell lines and not products given orally or products derived from microbial diploid or primary cell culture systems. The 10 nanogram figure was derived by considering data and theoretical calculations regarding the tumorigenicity of injected DNA. While CBER is attentive to WHO guidelines, CBER evaluates products on a case by case basis in determining appropriate limits for cell substrate DNA.

Moreover, it should be noted that for live

viral vaccines and other less purified products, it may not be possible to limit the total DNA to 10 nanograms. Thus, the question is raised what data would be required to provide assurances that any given amount of DNA for vaccines produced in novel cell substrates would be safe? And that, of course, would include neoplastic cells.

In thinking about potentially safe levels of DNA from neoplastic cells, we considered the possibility that these cells could contain infectious genomes or viruses. This slide presents some of the existing quantitative data regarding the potential infectivity of cloned or purified viral genomic DNA. Information on tumorigenicity is also presented. In general, from these and other studies, we noted that viral genomic DNA is at least as infectious as it is tumorigenic.

In these experiments that are published in the literature, cloned or purified genomic DNA was injected directly into various animal models as we see here, so mice, monkeys, marmosets and Syrian hamsters. On the right hand side of the table I've calculated the theoretical risk associated with a single dose of a product that contains one microgram per dose of cellular DNA that contains a single genome per cell.

These numbers are based on methods that Andy Lewis and I published last year. The calculation accounts for the dilution of the viral genome and the cellular genome and assumes that the viral genomic DNA is as infectious or tumorigenic when incorporated in cell substrate DNA as it is when linearized and injected directly.

The estimated risk of an infection associated with this theoretical product thus would range from as high as one in about 8,000 if it were a polyoma virus DNA to one in tens of millions for other DNAs. This information can be used to develop a worse case scenario for residual cellular DNA.

If you assume that -- assume that the DNA you have to worry about is as infectious as polyoma virus DNA is which is by far the most infectious DNA that anybody has reported on, assume that each cell has 50 copies of this infectious DNA. Assume that there's been absolutely no degradation of the cell DNA.

Assume that the virus DNA in the cell is proportionally able to induce tumors or infections based on its dilution in the cell -- in the cell genome which is also a very conservative assumption because of threshold phenomena associated with

tumorigenicity, then cell DNA which contains 50 copies of polyoma virus be expected to initiate about 6 times 10^{-3} infections per microgram. If DNA were limited to 160 picagrams, this would correspond to one infection per 10^{6} or per million doses.

These assumptions are meant to be conservative in order to provide extra assurance regarding the safety of injected residual DNA. In general, DNA administered orally or mucosally to animals is considerably less infectious.

Now Keith Peden at the Center for Biologics is the recent recipient of a national vaccine program grant to quantitatively study the influence of some of these factors or the infectivity of viral genomic DNA. His project will determine the relative infectivity of integrated versus nonintegrated retrovirus genomes and will determine the effect of DNA fractionation on the infectivity of integrated versus nonintegrated virus genomes. These are factors that probably influence the potential infectivity of virus genomes incorporated into cellular DNA, but have not yet been looked at quantitatively. And of course, we believe that this type of information will improve our understanding of what levels of residual DNA neoplastic cells are safe.

Moving on to prion related issues, according to the prion hypothesis, genetic mutations in PrP genes can lead to generation of infectious prions because neoplastic cells may exhibit greater genetic instability, including a greater tendency toward mutations. The question was raised whether neoplastic cells might have a greater risk of containing mutant PrP genes. This concerned was discussed at the September 1999 cell substrate meeting by Neil Cashman.

These mutant genes could, in turn, theoretically encode infectious TSE agents, transmissible spongiform encephalopathy agents. Of course, it is important to be sure that new cell substrates do not contain such agents. To study this issue, Konstantin Chumakov and David Asher sequenced PrP genes in Hela cells of different lineages. Hela cells, from multiple sources, were obtained for the purposes of this experiment. All of the listed cells hereon the lower part of the slide have been shown to be Hela cells, in some cases, due to contamination of original cell cultures with Hela cells. In red, to the left which is very difficult to see, is the year the cells were established. And in blue, to the right of the name of each cell line is the estimated number

of passages from the primordial Hela cell cultures.

Sequencing of the PrP genes revealed no point mutations among the different cell lineages. However, Dr. Chumakov noted two variants of the gene in Hela cells. One variant denoted PrP 4 on this slide, contains four octa peptide repeats near the C terminus of the prion protein. The other variant denoted PrP 3 contains three such repeats. Of interest, some Hela cells, those on the lefthand side are heterozygous at this locus, containing both the PrP 4 and the PrP 3 allele, while other Heal cells on the right hand side contained only the PrP 3 sequence. The most likely reason for this is if the original donor of the Hela cells was a heterozygote and that the chromosomal instability associated with Hela cells led to the loss of the chromosome containing the PrP 4 allele in some of these lines.

Now familial Crutzfeldt-Jakob disease has been strongly associated with increased numbers of these repeats. Of potential relevance, the PrP 3 allele has about a 2 percent population in the population, penetrance in the population and familial C-J disease has also been reported in PrP 3 homozygous.

