UNITED STATES OF AMERICA

FOOD AND DRUG ADMINISTRATION

+ + + + +

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

+ + + + +

VACCINES AND RELATED BIOLOGICAL
PRODUCTS ADVISORY COMMITTEE

+ + + + +

OPEN SESSION

+ + + + +

WEDNESDAY,
NOVEMBER 16, 2005

The conference convened in the Versailles Room of the Holiday Inn Select, 8120 Wisconsin Avenue, Bethesda, MD 20814, at 8:40 a.m., pursuant to notice, Gary D. Overturf, M.D., Chair, presiding.

COMMITTEE MEMBERS PRESENT:

GARY D. OVERTURF, M.D.	Chair
MONICA M. FARLEY, M.D.	Member
RUTH A. KARRON, M.D.	Member
PHILIP S. LaRUSSA, M.D.	Member
DAVID MARKOVITZ, M.D.	Member
CINDY LYN PROVINCE, R.N.,M.S.N.,M.A.	Member
STEVEN SELF, Ph.D.	Member
WALTER ROYAL, III, M.D.	Member
BONNIE M. WORD, M.D.	Member

This transcript has not been edited nor corrected, but appears as received from the commercial transcribing service. Accordingly, the Food and Drug Administration makes no representation as to its accuracy.

FDA STAFF PRESENT:

CHRISTINE WALSH, R.N. Exec. Secretary

CONSULTANTS:

JAMES COOK, M.D.
SETH HETHERINGTON, M.D.
PAMELA McINNES, D.D.S.
ROBIN ROBINSON, Ph.D.

AGENDA ITEM **PAGE**

CALL TO ORDER:
Gary Overturf 4

ADMINISTRATIVE MATTERS:
Christine Walsh 4

INTRODUCTIONS: 9

USE OF MDCK CELLS FOR MANUFACTURE OF INACTIVATED INFLUENZA VIRUS VACCINES

INTRODUCTION AND OVERVIEW:
Philip Krause 11
QUESTIONS 33

TUMORIGENICITY PRESENTATION:
Andrew Lewis 44
QUESTIONS 75

ADVENTITIOUS AGENT PRESENTATION:
Arifa Khan 96
QUESTIONS 115

ISSUES ASSOCIATED WITH RESIDUAL CELL SUBSTRATE DNA:
Keith Peden 120
QUESTIONS 146

MANUFACTURER'S PRESENTATION

CHIRON CORPORATION:
Rina Rappuoli 160
QUESTIONS 178

SOLVAY PHARMACEUTICALS, INC.:
Jeroen Medema 194
QUESTIONS 220

COMMITTEE/OPEN DISCUSSION: 231

ADJOURN:
Gary Overturf 296

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

8:40 a.m.

CHAIR OVERTURF: This is the meeting of the Vaccines and Related Biological Products Advisory Committee for November 16th. I don't have any special announcements. I think we are ready for a very full day of presentations. And before we start, I would like to turn the meeting over to Christine Walsh.

MS. WALSH: Good morning. I'm Christine Walsh, the Executive Secretary for today's meeting of the Vaccines and Related Biological Products Advisory Committee. I would like to welcome all of you to this meeting of the Advisory Committee. Today's session will consist of presentations that are open to the public. Tomorrow's meeting will consist of both open and closed sessions.

I would like to request that everyone, please, check your cell phones and pagers to make sure they are in the off or silent mode. I would now like to read into the public record the Conflict of Interest statement for today's meeting.

"The Food and Drug Administration is

convening today's meeting of the Vaccines and Related Biological Products Advisory Committee under the authority of the Federal Advisory Committee Act of 1972. With the exception of the industry representative, all Members and consultants of the Committee or special Government employees or regular federal employees from other agencies are subject to the Federal Conflict of Interest Law and Regulations.

The following information on the status of this Advisory Committee's compliance with federal ethics and Conflict of Interest laws, including, but not limited to 18 USC 208 and 21 USC 355(n)(4) is being provided to participants in today's meeting and to the public. FDA has determined that Members of this Advisory Committee and consultants of the Committee are in compliance with federal ethics and Conflict of Interest Laws, including, but not limited to, 18 USC 208 and 21 USC 355(n)(4).

Under 18 USC 208, applicable to all Government agencies, and 21 USC 355(n)(4), applicable to certain FDA committees, Congress has authorized FDA to grant waivers to special Government employees who

have financial conflicts when it is determined that the agency's need for particular individual services outweighs his or her potential financial Conflict of Interest, Section 208, and where participation is necessary to afford essential expertise, Section 355.

Members and consultants of the Committee who are special Government employees at today's meeting, including special Government employees appointed as temporary voting members, have been screened for potential financial Conflicts of Interest of their own, as well as those imputed to them, including those and their employers, spouse or minor child related to discussions on the use of MDCK cells for manufacture of inactivated influenza virus vaccines and the discussion of the development of new pneumococcal vaccines for adults.

These interests may include investments, consulting, expert witness testimony, contracts, grants, credos, teaching, speaking, writing, patents and royalties and primary employment. Today's agenda for Topic I includes a discussion of the use of MDCK cells for manufacture of inactivated influenza virus

vaccines. For Topic II, the Committee will discuss developing new pneumococcal vaccines for adults.

In accordance with 18 USC Section 208(b)(3), waivers have been granted to the following special Government employees: Dr. Ruth Karron and Dr. Steven Piantadosi. A copy of the written waiver statement may be obtained by submitting a written request to the Agency's Freedom of Information Office, Room 12A30 of the Parklawn Building.

With regard to FDA's guest speakers, the Agency has determined that the information provided by these speakers is essential. The information is being made public to allow the audience to objectively evaluate any presentation and/or comments made by the speakers. Dr. Matthew R. Moore is a medical epidemiologist, National Center for Infectious Diseases, CDC, Atlanta. Dr. Sandra Steiner is a microbiologist/immunologist, Division of Bacterial and Mycotic Diseases, CDC, Atlanta. As guest speakers, they will not participate in the Committee deliberations nor will they vote.

In addition, there may be regulated

industry and other outside organization speakers making presentations. These speakers may have financial interests associated with their employer and with other regulated firms. The FDA asks in interest of fairness that they address any current or previous financial involvement with any firm whose product they may wish to comment upon.

These individuals were not screened by the FDA for Conflicts of Interest. Dr. Seth Hetherington is serving as the industry representative acting on behalf of all related industry and is employed by Inhibitex Incorporated. Industry representatives are not special Government employees and do not vote.

This Conflict of Interest statement will be available for review at the registration table. We would like to remind members and consultants that if the discussions involve any other products or firms not already on the agenda for which an FDA participant has a personal or imputed financial interest, the participants need to exclude themselves from such involvement and their exclusion will be noted for the record.

FDA encourages all other participants to advise the Committee of any financial relationships that you may have with the sponsor, its product and, if known, its direct competitors." Thank you. That ends the Conflict of Interest statement. Dr. Overturf, I turn the meeting back over to you.

CHAIR OVERTURF: At this time, I would like to go around the table and have everybody introduce themselves and tell us where they are from. So I'll start with Dr. Markovitz.

MEMBER MARKOVITZ: Yes, I'm David Markovitz from University of Michigan and from the Division of Infectious Diseases and Department of Internal Medicine.

DR. HETHERINGTON: I'm Seth Hetherington. I'm the Chief Medical Officer and Vice President of Clinical Development for Inhibitex near Atlanta, Georgia.

MEMBER ROYAL: My name is Walter Royal. I'm a neurologist in the Department of Neurology at the University of Maryland School of Medicine.

MEMBER FARLEY: My name is Monica Farley.

I'm an Infectious Disease Specialist in the Department of Medicine at Emory University in Atlanta.