So to summarize the study, Hela progenitor

cells were heterozygous in the PrP C terminal for pica region. Different Hela cell lines have different PrP sequences. Sequence differences may have resulted from chromosome loss with repeated passage of Hela cells. No point mutations were observed. Although prions have never been shown to spontaneously arise in tissue culture, this study does suggest that there's a potential value to sequencing the PrP gene in neoplastic cells, human neoplastic cells proposed for use as vaccine cell substrates and to require those cells to possess a normal sequence that obviously one that has not been associated with familial CJD.

Now adventitious agent contamination of a cell substrate could impact on the safety of a product in several ways. First, if a product is not inactivated or cleared, it could lead to infection in product recipients and of course, if it were an oncogenic virus, this could lead to tumors. Second, it could interact with a vaccine virus to cause unintended consequences and third, it could lead to contamination of residual DNA in the product with potentially infectious genomes and as Dr. Huong pointed out, potentially also residual RNA.

Thus, based on the discussions at the Advisory Committee meeting in November 1998, the

potential risk -- actually, also the Cell Substrate Workshop in September, the potential risk from adventitious agents is perceived to be a major obstacle to the use of neoplastic cell substrates. This is because immortalized cells for which the mechanism of transformation is unknown are presumed to have a higher risk of containing oncogenic viruses. Because this risk could extend to viruses that have not yet been discovered, there is a need to develop new nonspecific methods to detect viruses.

Traditional, nonspecific assays to detect viruses are based on tissue culture and on animal injection. Expansion of the indicator cell lines for these tissue culture assays to include additional lines might improve the sensitivity of virus detection. Although fairly insensitive, electron microscopy is a nonspecific test that under appropriate conditions can detect essentially all viruses. Animal tests already in use include hamster antibody production, rat antibody production and mouse antibody product assays as well as injection of adult and suckling mice and general safety tests in which the ability of injected product to kill guinea pig and mice is examined.

Andy Lewis recently received funding from

the National Vaccine Program to investigate the sensitivity of various animal models for oncogenic viruses. These studies could help to interpret assays in which a cell substrate lysate or supernatant would be inoculated into susceptible animals. And the presence of an oncogenic adventitious agent would be assumed if tumors developed in the animals. Most known tumor viruses can be detected in this manner if three rodent species are used, mice, rats and hamsters. Although the sensitivity of the method for most viruses has not yet been studied and that's the point of trying to look at this.

Now Keith Peden has done a lot of work using PCR-based reverse transcriptase assays to nonspecifically detect retroviruses. Arifa Khan has been working on increasing the sensitivity of these assays to detect latent retroviruses either by co-cultivating the cells with other cell lines that might be more susceptible to infection by latent viruses or by adding various inducing agents that could provoke reactivation of latent retroviruses. In fact, induction could be used as an adjunct to any of these virus detection studies in an effort to improve the detection of latent viruses.

Konstantin Chumakov is starting to study

the use of DNA oligonucleotide arrays to identify cellular responses to infection and to determine whether there is cellular expression patterns that might be indicative of contamination with replicating adventitious virus.

In my lab, we recently received funding for the National Vaccine Program to study the use of consensus PCR primers to detect viruses that are related to those already in GenBank with the potential to also detect as yet undiscovered viruses that are related to existing viruses including those that might be in the latent state.

In my lab, we also have been working on completely nonspecific amplification of viral nucleic acid also funded, in part, by the National Vaccine Program. I'll show you some of our early results in the next couple of slides.

Our method is premised on the idea that it should be possible to physically separate viral and cellular nucleic acids and that the obtained viral nucleic acids could be amplified using nonspecific PCR based methods. So far we've concentrated on the tendency of our own nucleic acids which are enclosed in a protein capsid to be able to elude digestion by various nucleases. Cellular nucleic acids have no

such defense. We also have taken advantage of different densities to use ultracentrifugation to separate viral particles from cellular DNA and RNA. We then use nonspecific PCR methods to amplify the resulting nucleic acids and close and sequence the PCR products. We can then compare the PCR product with GenBank sequences to determine if they're related to any known viruses.

In this experiment we spiked one million Vero cells with either 5,000 or 500 plaque forming units of Varicella roster virus and used the described method. After 35 cycles of PCR, a faint band is visible with 5,000 plaque forming units and after 70 cycles we detected several PCR products and samples spiked with 500 plaque forming units of virus. We then cloned and sequenced these bands and found that all of them contained Varicella roster virus. We then performed additional work to improve our sensitivity of detection. This slide shows approximately what this method is capable of doing right now in our laboratory for different viruses, either spiked into cells or in the case of polio, in the polio vaccine directly.

For typical DNA viruses which are the first four on the list, we can detect between 10 and

1,000 infectious units spiked into one million cells. We haven't yet done enough work to define the sensitivity of a modified method that includes a reverse transcriptase step to detect RNA viruses, but we were able to detect and identify polio and influenza viruses at the lowest dilution we attempted.

With the exception of the direct amplification of polio vaccine, these experiments were all done using spiked Vero cells. Although our results are unpublished and should be considered preliminary, I find it somewhat reassuring, at least, that Vero cells do not contain adventitious agents using this method as well.

One limitation of these assays is that they can detect encapsidated viruses but cannot detect latent viruses. Of course at this early stage I can't either tell for sure whether there's some viruses that we cannot detect using this method, but our early results look promising.

We believe that with further work, however, we'll be able to further improve the sensitivity of this method and we'll have a method that can be used to nonspecifically determine whether potentially infectious viruses are contaminated in cell substrates.

So to summarize, we believe that the research that is on-going at CBER will help us better understand the issues associated with neoplastic cells and will enable us to make further progress in developing policy in this area. I would like to just acknowledge that the principal investigators in the laboratories whose work I've described including Konstantin Chumakov, David Asher, Keith Peden, Andy Lewis and Arifa Khan and thank you very much for your attention.

CHAIRMAN GREENBERG: Thank you, Dr. Krause, that was enlightening. I would just like to editorialize for a second. This Committee has on numerous occasions reviewed the downslide in funding for the intramural labs at the FDA to do their own research. I'd like to commend you, you listed two or three separate grants that you folks have gone out and gotten to maintain your ability to do this research. For sure, I feel and I think the Committee would feel that this is incredibly important. I don't know other government agencies, I guess there are, that are doing similar studies, but it is crucial that our ability to detect and move forward in detecting agents in vaccines and to test safety be maintained and I would hope that somebody in the public record let it be

known that the Committee is highly supportive of intramural research at the FDA to be done in this area.

Dr. Snider?

DR. SNIDER: Just to elaborate on that because I think that's a terribly important point. I'm very pleased that the National Vaccine Program which is in my office at CDC was able to provide some of the funding, but I do want to point out that the $6 million as some other folks know from which this money was taken is to address all unmet needs in vaccines for all the agencies and so it's not a stable source of funding for this kind of activity which, as you point out, is critically important to sustain.

CHAIRMAN GREENBERG: So again, my FDA colleagues, to the degree that you can remind us of how this point of view, which I think is a consensus among the Committee members can be taken to an audience that doesn't hear it as well as you do, please let us know. Now what I'll do is entertain questions around the table.

Dr. Wolfe?

DR. WOLFE: I saw something like this at the meeting at the end of 1998. But without meaning to seem a traitor to my former employer, NIH, which I

happily spent five years at, the NIH budget, as you all know, is upwards around $17 or $18 billion and I really am baffled as to why you have to go to the much lower funded CDC to get money to do things that should be in your own budget. I think the fact that you have to go somewhere else and that there isn't enough appropriate in your own budget is outrageous, given that money is available for doing things that are, I think, important but certainly no more important than what you're doing in the form of this almost geometrically expanding NIH budget. I'm glad the NIH budget is going up, but the same people that are causing that to happen with tiny fractions of that could be funding directly through the budgetary process what you're doing. I would strongly support that and if there's anything I can do, I will. Thank you.

CHAIRMAN GREENBERG: Thank you, Dr. Wolfe. Just to clarify, I think, Bill, you're in the audience, one of the issues to me that makes this so difficult is that the FDA, unfortunately, is not allowed to apply for that very large NIH budget which has always struck me as a classic government Catch-22 and I realize another way around that would be to directly fund the FDA.

DR. WOLFE: Right.

CHAIRMAN GREENBERG: But I want to get around it as fast as possible.

DR. WOLFE: I agree, whatever works.

CHAIRMAN GREENBERG: Ms. Fisher?

MS. FISHER: I agree. I think it's unfair for the FDA as a regulatory agency to be left holding the bag on these issues and not be given the money to address the serious scientific issues about these vaccines. So many are being developed and I think it's outrageous and I agree with Dr. Wolfe, that there has to be a concerted effort on the part of the public to try and get the money that you need to do this scientific research.

DR. WOLFE: Just a follow-up question. I mean how much request went into at least the budget that left CBER, if not FDA, for things like this? Or is it sort of a self-censoring thing? You've been turned down so many times that you don't even ask for it in the budget? I mean, what's going on with the budget request on these issues?

DR. KRAUSE: I actually don't have a good answer. I know Bill, do you have an answer to that?

CHAIRMAN GREENBERG: Bill, I think this is an important area. We could spend easily the next

week discussing it.

DR. KRAUSE: A few minutes, at least, though.

DR. EGAN: I mean there are a lot of issues related, the budget, decline in budget, etcetera. We've been over this many, many times. I would suggest that you talk with Dr. Heaney or others. There's precious little time that the Committee has to debate these issues. I would like to -- would prefer, I think it would be more -- I thank you for the effort, but I think the time would be more profitably spent if you could criticize or critique the experiments that Dr. Krause and others are doing and providing us with scientific guidance on what else we need to be doing and we'll try to do it as well as we can within those guidelines.