DR. McINNES: Pamela McInnes, Deputy Director, Division of Microbiology and Infectious Diseases of the National Institute of Allergy and Infectious Diseases.

MEMBER PROVINCE: I'm Cindy Province. I'm the Consumer Representative on VRBPAC and I'm the Associate Director of the St. Louis Center for Bioethics and Culture.

MEMBER LaRUSSA: Philip LaRussa, Division of Pediatric Infectious Diseases, Columbia University.

MEMBER WORD: Bonnie Word in the Division of Pediatric Infectious Diseases at Baylor College of Medicine, Texas Children's Hospital.

DR. COOK: I'm Jim Cook. I'm Chief of Infectious Diseases at the University of Illinois.

DR. MINOR: I'm Philip Minor. I'm head of Virology at the Institute of Biological Standards and Control in the United Kingdom and I have input into European affairs and the like.

MEMBER KARRON: I'm Ruth Karron, Center

for Immunization Research, Bloomberg School of Public Health, Johns Hopkins University.

MEMBER SELF: I'm Steve Self, head of Biostat and Biomathematics Program at Fred Hutchinson Cancer Research Center in Seattle.

CHAIR OVERTURF: Dr. Robinson, would you like to introduce yourself?

DR. ROBINSON: Robin Robinson from the Office of Public Health Emergency Preparedness at HHS and I'm head of the Pandemic Influenza Program at HHS.

CHAIR OVERTURF: And I'm Dr. Overturf. I'm the Chair of the Committee and Professor of Pediatrics and Infectious Disease at the University of New Mexico. Today's discussion, as I said, will be about MDCK cells and their use in possible manufacture of vaccines. And the meeting is going to be opened by a presentation by Dr. Krause.

DR. KRAUSE: Good morning. I'm Phil Krause. I'm the Acting Director of the Division of Viral Products in the Office of Vaccines Research and Review at CBER.

(Agency sound system feed interrupted.)

DR. KRAUSE: Some of the vaccines continue to use different scientific investigation. Vero cells at non-tumorigenic passages were introduced for the manufacture of highly purified, inactivated vaccines like inactivated polio vaccine and were introduced in the 1980s and that vaccine was approved in 1990 in the U.S. and it is the most commonly used inactivated polio vaccine, at this point.

And in the late 1990s, we came to the Advisory Committee to discuss the use of vero cells at non-tumorigenic passages for live-attenuated vaccines, so these cells are now used in investigational live-attenuated vaccines. And in the early 2000s then, we had discussions and there is now investigational replication-defective recombinant vaccines that are manufactured in in vitro-transformed human cells, currently, 293 and PER.C6 cells.

So the MDCK cell then represents to some degree a logical next step in this progression. But what are we talking about when we talk about the MDCK cells? I think it's important for any cell substrate to think back to the history of where the cell was

derived and how that cell line was developed. And one point to be made is that in 1958, Madin-Darby developed the MDCK cell line from a healthy female cocker spaniel. They determined soon after that that then those cells would be at the American Type Culture Collection.

Over time, different investigators and different people have used different versions of the MDCK cells that have involved varying numbers of passages and varying conditions of passages and Gaush, who developed one of these strains at the Univeristy of California described actually different MDCK cell strains that had somewhat different phenotypes. And so it is useful to think about the history of any individual cell line and recognize then that multiple, relatively independent derivatives of the cell line can be described and there may be some differences among them.

So why are MDCK cells being considered for use in manufacture of inactivated influenza vaccines? Well, you're going to hear more about this from the manufacturers a little bit later on, but some of the

clear advantages are that the virus grows much better in cells, which then makes it easier to manufacture the vaccine. There is also an advantage to the ability to do more rapid scale-up as compared with the egg-produced influenza vaccines that are currently being used. There is the ability to bank and thoroughly characterize the cells. And these cells will ultimately adapt to serum-free growth, which may then provide some advantages in eliminating concerns about the source of the serum.

Why then would somebody be concerned about using MDCK cells? Well, the major issue we're going to talk about today really relates to tumorigenicity and the neoplastic nature of the cell. And the original line of MDCK cells was described in the past as non-tumorigenic. However, some MDCK derivatives have been found to be highly tumorigenic. And highly tumorigenic cell substrates have never before been used to manufacture viral vaccines in the U.S. And highly tumorigenic cell substrates then pose regulatory challenges that we will be discussing today.

So I would like to point out though that the discussions that we have today go well beyond the issue of using the cells for the flu vaccines, because this is the natural next step in the progression of vaccine development in the United States proposed for possible diseases. Because the ability to make vaccines in tumorigenic cells would expand the repertoire of cells that can be used in development of new vaccines.

This includes various genetically engineered viral vectored vaccines that show some promise. It could well have some real advantages in the manufacture of HIV vaccines and, of course, the topic that we are focusing on today is the idea then of making either annual or pandemic influenza vaccines in these kinds of cells.

So what are the concerns about tumorigenic cells? Well, as this has been discussed and I will summarize this discussion as this introduction goes on, there is the potential for increased risk of adventitious agent contamination in tumorigenic cells. There is a potential for increased risk associated

with residual DNA. There is a potential for increased risk associated with virus/cell interactions.

There may be a potential for other increased risks and I think perhaps importantly, and one of the reasons we feel that it is very important to have this discussion in open session today, is the fact that there may simply be a perception of increased risk, even if we can address all of these other risks.

So what I would like to do next is go over the last 10 or so years of CBER thinking about the introduction of neoplastic cell substrates, because this really is just your next step in a progression of thinking about how it is that we can use new types of cell substrates in order to manufacture vaccines. And so I'm going to take you back to 1995 and tell you what kind of cells were being used to produce biologicals, at that time.

Well, Namalwa cells, which were derived from a human burkittsville lymphoma and were transformed by Epstein-Barr virus were, at that time, used for production of interferons. Those cells were

tumorigenic. Rodent cells were being used for monoclonal antibody productions and hybridomas, various therapeutic proteins, which were being made in Chinese hamster ovary cells and baby hamster kidney cells, and Chinese hamster ovary cells, I told you earlier, were being used for some investigational protein subunit vaccines.

And these cells are all tumorigenic. These cells also have the property that they produce non-infectious retroviruses. And so in order to be sure that they could safely be used, the regulatory process involved making sure that high amounts of viral elimination or inactivation were achieved in the manufacture of these vaccines. And in general, the standard has been that there should be at least 6 logs of clearance in excess of any known retrovirus burden.

And because that, in the case of some of these cells, would require showing in some cases the ability to clear as many as 12 or 13 logs of virus, this could generally only be demonstrated by having multiple independent steps, each of which was capable of clearing a defined amount of virus. And this was

the case, because in many cases it wasn't possible to spike the product with large enough amounts of virus in order to prove that the production process could remove as much as one would have liked to be able to show. And so this was a well-accepted procedure for doing this.

And then vero cells, I told you, at non-tumorigenic passages were being used for production of inactivated polio vaccines. And at that time, there were stringent limitations on DNA content and these cells were being used only for inactivated vaccines.

So as CBER OVRR recognized the need to expand the repertoire of cells that were being used for vaccine production, we engaged the VRBPAC in a number of these discussions. And all of these discussions, including the one today, were based on the premise that full public discussion of the transition to the use of neoplastic cell substrates is important.

And I'm just going to summarize four of these discussions for you right now. One of them is an initial discussion we had with the Committee back

in 1998, we then, based on that initial discussion, cosponsored an international cell substrate meeting and reported back to the VRBPAC on that in 1999. In the year 2000, we discussed the use of vero cells with the VRBPAC. In 2001, we discussed the use of 293 and PER.C6 cells.