CHAIRMAN GREENBERG: I do think, however, is each of you to right your conscience and just perhaps take this moment to -- I personally have not written the Commissioner on this issue and I will make a note of that and do it and if any of you feel similarly, I can't but believe that separate letters from each member of this panel with this specific meeting in mind won't have some effect on how she thinks about things.

DR. EGAN: I guess Dr. Heaney will love me for this suggestion.

DR. FAGGETT: Harry, let me go on record saying the National Medical Association, as an organization, will be writing a letter.

CHAIRMAN GREENBERG: Okay, so let's move on and talk about some of the area -- any questions to Phil about the actual science here?

Diane?

DR. GRIFFIN: I think the PrP question is an interesting one and an important one and certainly sequencing the genome in that gene is one approach, but I wonder if an additional approach wouldn't be to if the actions or reagents are available to look for protease resistance of the protein because that's the biological phenotype of the protein and we know a lot of mutations that can predispose to that phenotype, but it's always possible you don't know all of the mutations and so screening for the ability of the cells to produce a protease resistant form would also seem to be relevant.

DR. KRAUSE: That's an interesting idea. Of course, the gold standard for that type of thing would be to actually inoculate animals, but the trouble is if those kinds of experiments take so long

to and --

DR. GRIFFIN: No, but all of those correlate with the presence of protease resistance form.

DR. KRAUSE: Right.

CHAIRMAN GREENBERG: Is there any information available about people who -- Phil, is there any information available about spongiform encephalopathies occurring in people who have cancers so if PrP mutations were associated with tumorigenesis I would expect that you might -- that that might be seen biologically or is this -- Diane is looking at me like I'm --

DR. GRIFFIN: It takes a long time to develop.

CHAIRMAN GREENBERG: There are cancers that don't kill you right away.

DR. GRIFFIN: Phil has to have a tremendous background of knowledge in this area. He comes from the right country.

DR. MINOR: Well, as a Brit, of course, this really wouldn't be my main source of concern about TSEs. I mean I think they're totally separate issues, okay? I'd be very, very surprised if you actually found a continuous cell line that would

really support PrP a form of the disease. I think if you've found one you should patent it because it will make a lot of money for you.

The two things, as far as I know, they're not linked in terms of like the tumor DNA aspect and the PrP aspect at all.

CHAIRMAN GREENBERG: Other questions? Dr. Snider?

DR. SNIDER: I just wanted to ask you because you went over it pretty fast, what you see in the future as a potential for the DNA chips to contribute here. I think at least to me it's quite intriguing what the possibilities might be and could you just say a few more words about that?

DR. KRAUSE: Sure, of course, that's very exciting technology and Dr. Greenberg already suggested earlier one idea which is to sort of try to understand the difference between similar cells with different phenotypes, although I suspect that once those experiments are done, we'll find a lot of differences in a lot of genes and we'll still be scratching our head regarding what they mean. It would be nearly impossible to then take each of those genes and then put them back into cells to see exactly what they're doing. But that doesn't mean those

experiments are not worth doing.

CHAIRMAN GREENBERG: I actually was thinking about looking at one cell type Vero and looking at transcriptional arrays as it moves from nontumor producing to tumor producing and you may not figure out the mechanism, but you may find out a signature of transcriptional responses that would allow you to monitor yourselves and know what you don't want when you're making a vaccine.

DR. SNIDER: I agree that that's a useful experiment and Dr. Chumakov is actually planning to do that.

There also is the idea of simply taking essentially all known viruses and putting some sequence from them on a chip and using that as a way to rapidly screen products and of course the advantage of these chips is you can put lots and lots of DNA sequences on them and it doesn't get you at the problem of how to detect unknown viruses although depending on how you did that you might be able to put nonspecific sequences on there and you might not achieve, at least with current technology, the sensitivity that one might with some of the PCR-based methods, but one I think that's potentially useful as well.

And then the third idea is one that has actually been done for bacterial infections is try to understand host response to viral infection and if one can identify specific genes that are expressed in cell substrates or in cells when there are viruses present in those cells or that suggest that viruses might be there, that could be sort of a red flag, without telling you what the virus was that there might be something to be a little bit more concerned about with those cells and I think that that's analogous to the idea with the Vero cells.

DR. SNIDER: Let me just follow by saying that to me there's a connection between these exciting kinds of studies and the topic of funding we were just talking about.

CHAIRMAN GREENBERG: Yes, they take money.

(Laughter.)

DR. SNIDER: Yes, but also they excite people who are in the area or in a related area who might be working on something a little bit different to move over into working into vaccine safety issues.

CHAIRMAN GREENBERG: Dr. Huang?

DR. HUANG: Just in terms of perspective, Dr. Lewis mentioned at the meeting in 1998, 1999 that primary cells are more dangerous in terms of the

probability of adventitious agents and I'm wondering as we discuss this, how much of the scrutiny is currently being applied to the primary cells that we're using routinely?

DR. KRAUSE: The answer is that right now actually, to my knowledge, there is very little being produced in primary cells in the U.S. It's sort of the one classic primary cell vaccine was the oral polio vaccine produced in primary African green monkey cells, but that vaccine is really not in general use any more and due to change in recommendations by the Advisory Committee on Immunization Practices. So I think that -- and of course, the major reason for moving away from that is probably not the cell substrate risk, but it's the risk of the vaccine associated, paralytic polio.

I think all of us have some concerns about the idea of introducing new vaccines into new primary cells and although historically that's, of course, where the vaccine business started, I think it's one of the reasons why people are willing to consider some of these neoplastic cells as alternatives to that.

The other comment in terms of adventitious viruses, to follow up, I think what Dr. Snider said, was that of course if you can propagate cells in the

absence of serum then one might reduce theoretical risks from adventitious agents from cows including, for instance, bovine TSE agents and so there's always a trade off on these kinds of things.

CHAIRMAN GREENBERG: Ms. Fisher?

MS. FISHER: I have a question, follow-up to Dr. Huang's comment, was there a threshold for adventitious agent contamination in those primary -- in the production of the OPV as well as residual DNA?

DR. KRAUSE: From what I'm aware of with no threshold to find, but what was defined at the time were the best available tests to detect these particular agents. And so if one were to look back and examine what could the sensitivity of those tests have been, then one could define some kind of a threshold. That wasn't a part, I think, of the conscious decision making when that product was licensed.

The other thing to keep in mind is because this was a such a concern, in fact, in the early days of the polio vaccine including through manufacture at the very end, 25 percent of all of the cells were kept as controls in order to try to do one or another kind of adventitious agent testing on it. So if you can imagine, almost as many cells are used simply to look

for adventitious agents as were used to produce the vaccine.

MS. FISHER: Was that testing with the FDA or the manufacturer?

DR. KRAUSE: That testing was done by the manufacturer. Those were controlled -- and they would have to be because they have to run those tests at the same time as they make the product.

MS. FISHER: And the FDA then tested to make sure that their tests were correct?

DR. KRAUSE: The FDA has on various occasions done different kinds of adventitious agent testing on polio vaccines and there was a lot of interest in the 1970s, for instance, when Paul Parkman did a lot of long-term cultures of polio vaccines in various cell lines.

MS. FISHER: The reason I'm just pursuing it is because as a matter of precedent, if there's going to be a threshold for -- with these cell substrates for adventitious agents or residual DNA then it makes it very important for there to be follow up testing if that indeed is going to be.

CHAIRMAN GREENBERG: It's my impression that still in many parts of the world oral polio is, in fact, made in primary African green monkey kidney,

however, for example, in Vietnam, very large amounts. So primary cells are still used for much of the world to make vaccines and I do think that all things being equal, I'd rather have something better controlled than primary cells.

Any other questions?

DR. SHEETS: The other primary cells that are used for vaccine manufacture are eggs and those are still being used and there are both inactivated vaccines that are licensed and proposals to make live viral vaccines in eggs.

CHAIRMAN GREENBERG: If there are no other questions, I would like to move on to the Committee discussion that would be led by Dr. Lewis. Are there any other questions of Dr. Krause? Okay, then -- yes?

DR. KIM: Just for completeness, I knew you talked about PCRs to detect viruses. Have similar attempts been made to detect nonidentifiable or codable bacteria?

DR. KRAUSE: The answer to my knowledge is not directly in vaccines by PCR methods, you know, David Wellman at Stanford University has done a lot of work to define bacteria ribosomal or in any sequences that are essentially conserved across all bacterial species. One of the problems you run into is that

bacterial DNA is so ubiquitous that those kinds of assays have a potential to be positive, no matter what you test. And of course, a positive result by an assay like that doesn't mean that there's a live bacterium there. But at the same time there are clearly limitations on the ability of various kinds of culture methods to find bacteria and that's an area in which some further work could potentially be done in a way in which I don't think is specific to neoplastic cell substrates, but which might provide further assurances about cell substrates.

CHAIRMAN GREENBERG: Well, I would just say that as Ms. Fisher said, adventitious agent testing is not specific to neoplastic -- she was making this point too, I mean, at all times adventitious agents should be absent to the best of our ability to detect them. And the better you can detect them, the more certain we are of that.

Andy, would you like to come up here now and lead the troops through this?

So the way we're going to do this, this is not a vote situation. This is, as I understand it, the FDA and Andy are seeking our advice and thoughts about where he stands and where the FDA stands on this issue of moving forward and better defining how we can

use new cell substrates for new vaccines. And so he's going to pose some questions to us or thoughts and then what I'm going to do is just ask you folks to muse about your responses.

I just got a note from my colleague, Dr. Egan, saying that some of you need to check out, that is check out of the hotel.

(Laughter.)

I was making no remarks about more global checking out. Is that the case? I think, Bill, if somebody could just ask for an hour's extension I think that's what -- let it be recorded that Diane made that recommendation, not me. Are people in their rooms still and need to check out or not? I'm seeing -- I don't see any widespread feeling for that, Bill, so what I'm going to do is -- Bill, do you need to check out? Okay, while this is a very good idea, it doesn't look like the panel is telling me they need to do it. So I think we'll just continue.