So at this discussion which occurred in 1998 with the VRBPAC, and I would just point out each of these discussions generated a transcript of somewhere between 200 and 300 pages, and so I'm going to summarize the major results from each of these. But, of course, it's not possible to distill each of these transcripts down to a single slide and give true justice to the depth and the quality of the discussions.

But at this initial discussion, the Committee recommended that OVRR CBER develop a document that described a proposed approach to addressing the use of neoplastic cells in vaccine manufacture. They recommended that CBER sponsor a workshop to obtain public discussion of this document and additional scientific input into these issues.

They recommended continued dialogue with the Advisory Committee and also recommended research to provide a scientific foundation for decision-making regarding the use of neoplastic cells in vaccine manufacture.

So as a result of that encouragement by the Advisory Committee, CBER then cosponsored along with the International Association for Biologicals, the National Institute for Allergy and Infectious Diseases, the National Vaccine Program Office and the World Health Organization an international meeting, which was entitled "Evolving Scientific and Regulatory Perspectives on Cell Substrates For Vaccine Development."

And then soon after that meeting, actually in the same month, we summarized the results of that meeting to the VRBPAC. The key goals of this meeting were to, in a scientific sense, identify the concerns and issues associated with use of these new cell substrates and identify approaches to determine levels of risk that might be associated with those issues. And the other thing is that at this meeting, there was a discussion of a CBER document that had been prepared

in response to the November 1998 VRBPAC and that involved then a presentation of a Defined Risks Approach, as a conceptual framework for considering the issues.

So what do we mean by Defined Risks Approach? Well, a Defined Risks Approach represents an attempt to establish, where possible, a quantitative conceptual framework for estimating upper bounds on potential risks, so that we could understand what the risks of any of these individual issues might be. And so the basic steps of that involved identifying a possible risk event, based on the list that I showed you earlier; estimating or determining the frequency with which the risk event might occur or has been observed to occur, either in nature or under experimental conditions; estimating the possible frequency of the risk event per dose of the vaccine; developing and determining the sensitivity of one or more assays that could be used to detect the risk event; and then or developing and validating one or more processes that could be used to establish a product-specific safety factor.

And so by going through these individual possible risk events then, the thinking was we could develop an approach that would allow us to assure that with respect to each of these issues vaccines made in each of these cell substrates would be safe.

The scientific conclusions of this meeting were as follows, and this also was a very lengthy meeting and involved the publication of an entire booklet or actually full book of papers and conclusions and discussions, and so again, this one slide doesn't do full justice to that.

But the major conclusions were that the multi-factor nature of carcinogenesis suggests a very low risk of oncogenicity from cellular components other than oncogenic viruses. In that context, it was thought that unrecognized adventitious agents may be the major concern with neoplastic cell substrates, but it was clearly recognized that primary cells present a greater risk for adventitious agents than do neoplastic cells.

Risks from residual DNA were perceived to be low, although, the meeting concluded that there was

a need for more scientific data to verify that perception. And with respect to virus/cell interactions, the participants of the meeting concluded that risks must be considered based on specific virus/cell substrate combinations, as well as any selective pressures in the cell culture system.

The concern was raised at this meeting that neoplastic cells might contain abnormal PrP genes of unclear significance. And there was also an interesting discussion about the idea of designing cell substrates using defined mechanisms of transformation and the suggestion that that could be considered as a way to address some of these potential issues.

In 2000, OVRR came back to the VRBPAC to discuss issues and topics regarding the use of vero cells for vaccine manufacture. Now, vero cells are non-tumorigenic, in general, but they have the capacity to become tumorigenic upon repeated passage. The mechanism of transformation of these cells is unknown, but substantial experience did exist at that time and continues to exist using vero cells in

research and diagnostics. And a high level of testing detected no evidence for the presence of adventitious agents in vero cells.

The Committee recommended that it was important to assure the removal of intact cells from vaccines. They expressed, in general, more concern about parenteral, the mucosal vaccines produced in vero cells. There was significant concern expressed about the use of vero cells at tumorigenic passage levels and I think that is partly because it was not understood why it was that vero cells may become tumorigenic. In fact, that's still not understood.

Some members did express concern about using cells with the potential to become tumorigenic, but overall the conclusion was that if the DNA quantity was limited to 10 nanograms for vaccines produced in vero cells at non-tumorigenic passages, that it would be all right to use these cells.

In 2001, we came back to the VRBPAC to discuss the use of in vitro-transformed neoplastic cells to produce replication-defective vaccines. And so this is the strategy that came to some degree

almost directly out of the recommendations of the international meeting. It is to take cells and to transform them using a defined mechanism so that we would know why they became tumorigenic and thus could be sure that the reason they became tumorigenic was a risk that we could manage and something that we could understand.

And the cells that were really discussed in detail there were the 293 cell line and the PER.C6 cell line, which had been used for gene therapy products and were being proposed for the use in propagation of investigational live adenovirus vectored vaccines. And these cells allow replication of defective adenovirus vectors and PER.C6, in particular, is designed to minimize the formation of replication competent adenoviruses, which can be a problem when one is trying to replicate those kinds of vectors.

These cells have a defined mechanism of transformation, the E1 gene of adenovirus type 5. These cells are weakly tumorigenic and extensive testing detected no evidence of the presence of

adventitious agents.

The Committee discussed the value of these cells for manufacturing vectored viral vaccines. They discussed the role of the known mechanism of transformation and there was some skepticism that this provided the clear safety margin. They discussed the importance of minimizing steps, that is initiation events, toward oncogenesis in vaccine recipients. Even if an oncogenic outcome is not directly correlated with the use of neoplastic cells, it was considered to be important to assure that vaccine recipients are not primed. And that's something that we'll come back to a little bit later on in the presentations.

There was a discussion of the adenovirus E1 gene, including the fact that there was a very low likelihood that it would be taken up in a significant number of cells; the fact that this particular gene had involvement in apoptosis, which was considered to provide some additional safety factors; and also, the point was made that it was very unlikely, given the large number of cells required to form tumors even in

immunosuppressed animals, that that number of cells would take up this gene and reach the tumor cell threshold dose necessary for clinical impact.

There was broader discussion of whether the degree of tumorigenicity of these types of cells was important and there were varying opinions expressed on that. There was a discussion of the approach to TSE issues in neoplastic or retinal cells and because retinal cells have some neuronal derivation, the principle was established that it would be useful to sequence the PrP gene in these cells and make sure that it had a normal sequence. But the conclusion was that these cells could be used for manufacture of replication-defective adenovirus vaccines with appropriate limitation on residual DNA.

So just to summarize then, I'm going to go through the concerns that I listed before and how we have addressed them to date with the use of new neoplastic cell substrates.

So an obvious concern about the use of neoplastic or tumorigenic cells is the idea that tumorigenic cells may form tumors if they were

transferred to a recipient of a vaccine, and that has actually been reported with human cells that have been given to humans.

However, if the cells are non-human, there are immunological xenograft rejection mechanisms that should prevent this from happening. And, of course, the other thing that is done in vaccine manufacture is assuring via validated methods that there are no intact cells in the final product. And that provides an enormous margin of safety and assurance that there aren't any tumorigenic cells in vaccines that are made in these kinds of cell substrates. And so this generally is considered to address this issue.

There are special considerations regarding the potential presence of adventitious agents in neoplastic or tumorigenic cells, and there is the concern that adventitious agents that may have induced the original neoplastic or tumorigenic phenotype may be present in the cells and, of course, some viruses are known carcinogens in humans and in animals. And so there is a real possibility that some cells may have been transformed by viruses that could still be

present, especially if we do not know the mechanism of transformation.

There is also the potential that neoplastic or tumorigenic cells may have expanded capacity to support viral replication as compared with other types of cell substrates and, thus, in that sense may be more likely to contain agents.