This thing about lunch is becoming more of an issue than I thought. My feeling is the lunch break was an hour and we were scheduled to end at 2:45. That's when the adjournment was scheduled. So I feel if we continue on we'll end at 1:45 and I imagine there are no infants in this audience and I'm

making a decision that most of you can last until 1:45. If you can't, I would suggest you run down and get a little glucose, but that -- and there are cookies over there. So I think that people can manage this amount of flexibility in their timing of the midday.

Go ahead.

DR. LEWIS: I think I should probably start off by apologizing because in the rush to get the stuff together, I've overlooked making a slide of the questions, but I think everyone has these questions as a handout. I hope the audience has them as well.

CHAIRMAN GREENBERG: For everybody, this is the -- excuse me, yes. I have it here. Can you hold it up, Diane, while we try to find it. It's the small one.

DR. LEWIS: It's the small one, just three questions on a page.

CHAIRMAN GREENBERG: For Committee discussion on May 12, 2000, issues regarding draft policy proposals.

DR. LEWIS: That's correct.

CHAIRMAN GREENBERG: Go ahead.

DR. LEWIS: To fulfill its obligation to

regulate vaccine safety and efficacy one of the Office of Vaccines' mission is promote the development of new and better viral vaccines. Several current public health challenges, including AIDS, the experience of new strains of -- the emergence of new strains of influenza in chickens and the threat posed by bioweapons require new approaches to vaccine development. New technologies of making development of new vaccines possible, and the successful application of these technologies can be greatly facilitated by the use of neoplastic cell substrates for virus propagation.

Tumor cells have been prescribed as use of vaccine substrates since 1954. However, over the past four decades advances in understanding neoplasia in neoplastic development and in vaccine regulation, it permitted a reassessment of the prescription against the use of all types of neoplastic cells as vaccine substrates.

This reassessment has been underway in the Office of Vaccines since 1998 and will continue until working policy proposals regarding the use of neoplastic cells as vaccine substrates are in place. To meet this obligation to sustain the continued application of new technologies to do vaccine

development, the Office of Vaccines has developed draft policy proposals and it's in the spirit of stimulating and focusing the discussion and not yes or no policy decisions, that we have presented these proposals to the Committee today. So it's in this spirit that we ask the Committee to please comment on the three questions that are on the hand out. They are first the OVRR CBER's draft policy proposals regarding the use of neoplastic cells as vaccine substrates. Second, any of the concepts or perspectives that were used in the development of these policy proposals. And third, any of the issues that CBER is considering or may need to consider in the opinion of the Committee regarding the use of neoplastic cells as substrates or viral vaccine manufacture that the Committee finds appropriate.

CHAIRMAN GREENBERG: Thank you, Andy. These are relatively broad questions or queries to the Committee and I'm not quite sure how to guide you through this so I'm just going to solicit your opinions. I also think it will be useful as you think about them since the actual proposal is broken down into 1 through 5, that you think about those specifically and if you have any thoughts about that way of thinking about the draft proposals or the

specific groupings 1 through 5, that you share those with Andy.

So I'm just going to -- David, you're in the -- good, I won't do it this way. If people have something to say, Dr. Huang. You got off, David.

DR. HUANG: I thought it would be useful to focus on cell passages and providing the ability of one kind of, one master batch that would be uniformly used by manufacturers and really studying that and knowing it well. On the other hand, and I'll go into that in a little more detail, but on the other hand, I don't think just having one Vero cell master batch is going to be the answer. Obviously, we're going to need several and probably of different origins.

First of all, we must get a way from passage level. I mean that's really anachronism these days in the sense that we can passage cells 1 to 2 or 1 to 8 and the days between passage can be a couple of days to 7 days to 10 days depending on how people do their protocols. So I think that we really need to begin to think about doubling times and doubling generations of cells so that we can define them better, but obviously if we have a master batch and we delineate the manufacturers should use these in particular ways and passage them in particular ways,

there will be a certain amount of uniformity.

I certainly support the concept of using cell lines. I think that it just gives you so much more comfort that you've got these things around to study them and as new tests develop, we can have more and more sensitive approaches to detecting adventitious agents and other problems that may be part of the cell lines.

CHAIRMAN GREENBERG: Good. Dr. Kim and then Dr. Stephens.

DR. KIM: Along the line I again, I'd like to see some information on the reproducibility of the data which was provided to us earlier regarding tumorigenicity that whether -- I know there was one extensive study done by Pasteur Institut in the 1980s and I'd like to see that being repeated by somebody and then see whether indeed, same observation can be made or same kind of data can be generated or this relates to the particular batch and I'd like to see that. Certainly, reproducibility is an important issue in science that I'd like to see that.

CHAIRMAN GREENBERG: Thank you, Dr. Kim. I want to remind the Committee that we will get back to the specifics of Vero cell as a substrate later and at this point although Vero cells are certainly the

highest up on our plate, we're going to talk about those as a specific problem coming up and this discussion is more generic to that whole litany of types of possible cell substrates that Dr. Lewis talked about.

Dr. Kohl and then Dr. Snider. I'm sorry, Dr. Stephens was first there, excuse me.

DR. STEPHENS: I think that there are clear advantages to using transformed or quote neoplastic cells and those have been well outlined. I think that coming at this from somewhat of a novice in terms of my understanding, sometimes I get the feeling that the assumption in the past has been what you don't know won't hurt you and I think that's not the case in this particular situation. I guess I'm impressed with the lack of some of our sophistication in terms of where we stand in understanding this area and would certainly urge additional funding and would like to kind of join you, Harry, in terms of a letter from this Committee. I think that would potentially be a very, very powerful statement to increase our understanding of -- at a sophisticated level and at a molecular level of these transformed cell lines because I think that that's really the area that needs a great deal of work from my perspective and would

hope other members of the Committee would join you in that letter.

CHAIRMAN GREENBERG: Thank you. Dr. Kohl?

DR. KOHL: I'd like to join in that letter, Harry, especially if you write it.

(Laughter.)

I'm sure you can get most of us to sign on. I'm looking at summary draft proposal table, the 1 to 5 and I'm concerned with what I think is a logical inconsistency in the table which has implications on how you determine 1 to 5 and that is under adventitious agent status. You've got -- we've got four nondetected and then the last one says difficult to determine with current technology. Well, that kind of washes out the significance of the nondetected all across the column. So somehow I know what you're saying, but I don't think it's logically consistent and there's got to be a better way you could say it or to rework the table and then that has implications on the group because then maybe we shouldn't have such a strong discouraged statement under No. 5 because with current technology, in particular, even though we've got a large experience with Vero cells, current technology may bring something from 5 up to even a more sophisticated level

than we currently have with Vero cells or tomorrow's technology.

DR. LEWIS: Yes, I think your -- I don't disagree with what you're saying. It's important to point that out. I think that what -- when we, of course, when we put this draft together and these drafts together, these are hypothetical situations. So we were assuming that using current technology we could not detect anything here and that in these situations, especially with regard to draft proposal 1 with regard to inactivated substrates, if we couldn't find anything by current technology, then you can use clearance and inactivation to say that there's nothing there with the level at which you can clear model viruses. So I think it was in that perspective that these things, these not detected was assigned to certainly to category 1.

Now you have and Dr. Sheets will present to you what's known about the ability to detect or not, to detect adventitious agents in Vero cells. The category 3, I think the models that were in our mind there, again, have been pretty extensively tested and there's been no evidence that, for example, that there's a contaminating agent in 293 cells.

With regard to category 4, it is more

hypothetical in the sense that there are not any -- we don't have any good examples there of things that might be used. But certainly when you push the envelope, we would be certainly concerned with category 5 that it would be very difficult to be sure with the current technology that we could get to the threshold we're comfortable with. So I think that's sort of the flavor, but I understand what you're saying about the logical inconsistency and we will need to think about that.

CHAIRMAN GREENBERG: I would also say that I'm not sure this table is the most clear way of presenting this data. I'm not sure I can draw it out for you right now, Andy, but you might think of a better way of tabularly describing your categories.

Dixie, did you have your hand up? Yes.

DR. SNIDER: Yes. I would like to join others in supporting the use of transformed cells as substrates. I think we've had some discussion around all the problems that we get into with primary cell cultures, so I think it's important to have transformed cells as substrates and to have them well characterized. I think we don't want to -- I would prefer not to put all our eggs in one basket. Obviously, I don't think we can. I mean Vero cells

won't necessarily support everything that needs to be grown and also even though as has been pointed out Vero cells have been extensively studied, there are still things that we don't know about them. And so I think having multiple sources is important not only for the spectrum of agents we might want to use to develop vaccines, but also to have other things that we can use in case we detect troubles with Vero cells down the road, for example.

With regard to the concerns, I think it's appropriate to be concerned about these adventitious agents and their potential impact on the development of tumors. I think it's important to be concerned about TSE's, transmissible spongiform encephalopathies. Of course, HIV and other agents have shown us that they can manipulate immune system and I think comments that people have made about general concerns about impact on the immune system should not be dismissed and that that's an area that we really have looked at, perhaps as much or as carefully as we ought to and I think that should be on our radar screen, that there's the potential that some adventitious agents may be able to somehow modify our immune system in ways that we would not desire.

CHAIRMAN GREENBERG: Dr. Griffin?

DR. GRIFFIN: I'd like to pick up on the theme that Dr. Kim started which is I can understand the concern about adventitious agents and I think that that's probably where we're most able to address, even though there's always things we may not know are out there, but I think all this is predicated on the fact that there aren't any cells that are present in what's being given because then -- so then you have the possibility of infection from these adventitious agents, but if you're actually transferring cells themselves then you have to add in the additional concern of whether the cells themselves can cause tumors and most of the studies that have been done were limited to 1980 technology as far as I'm concerned as far as these cells and in fact, I think some of these you would acknowledge probably are oncogenic -- would cause tumors in nude mice or whatever an dhow much concern or testing or -- would be necessary to make sure that the cells themselves are not in what you're giving and couldn't themselves cause tumors, independent of whatever reason that they're --

DR. LEWIS: Yes. The issue of residual tumor cells, especially human tumor cells in vaccines was -- that was one of the five major issues that we

presented before the Committee in 1998 and it was on the agenda at the cell substrate meeting in September and it's something we have been concerned about.