And so far this issue has been addressed by limiting the use of tumorigenic cells to investigational inactivated vaccines for which high levels of purification is performed with the exception of the PER.C6 and 293 cells for which we also have additional information about the mechanism of transformation, as well as expanded testing for oncogenic and other agents.

A third concern about the use of neoplastic or tumorigenic cells is that the residual DNA from the cells that is inevitably present in a vaccine might be infectious or oncogenic. And Dr. Peden is going to discuss this in some more detail later on, and I failed to mention that Dr. Khan will discuss the adventitious agent issues a little bit

more later on.

But this issue has been addressed to date by doing in vivo oncogenicity testing on the cell substrate DNA to be sure that the cell substrate DNA doesn't have this activity; by limiting the quantity of residual DNA that might be present in a dose of vaccine; and by creating limitations or by limiting the biological function, for instance, by looking at the size or other properties of any residual DNA.

In our international meeting there was a robust discussion about virus-host and virus-cell interactions and one of the ideas there, for instance, is that a vaccine virus might package cell DNA or incorporate cell elements that could be oncogenic, thus limiting the ability to eliminate those theoretically oncogenic agents from a vaccine.

And to date this issue has been addressed by demonstrating that final vaccine preparations don't contain transforming DNA. And I point out that this is not an issue for cytoplasmic RNA viruses like influenza which, of course, is what we're discussing today in the context of the MDCK cells. And in some

cases inactivation of the viral vaccine would certainly eliminate this concern as well.

There are other potential concerns about the use of neoplastic or tumorigenic cells, which I'm just describing on this slide and these are in general considered to be much less likely. And there is, however, the idea that some other mechanism, for instance oncogenic proteins, RNAs or some other factor that could induce a heritable epigenetic change that is associated with immortalization or tumorigenicity of a cell substrate, could present a risk to the recipient of a vaccine manufactured in tumorigenic cells.

And this issue has been addressed to date by the scientific consensus that such other mechanisms are very unlikely, by the use only of weakly tumorigenic cells, as well as by in vivo testing of cell lysates to make sure that these kinds of elements are not present in vaccines.

There is also the concern as we move toward the use of tumorigenic cells that our previously used tumorigenicity assays may not

adequately define the tumorigenic phenotype or the risk associated with the use of tumorigenic cells, and Dr. Lewis is going to talk a little bit more about how we might use or how we're recommending that tumorigenicity testing be done in order to address these kinds of issues.

So today's talks are going to be by Andrew Lewis. He will be discussing the regulatory implications of neoplastic cell substrate tumorigenicity, by Arifa Khan who will be discussing adventitious agent testing of novel cell substrates for vaccine manufacture, Keith Peden who will discuss issues associated with the residual cell substrate DNA.

We're fortunate to have with us today manufacturer's vaccines in MDCK cells, Chiron and Solvay, and I really want to take a moment out to applaud them for coming here and presenting their data. There is no closed session associated with this meeting.

This is an open session with the idea that to the degree that we can get this discussion out into

the public, then people will really understand what it is that we're doing and how it is that these scientific issues are being addressed, and the data that they are bringing to us today will be very helpful in doing that.

And so then after those presentations, we're going to ask the Committee to help us meet the following goals, to have a discussion of the use of MDCK cells, including those that are highly tumorigenic in the manufacture of inactivated influenza vaccines, a discussion of OVRR's overall approach to evaluate the safety of tumorigenic cells for use in vaccine production, and the discussion of any additional steps that you would recommend that CBER should take to address issues associated with any use of neoplastic cell substrates either in the context of MDCK cells or in the future. So thank you.

(Applause)

CHAIR OVERTURF: We have a few minutes so I will take -- Dr. Krause can take questions from the Committee before we proceed. Yes, Dr. Markovitz?

MEMBER MARKOVITZ: Yes. Dr. Krause, I

don't know if you want to punt this to Dr. Khan, but basically the adventitious agent issue, you presented really two different scenarios.

In one case you suggested that a previous group had said that adventitious agents were much more likely to be in primary cells than in neoplastic cells, but then later you emphasized how neoplastic cells may contain adventitious agents which might have caused their transformation or subsequently acquired them due to their ability to proliferate better in those cells.

Are there any data to actually address this?

DR. KRAUSE: So you will hear about some data that addresses this. I think in general, because you can bank a neoplastic cell line and can really test it very carefully, the ability to make sure that viruses that we know about at least are not in there is very good.

With primary cells that's much more difficult to do, because these cells are taken each time from a new lot of cells or a new animal. And, of

course, some of the most concerning episodes in vaccine manufacturing history, I'm thinking specifically about the contamination of early polio vaccines that were contaminated with the SV40 virus.

So I think that the scientists who have looked at this recognize that the ability to bank these cells and test them provides some real advantages over using primary cells and, overall, I think most scientists who have thought about this would place primary cells at a greater risk for adventitious agents than they would neoplastic or tumorigenic cells.

But the question then is are neoplastic or tumorigenic cells at a greater risk than, for instance, human diploid cell strains or other cells that don't have the neoplastic or tumorigenic phenotype, and what can we do to make sure that these cells are as safe as possible or completely safe for making vaccines.

MEMBER MARKOVITZ: Yes. I guess the real question, just as you have said, is diploid versus neoplastic cells and data there, is there any

indication that there is any difference in terms of adventitious agents between neoplastic cells and diploid cells?

DR. KRAUSE: So there certainly are examples of neoplastic cells that have viruses in them and you will not find that example, and the presence of those viruses is related to the immortalization of those cells. There also are examples of neoplastic cells and it may just be that because these cells don't senesce and because they can be passaged for long periods of time, this gives them more opportunities to be contaminated throughout their long history.

But it also is the case that many viruses rely on cellular mechanisms for part of what they do and cells that are dividing more rapidly are more likely to have nucleotides in them that the virus can take advantage of and use for replication. And so many viruses do grow better in neoplastic or tumorigenic cells, and I think that Dr. Lewis and Dr. Khan will have some examples of those kinds of things.

CHAIR OVERTURF: Yes, Dr. Minor?

DR. MINOR: I was looking at your slides on the way over, Phil, and I think you have summarized very well the evolution of views on the nucleic acid issue, okay, that initially the idea was that you would only use normal cells and then after that you would use tumorigenic cells provided you could show there was no DNA there, and then the amount of DNA gradually crept up, if you might. And I think what we're now faced with is looking at highly tumorigenic cells potentially and asking the question does it actually matter.

Is it your view that the change in attitudes to nucleic acid have actually been based on science and, if so, what science has it been or is it just a question of people getting used to the idea that these things are maybe not as drastic as everybody thought they were? Is this a fair question? No, never mind. Never mind the second question.

DR. KRAUSE: Well, so clearly one of the concerns, and this was expressed at the 1999 meeting, was that as people were using more and more, allowing more and more residual cell DNA. In fact, although

there was a general scientific consensus that this was probably okay, that consensus wasn't really based on any data.

And one of the things that was recommended was to obtain more data about what amounts of residual cell DNA of different types could be considered safe with respect to different issues. And Dr. Peden actually will be presenting some of that additional data and, as you know, some of those data have been generated and some of those data, in fact, have been generated with the support of NIID, which has been very generous in funding some of these studies.

CHAIR OVERTURF: Yes?

MEMBER ROYAL: I just have a question, Walter Royal, University of Maryland, a clarification. When you talk about viral DNA are you talking about a complete viral genome as opposed to a fragmented genome that might be incorporated in various places within the host cell?

DR. KRAUSE: So Dr. Peden will describe this in more detail but, of course, either could potentially be a concern. If a virus contained an

oncogene that could integrate in some location, you wouldn't need the entire viral genome. And so that is where we think about oncogenic events that could theoretically be due to viral genomes or other oncogenes that might be present in a neoplastic or tumorigenic cell substrate.

There is also, however, the concern that if an entire viral genome were present either epigenetically or integrated into the genome of a cell substrate that that entire genome then, if that DNA were inoculated into a recipient of a vaccine, could then recover the virus and then give rise to the kind of infection that that virus would cause in nature.

And so I think we have to consider all of those possibilities and Dr. Peden will describe our strategy for doing so.

CHAIR OVERTURF: Dr. Self?

MEMBER SELF: If I understand this, there are lines of these cells that are more and are less tumorigenic. Will there be data presented to tell us something about what is known of the mechanism for these changes that have occurred?

DR. KRAUSE: So I think there probably will be some discussion as to why it is that cells become tumorigenic. My own conclusion from looking at that literature is it's not very well understood.

There do exist some reasons clearly why cells become tumorigenic that might not provide any particular risk to a vaccine recipient, among them if a cell develops the ability to escape immune surveillance that may increase the likelihood that it's tumorigenic, but it's unlikely then that even if one could confer that ability to an otherwise non-neoplastic cell in a vaccine recipient, it's unlikely that that would cause any problems and those cells would just senesce anyway.

But I think that these are the kinds of discussions that we're hoping that the Committee will have and some of those data will be presented including, I think, by the manufacturers.

MEMBER SELF: I wasn't thinking sort of generally, but very specifically in these particular cell lines.

DR. KRAUSE: So I do not know the

mechanism by which these particular cells became tumorigenic.

CHAIR OVERTURF: Dr. Krause, you mentioned among the concerns a question of a perception of risk and actually from my standpoint as a clinician, I am particularly concerned about that issue.

Has the FDA considered plans or talked about plans for how they wish to convey the risk to try to allay that perception among the users or the receivers of vaccines?

DR. KRAUSE: So, obviously, these kinds of open Advisory Committee Meetings are a big part of that process, but we certainly will welcome whatever suggestions you have in that regard as well.

CHAIR OVERTURF: Yes?

MEMBER LaRUSSA: Just an expansion on one of the previous questions. I was also interested in the genetic correlates, the tumorigenicity phenotype for the MDs, MDCK cells. Is the original cell line still available?

DR. KRAUSE: So --

MEMBER LaRUSSA: Is that something we

could go back and look at now?

DR. KRAUSE: So Madin-Darby did after a fairly small number of passages bank the original cells with the ATCC, and I believe that what you get from the ATCC if you now order it is a few passages expanded beyond that, which is what they need to do in order to be able to continue to send it out. And so, in fact, one can look at at least that representative of the original cells.

CHAIR OVERTURF: Dr. Royal?

MEMBER ROYAL: Using that vero cell line as an example, is it known what happens when it goes from being non-tumorigenic to tumorigenic?

DR. KRAUSE: So that is an issue that Dr. Lewis is actually studying fairly vigorously in the laboratory. I don't think he has any final conclusions, but that is something that we would like to understand.

It's our sense that at least some of the mechanisms by which a cell line can become tumorigenic are mechanisms that are really related to the tumorigenicity assay and what it is that's measuring

and don't necessarily translate into a direct risk to a vaccine recipient.

But, of course, it's very difficult then to say that all of the possible mechanisms by which a cell can become tumorigenic would have that property. And so I don't think that we can say that.

CHAIR OVERTURF: Dr. Robinson?

DR. ROBINSON: Phil, could you give us the Agency's position or policy on the sliding scale from non-tumorigenic cells to weakly tumorigenic to highly tumorigenic relative to cellular DNA residual?

DR. KRAUSE: So, of course, we always look at each product individually and so I can tell you what we have been recommending and what we have attempted to follow with the particular cell substrates that I have described.

So for vero cell produced vaccines that are intended to be given parenterally, we would like to see fewer than 10 nanograms per dose. The same is true for the vaccines that are produced in the 293 or PER.C6 cells. And, of course, the vero cells are not tumorigenic. The 293 and PER.C6 are tumorigenic but

we believe that we understand the mechanism by which those cells became tumorigenic and vaccines produced in those cells can be studied to make sure then that they don't contain whole copies of the gene that transformed them. And so there are additional things that can be done there.

But this really is the next step, and so we do not have as of this time today a number that we believe is necessarily the right number for a highly tumorigenic cell. You will hear from the manufacturers, I think, how it is that they are approaching this and so then the question obviously will be is that the right way to do this.

And, of course, that strategy that they are using is one that has been developed based on this entire series of discussions and with an idea of trying to mitigate these specific concerns.

CHAIR OVERTURF: Any further questions? I think we'll proceed then to the second speaker who is Andrew Lewis who will provide a tumorigenicity presentation.

DR. LEWIS: Good morning. I'm Andrew

Lewis, Chief of the Laboratory of DNA Viruses, the Office of Vaccines, the Division of Viral Products. My responsibility to the meeting today is to consider the regulatory implications of neoplastic cell tumorigenicity.

Now, in addressing these regulatory issues that are posed by the tumorigenicity of cell substrates, I'm going to attempt to first define tumorigenicity and oncogenicity, attempt to review the regulatory concerns associated with the tumorigenic cell substrates, especially cell substrates that are highly tumorigenic, review tumorigenicity testing, that is how tumorigenicity testing is evaluated, how highly tumorigenic cells can be identified, how uses of expanded models of tumorigenicity testing and their contributions or the possible contributions that these models can make to cell substrate evaluation and, finally, to review the mechanisms of neoplastic development and their implications for neoplastic cell substrate evaluation.

Sorry. I think to get started it's important to define and explain the process of

tumorigenicity and oncogenicity. Phil Krause has already alluded to some of these definitions, but the differences in these processes can provide useful information on the regulatory management of neoplastic cell substrates.

So tumorigenicity is actually the process by which neoplastic cells growing in tissue culture form tumors and the key word here is form tumors when they are inoculated into animals. Now, if you think about the terms tumorigenicity and oncogenicity, in the literature these terms are frequently used interchangeably.

But for purposes of regulatory management and dealing with regulatory concerns, it's necessary to come up with rather precise definitions of these terms, because the differences in the definition provide us with opportunities to use these processes for regulatory purposes.

So during the process of tumorigenicity, as I have just mentioned, the inoculated cells grow into tumors. But during oncogenicity, oncogenic agents transform the cells of the injected species in

the neoplastic cells that grow into tumors. So, obviously, if you find large numbers of host cells, that is from cells the species are injected, at the inoculation site of a cell substrate, this may indicate the presence of oncogenic virus or an oncogenic factor in the cell substrate itself. And certainly that would have regulatory implications.

Now, when we're thinking about the regulatory concerns associated with the use of neoplastic cell substrates, these concerns were first presented to the Advisory Committee in 1998, has been reviewed by Phil Krause, these concerns were developed into a paper which we entitled "A Defined Risks Approach to the Regulatory Assessment of Use of Neoplastic Cell Substrates for Viral Vaccine Manufacture."

This paper was presented at the cell substrate meeting in 1999 and was published along with the proceedings of this meeting in 2001. These concerns are summarized in this slide and is somewhat a repetition of what Phil has had to say. But, I think, it is important because there are a few

additional details.

The first concern is induction of tumor allografts. There were reports in the 1950s of surgeons who were operating on people, patients with cancer who actually inoculated themselves by surgical error, cut themselves with a scalpel that had been used to excise the tumor or to remove tissues around the tumor and they engrafted themselves with human tumor cells with fatal consequences. There weren't a lot of those cases, but they are out there.

The second concern is a transfer of known or unknown oncogenic viruses. For example, SV40 was a classic example of oncogenic virus being transferred by a viral vaccine, but most people don't recognize it. Lymphocytic choriomeningitis virus has been detected in cells. It has been isolated from human breast carcinomas and, in fact, human sarcoma. There are a variety of agents, such as herpesviruses, retro viruses, polyomaviruses and papillomaviruses that are present in human tumors. Some of these viruses are present as etiologic agents in these tumors. Some of them is passenger viruses that have found a nice place

to live.