I think most people feel that with filtration technology, it's possible by validated procedures to rid any vaccine of the neoplastic cell. But you're absolutely correct. If viable neoplastic cells remained in vaccines, especially human cells, that there are reported cases of allografts, tumor allografts in humans as a result of inoculating humans with tumor cells, so this would have to be, they certainly have to be removed. That's absolute.

DR. GRIFFIN: That's a given?

DR. LEWIS: That's a given. There's no question about that.

DR. GRIFFIN: Okay.

CHAIRMAN GREENBERG: I'm in agreement so that has to be a core value of vaccines that -- okay. Other?

DR. BLAIR: I just wanted to comment. It seemed to me that classes 3, 4 and 5 are more of a psychological difference than a real difference in the sense that with the exception of perhaps cells transformed by oncogenic viruses that can spread within humans or could be rescued and spread that

basically they all present the same problem in that you have to find, if they have adventitious agents in them and tests and you presumably find there are none, otherwise, they're all transformed. They will all make tumors in animals and so I'm not sure that differentiating among the spontaneous versus nonspontaneous or oncogene transformed is really necessary. In a sense, Vero cells can move from class 2 to class 5 between passages 161 and 191, as we've heard. And in a sense, the cells transformed by known oncogenes have an advantage and a disadvantage. One advantage is you know they contain something you can transfer and it will transform a cell. On the other hand, you know what that is and you can look for it and the experiments, the proposals that are being done to look at DNA activity would argue you can really determine specifically for the agents you know can be transferred to the sequences you know what the relative sensitivity is. So in that sense they may offer an advantage over some of the others, but otherwise it just seems like they're basically the same problem and they perhaps might be treated more equally than not.

CHAIRMAN GREENBERG: Hold on. Were you going to respond to that, Andy, or you were just

absorbing?

DR. LEWIS: No, I was alluding to Phil.

CHAIRMAN GREENBERG: Phil?

DR. KRAUSE: I guess in thinking about these issues though the question that came up for us was what is it about the fact that this cell is transformed with the fact that the cell is immortal or neoplastic that has us worried? And it comes down really to two possibilities. One of them is and what makes us more worried about this cell than we would be about another cell that wasn't neoplastic. And of course any cell could potentially have adventitious viruses in it and of course every cell should be tested as extensively as possible as we talked about before. But the fundamental question then I think still comes up of whether -- if one's concern is adventitious viruses whether there's a substantial difference between a cell whose mechanism of transformation is known and it is known not to be due to an adventitious virus and a cell whose mechanism of transformation is completely unknown and it is presumed based on history that it could have something to do with an adventitious virus.

So in terms of thinking about adventitious viruses, I would argue that categories 3 and 4 are

quite different from category 5. You could make an argument that there might be some similarities in terms of thinking about the DNA, but as I pointed out before, I think your concerns about the DNA may reach different levels depending on whether the DNA concern is associated with its infectivity or its tumorigenicity. And clearly, we need to learn more about that, but at least a draft proposal, I think, is based on those basic ideas.

CHAIRMAN GREENBERG: Dr. Minor?

DR. MINOR: Firstly, I think from the point of view of adventitious agents, a transformed cell wins hands down over almost everything else to me because you have all the time in the world once you look at it and make sure that it's not there. Okay? So I think from the adventitious agent point of view, this seems to me to be a good line to be using.

There are a couple of things that I'd quite like some clarification on on the draft policy proposals. In Section 1, your last two columns say highly purely/deactivated virally vectored subunit. That's the first column. And the next one also has virally vectored. Is the implication that you've already vectored stuff in the first column, would that be a highly purely viral vector?

DR. LEWIS: Yes.

DR. MINOR: And can that really be done to something like a vaccinia? Can you really get the DNA, for example, if that's what you're talking about, can you really get the DNA down so low that you regard it as a completely negligible factor?

DR. SHEETS: It depends on the vector.

DR. MINOR: Right.

DR. SHEETS: And so these are separated into different categories. For vaccinia, you may not be able to purify it, but for an adeno viral specter or an AAV vector you might be able to purify it.

DR. MINOR: Okay. Maybe that should be pulled apart to make it a bit clearer because I think what you're saying in your draft proposal 1 is that absolutely anything goes, okay? Any kind of neoplastic cell of any description, no matter how good, bad or indifferent it is, is acceptable, provided you purify it and I've some sympathy for that, but I think you've got to define what you mean by purify it and also what you mean by the vector.

DR. LEWIS: Yes, I don't think we take exception to that.

DR. MINOR: Okay, the second thing was in your category 3, provided that you can actually