The third possibility or concern is the transfer of oncogenic viruses. As I mentioned, the SV40 problem with the polio vaccine, but there are, in fact, reports in the literature about SV40 transformed human cells. In one case a meningioma cell, when it was inoculated into the nude mouse, the mouse host cells were transformed into fiber sarcomas or lymphomas that contained SV40 DNA. So this is an example of the transfer of oncogenic activity from a cell line forming a tumor to the host in which the tumor is being formed.

And the final concern deals with the transfer of cell components. It might initiate neoplastic processes. An example here is that a number of human tumors contain ras oncogene, activated ras oncogenes. But there is a report in the literature about the possible induction of tumors in mice by such an oncogene.

So in considering neoplastic cell tumorigenicity, it's generally recognized that some neoplastic cell lines are weakly tumorigenic and I

think Phil has already mentioned this. That is they express a weakly tumorigenic phenotype and they have a limited capacity to form tumors in animals, while other cell lines are highly tumorigenic and exhibit an enhanced capacity to form tumors in animals. And the issues that are associated with weakly tumorigenic cells, as has been noted, was discussed with the Committee in 2001.

The issues that we are going to be considering today represent the issues that are posed by highly tumorigenic neoplastic cell substrates. And the concerns that are generated by these types of substrates are listed in this slide. First, as a general perception, the more tumorigenic or the more clinically aggressive the neoplastic cell, the greater the risk of its components of inducing neoplastic processes.

Second, the factors that actually contribute to the highly tumorigenic phenotype require further explanation and I think this gets at the question that was just asked to Phil Krause. There have been no attempts to correlate oncogenic activity,

the cell substrate DNA with the aggressiveness of their tumorigenic phenotype. And then finally, the fewer cells that are required to produce a tumor, the smaller the safety factor can be attributed to the transfer of factors that might induce neoplastic activity.

For example, if you have a cell line that requires a million to 10 million cells to form a tumor, the possibility of transferring an oncogenic activity from those cells compared to a cell line that requires only a few 10s of cells to form tumors is quite significantly different.

Now, in this table I represented our estimations of the relative risk posed by different types of neoplastic cell substrates with primary cells and diploid cells in this strain. And focusing first on the footnotes, we looked at weakly tumorigenic cells, which again these are cells generated in a laboratory. The weakly tumorigenic cells that I'm aware of are transformed by the non-oncogenic adenoviruses Type II and V or possibly SV40 in every species, but the hamster.

But these cells require very high doses, a million to 10 million cells, to form tumors in animals and the animals that they form tumors in need to be immunosuppressed. For example, adeno two transformed syrian hamster immunocells formed tumors in newborn hamsters, they do not form tumors in adult hamsters. And such is the case with other types of SV40 transformed mouse cells or rat cells as well.

There is actually no reports that I'm aware of of the recovery of dominant cellular oncogenes from these types of cells. And with defective adenovirus vectors replicating in some of these weakly tumorigenic cells, such as the 293, you can get the formation of replication, competent adenoviruses as Phil has alluded to.

Now, if we look at highly tumorigenic cells, their capacity to form tumors is increased from a few millions of cells to 10 to a few hundreds of cells in most cases and in some cases 10,000 or more. Oncogenic viruses and dominant activated cellular oncogenes have been and can be recovered from highly tumorigenic cells and there are any number of reports

of these types of cells containing adventitious agents.

Now, if we focus on the data in the table, primary cells actually are generally considered to pose the greatest risk. And I think Phil has expanded on that a bit. Diploid cells range pose little or no risk of transferring oncogenic activity by way of cell components. But because they are laboratory-derived, weakly tumorigenic cells also are believed to represent less of a risk of transferring oncogenic activity compared to highly tumorigenic cells.

Now, I would hesitate to, I won't hesitate, I'll mention very frankly that these estimations are based on our best judgment of looking at the scientific literature and trying to make interpretations of what we think is going on out there. As our experience with monitoring and measuring and trying to understand these types of cell substrates evolves, we very well may need to change the way we are thinking about these data.

Now, the next topic I would like to get into is addressing the questions of how the

tumorigenic phenotype expressed by neoplastic cell substrates is actually evaluated. There are several different assays for determining whether neoplastic cells have the capacity to form tumors in animals or in vivo. Some of these assays are used to evaluate cell substrates and some are not.

The assays that are currently used to evaluate cell substrates include inoculation of athymic mice or rats, the inoculation of newborn mice or rats that have been treated with either radiation or antithymoctyte globulin. The other way of assessing where the cell lines are tumorigenic or not is if you have cells, especially rodent cells, that are transformed from cells of an inbred strain that are transformed by an oncogenic agent, you can put those cells back into the animals from the inbred strain and determine whether tumorigenic or not. But these types of assays are generally not used for regulatory purposes.

Now, the role of cell substrate history has played a very significant role in tumorigenicity testing for regulatory purposes. The concerns about

neoplastic cells as vaccine substrates were first voiced in 1954 by the Armed Forces Epidemiology Board with the recommendation that only normal cells be used. Now, prior to 2000, with the exception of the experimental vaccines that Dr. Krause mentioned, only cells that were shown to be non-tumorigenic were used in the manufacture of viral vaccines.

Although, there has been considerable controversy as to what the Epidemiology Board actually meant by normal cells, the affect of this recommendation was that neoplastic cells were excluded as substrate for vaccine manufacture for decades and neoplastic cells that were tumorigenic were, for the most part, excluded until 2000, 2001.

Now, the tumorigenicity assays that were recommended by OVRR CBER prior to 2000 were single-dose assays. And these assays were designed to rule out the capacity of cells to form tumors. The assay basically consists of inoculating the animal, generally a nude mouse, with 10 million cells. The types of animals that were used were either nude mice, 10 animals, or newborn rats, newborn mice or newborn

hamsters that had been immunosuppressed with antithymoctyte globulin or possibly mice that were thymectomized and radiated and reconstituted with bone marrow from healthy mice.

The observation period of these assays ran for three weeks for half the animals and 12 weeks for the other half, unless some of the animals got significant tumors and they were sacrificed beforehand. At the end of the observation periods, the animals were sacrificed and necropsied and histopathology of the injection site, the tumors, lymph nodes and organs were taken to look for tumor growth or evidence from metastases.

The endpoints of these assays was tumor incidence. That is the number of animals tumors over the number of animals that actually survived. Now, these types of single-dose assays have some limitations. First, they are appropriate for documenting the lack of tumor form and capacity, but they provide only a single data point. These types of assays become less useful when you're looking at cells that possess a capacity to form tumors.

And single-dose short-term assays, especially assays that only run a few weeks, can give data that is unreliable on the ability of some neoplastic cells to form tumors. An example of that is presented in this slide. If you look at SV40 transform biopsied mouse embryo cells, two different lines, now, these lines are independent derived from different transformation events. They are cloned.

In two out of two experiments, after a five week observation period, none of these cell lines, neither of these cell lines produced tumors in animals. So they were being determined as non-oncogenic. After 10 weeks, however, this cell line produced tumors in 100 percent of the animals, while this cell line produced tumors in none of the animals. So this cell line then could be considered highly oncogenic and this cell line non-oncogenic.

After 15 weeks, however, the second cell line now has produced tumors in 50 percent of the animals, so it might be considered tumorigenic or perhaps weakly tumorigenic. By 20 weeks and 25 weeks, however, these data are indistinguishable, so these

lines are -- the tumor forming capacity to these is equivalent.

Now, about five years ago to better address issues presented by highly tumorigenic neoplastic cell substrates, we believe that our recommendations for tumorigenic testing needed to be revised. The reasons of these revisions are listed in this slide. First, induction of highly tumorigenic cell substrates in the manufacture of viral vaccines sets new precedents.

Second, the presence of unknown agents are factors in highly tumorigenic cell substrates represents the greatest risk. Third, the detection of unknown agents are factors that could transfer oncogenic activity can be enhanced by expanding the tumorigenic testing methods and evaluating the data available from such assays.

And finally, I think most would agree that almost every technique practical needs to be used to eliminate or to assess a risk of transferring infectious or oncogenic agents by vaccines.

Now, our new recommendation for

tumorigenic testing and its potential impact on cell substrate characterization in vaccine safety are presented in the next series of slides. In this slide, I'm going to show how expanded tumorigenic testing can enhance the regulatory management of neoplastic cell substrates.

First, the tumorigenic theme type of the cell substrate can be defined by evaluating the kinetics or actually the dynamics of tumor formation at doses of 10 million, 100,000, 1,000 and 10 cells per adult nude mouse. By determining the tumor forming capacity, we can establish some idea of the level of tumorigenicity clinically or the level of aggressiveness that is expressed by the tumorigenic phenotype.

Unrecognized oncogenic agents can be detected by identifying the species of the cells that grow into tumors across a range of tumor forming doses and evaluating any spontaneous tumors that appear for evidence of DNA from the cell substrate. This gets back to our definition of the difference between tumorigenicity and oncogenicity. And finally,

unrecognized oncogenic agents can also be detected by looking for aberrations in the kinetics by which tumors are formed by the cell substrate.

Now, determining the dose response characterizes neoplastic cell tumorigenicity is the key to identifying cell substrates that are highly tumorigenic. And the key to developing dose response data as it changes over the course of the tumorigenicity assay is by expressing the tumor incidences that develop as tumor producing doses or TPD_{50} values.

TPD_{50} value represents tumor producing doses at a 50 percent endpoint. This provides useful data on the number of cells that are required for tumor development. The fewer cells required, the more aggressive the phenotype. It provides information on tumor latency. The more rapidly the tumors appear, the more aggressive the phenotype. And then if we look at histopathology, those tumors that metastasize also would indicate, would imply that they are more clinically aggressive and it contributes to our understanding of those phenotypes.

Since the TPD_{50} values are not generally used to report data on tumorigenicity assays, I felt like a little more explanation might be useful. And as I have said, the tumor producing TPD_{50} equals tumor producing dose at the 50 percent endpoint. This is the number of cells that are actually required for tumor formation in half the animals. These TPD_{50} values all were changed as the tumor incidence changes during the observation period until they reach the limit of the capacity to cells that form tumors. And these values are best determined by the Spearman-Karber Estimator of 50 percent endpoints.

Now, the type of data that can be generated by these dose response assays are presented in this table of tumor formation by HeLa cells. If you look at the first column here, this is the time, the observation period from one week to 12 weeks and animals are injected with either a million, I mean, 10 million, 1 million, 100,000 down to 100 cells. And if you look at the first week, after one week in animals, these are nude mice now, inoculated with HeLa cells, 100 percent of the animals have tumors that are

inoculated with 10^7 cells. In 10^6 cells, none of the animals have tumors.

The TPD_{50}, at this point in time, is 6.5. By the second week, however, the situation has changed. 100 percent of the animals have tumors at 10^7 and 10^6 cell doses, but only four of 13 of the animals have tumors at 10^5 cells per animal. The TPD_{50}, at this point, is 5.19.

From the third week through the seventh week, the TPD_{50}, based on the tumor incidence as tumors develop, evolves from 5.19 to 4.75. And by seven weeks, the tumor forming capacity of this cell line is spent and the TPD_{50} value remains flat through the 12 week observation period.

Now, you can take the dose response data from assays like this and you can graph it as shown in this figure. These curves represent the manner, a visual presentation of the manner in which the TPD_{50} evolves over the course of the assay. And I think you can see here it starts at 6.5 at one week. By the second week it is 5.2. And then it flattens out over the remaining course of the assay.

Now, TPD_{50} evolution curves are an intriguing response. My colleague, Dr. David Allen, at the National Institute of Health pointed out that the TPD_{50} evolution curve actually represents a survival curve of average tumor latency. Now, converting these data into survival data allows this type of data to be analyzed statistically as a survival function and this simplifies considerably the method of looking at this type of information.

Now, in this slide, you can see the differences between the dynamics of tumor formation by weakly and highly tumorigenic cell lines. The upper curve represents 293 cells, which are adenovirus transformed human embryonic kidney cells, which have a TPD_{50} of 6.5. It takes those cells roughly three weeks to begin to form tumors and its only about five or six weeks before the curve flattens out at about 3 million cells.

Whereas, if you look at the lower two curves, this curve is HeLa cell data, that I just talked about, this curve is data on BHK-21 cell line, which is a spontaneous cell, it's a hamster kidney

cell that's spontaneously transformed. HeLa cells have the capacity to metastasize. They are cells that are derived from a human papillomavirus Type 18 induced carcinoma in humans and they have been around for many years and they are generally certainly considered by all to be highly aggressive and these cells have the capacity to metastasize. BHK-21 cells also have the capacity to metastasize.

So the difference in the time in which the tumors appear, the weakly tumorigenic cell line was much delayed higher TPD_{50}, these cells come down quite rapidly, lower TPD_{50}s. This allows us to distinguish between these phenotypes.

Now, the bars in this figure show that a range of TPD_{50} values expressed by the tumorigenic cell line from three different species, including humans, mice and hamsters, the value of these cell lines established with these species range from 10^{100} to 10^{6} to 10^{7} across the species. These data, at least to me, imply that the TPD_{50} vales are most likely a fundamental characteristic of the tumorigenic phenotype across species.

Now, in the next series of slides, I'm going to consider an attempt to critique the dose response tumorigenicity assays by examining factors that affect tumor formation that can alter the TPD_{50} values. If we're going to be recommending expanded tumorigenicity assays for the regulatory management of neoplastic cell substrates, it seems reasonable to ask questions about how good they are, what type of information they might miss, what factors might alter the type of date they provide and how these data could be used for the regulatory management of neoplastic cell substrates.

This slide presents data on four different studies that found that of 134 cell lines that were tested, 119 of these cell lines had capacity to form tumors in nude mice at doses of 10 varying from a million to 10 million cells per animal. Interestingly, cells that were established from carcinomas of pancreas and breast, gliomas in humans as well as lymphomas and leukomas, failed in about 25 to 50 percent of the time to form tumors in animals, in adult nude mice. But most of these cell lines

would form tumors in newborn nude mice, implying that there is a difference in the sensitivity between these two systems.

Now, there are a number of factors that have been shown to modify the tumor forming capacity of neoplastic cells growing in tissue culture. And some of these factors are listed on this slide. First, the contamination of the cell substrate with viruses and bacteria. The second is the infection of rodent host that are using the tumorigenicity testing. And finally, as I have alluded to, the level of immunocompetence of the rodent host itself with syngenetic adults being more resistant than syngenetic newborns, syngenetic newborns being somewhat more resistant than adult nude mice and adult nude mice being somewhat more resistant than newborn nude mice.

If we look at the impact of viral contamination of cells, of viral infections of the host on tumorigenicity assays, what we can see is that if you have the BHK-21 and HeLa cell models, which I have just shown you, at these 10^6, 10^7 cells per animal, these cells produce tumors in 100 percent of

uninfected animals. However, if you infect VSV, I mean, if you infect BHK-21 or HeLa cells with vesicular stomatitis virus, this virus produces a chronic infection in these cells. The cells are not lysed and if you didn't know you had infected them, you might not know it was in there, unless you tried to test for it.

But when you do that, it eliminates the capacity of either of these cells to form tumors in mice. Mumps does the same thing for BHK-21 and so does influenza. Whereas, with the HeLa cell, VSV eliminates its single forming capacity, but also Measles infection.

Now, if you look at human melanoma cell line, SH-Me, in normal mice this produces tumors in 100 percent. But if you look at nude mice that are infected with hepatitis virus, the capacity of these cells have very high concentrations to produce tumors is reduced by almost a half. So these type of activities can affect the tumor forming capacity of animals, of cells in animals.

Now, having looked at the possible

problems with dose response tumorigenicity assays have these types of aberrations, may be indicative of having contamination as a problem with the animals. I think next I would like to review what is known about the mechanisms of neoplastic development and how these mechanisms influence our thinking about the safety of neoplastic cell substrates for vaccine manufacture.

In this slide, we are looking at the mechanisms involving neoplastic development in tumor formation experimental animals. These models of neoplastic development were developed over the past 30 or 40 years in three different animal systems. The most extensively studied is the mouse skin model, the rat hepatoma model, the mouse mammary carcinoma model is also one of the systems that has been used. And these models, basically, are developed from treating animals with carcinogens.

Some carcinogens can induce neoplastic activity and others initiate the formation of neoplastic activity, other carcinogens are applied and they promote neoplastic activity. These models are somewhat complex and I'm not going to -- time doesn't

really permit going into the details. But anyway, by applying selective chemicals at various times, the process of neoplastic development can be broken down into three states.

The stage of initiation, which begins as apparently irreversible, represents and is believed to represent a single, possibly single genetic change. Once the tissue is initiated, you come along with a promoting agent and this produced changes in this initiated tissue which include dysplasia, hypoplasia, papilloma formation and possibly the development of carcinoma in situ.

These changes represent additional oncogene activation and tumor suppressant gene deactivation. And as a result of additional changes then, you go from promotion through the process of progression, which represents the final genetic changes that result in tumor formation invasion and metastases. Now, these are in animal models.

In human models in neoplastic development there are, basically, two fundamental systems. The somatic mutation model for the progression of colon

carcinoma that was developed by Vogelstein and his colleagues at Johns Hopkins in which they showed that the progression from adenomas of the colon to invasive carcinomas of the colon were accompanied by four to six genetic events, which they could detect. That this system has also, I think, been applied to several other human tumors, but with -- somewhat less extensively than the work that Vogelstein did.

Now, when you talk about transforming human cells and tissue cultures, it has been notoriously difficult to immortalize human cells and produce cells that, in fact, are tumorigenic in animals. Hahn and Weinstein at MIT changed this perception in the late '90s when they found that if human cells contain the SV40 T antigen, were transfected with -- these cells were non-tumorigenic, if they transfected them with the H-ras oncogene and with the h-TERT telomerase gene, they could then convert these cells into cells with actually formed tumors in nude mice. So this led to the development of the STRE gene model of neoplastic transformation of human cells in vitro.

Now, the mechanisms that are involved in neoplastic development and how they impact the regulatory management of cell substrates is, our thinking, sort of outlined on this slide. First, tumor development is a multi-step process that requires somewhere between three or six independent alterations involving different genetic loci. Every neoplastic mutation, which is independently determined and in a different locus, represents above -- every mutation above 1 decreases the possibility of transferring neoplastic activity by the power of the mutation number.

Tumor development represents that the end stage of neoplastic development that begins with an initiating event. Transfer of viral oncogenes or dominant activated oncogene activity that is capable of inducing neoplastic activity results in tumor formation and can be detected in animal models. The sensitivity of these animal models, however, to detect such oncogenic activity is low. Initiating events can represent single genetic processes. They do not appear to be reversible and they may or may not evolve

along the path of the neoplastic development during the life of an individual. Currently, there is no way to detect substrate components for neoplastic initiation.

Now, based on our evaluation of the safety issues that I presented, OVRR has developed the following recommendations for characterizing the tumor forming capacity of neoplastic cell substrates that are expected to be tumorigenic when injected into animals. First, we are asking or we are recommending that people evaluate and analyze for aberrations and dynamics of tumor formation by determining the tumor incidences of doses of 10^7, 10^5, 10^3 and 10^1 cells in adult nude mice.

The incidence of visible/palpable tumors as recorded at weekly intervals over a four to five month interval, the species of origin of the cells and the tumors across the range of tumor forming doses is determined with particular attention to tumors at the limiting cell dose. At the end of the observation period, all the animals are sacrificed and histopathology is obtained on the tumors, the

injection sites and internal organs. Any spontaneous tumors that develop are examined for evidence of DNA from the cell substrate.

Now, our expanded model of tumorigenicity testing provides information that's useful for regulatory decisions in the following ways: First, the data on tumor formation reveals weakly and highly tumorigenic phenotypes which influences the level of concern over adventitious agent contamination and oncogenic activity and for infectivity activity of the cell substrate DNA.

Data on aberrations in tumor formation, especially at high cell doses, may be indicative of cell substrate contamination with known or unknown agents. Data on the species of the origin of the cells that form the tumors at injection sites or distant sites, possibly to include spontaneous tumors, determine whether oncogenic activity can be transferred from the neoplastic cell substrate to the host. And histopathology on injection sites, tumors and organs establishes and possibly confirms the identity of the cell line and its possible

aggressiveness.

My summary slide seems to be missing, but I think that it has -- I can just briefly go back through what I said. The tumorigenic phenotype can be determined. We can determine whether cells highly tumorigenic or weakly tumorigenic. We can have some idea of whether they contain oncogenic agents that may or may not be detectable. The tumorigenicity testing assays in adult nude mice can detect tumor forming capacity of 9 out of 10 of the cell lines tested. The newborn nude mice offers as an alternative, if we have reason to believe that the adult nude mouse model is inadequate.

Tumor formation represents the in-stage of neoplastic activity at the end stage of the multi-step process of initiation for motion and progression. And with the exception of initiating events, which cannot be evaluated, the multi-step process of neoplastic development makes it highly unlikely that neoplastic activity could be transferred by cell components other than oncogenic viruses. And I think that's the end of my remarks.

(Applause)

CHAIR OVERTURF: Dr. Lewis, I have one small question regarding your slide on the impact of viral contamination on viral infection. You mentioned that both Measles and Mumps viruses decreased the oncogenic potential. The source of those viruses, were they the vaccine viruses that infected those cells or were they others?

DR. LEWIS: No, they were not vaccine viruses. No, sir.

CHAIR OVERTURF: Okay. Other questions? Dr. Krause? Dr. LaRussa, I'm sorry.

MEMBER LaRUSSA: Could you expand a little bit upon the decision to continue with the adult nude mice instead of using the neonatal mice? I guess, aside from the practical aspects of what that would entail.

DR. LEWIS: Yes, I think the use of the adult nude mouse goes back over a number of years. And most folks are quite comfortable in dealing with this model. The use of the newborn nude mouse represents a recent innovation. And, in fact, it is

being used in testing cell lysates and testing cell DNA as Dr. Khan and Dr. Peden will talk about. But it presents some problems.

First, remember that the nude mouse litter is heterogeneous. About half the animals will be nude and about half of them will be haired. And the haired animals have a thymus. And so they will not participate at the level of tumor formation that the other individual ought. So you have to segregate these animals. They have to be weaned and segregated, which represents husbandry problems.

And I think from our perspective, the adult nude mouse represents an adequate level of sensitivity. However, if we had a cell line, and this is just a hypothetical example, in which we were worried about the possibility that we were missing something, we would all -- we could, in fact, recommend that they look at newborn nude mice. And I think we are gaining some experience with the newborn nude mouse model with the lysates and with DNA that will help in making any adjustments that may need to be necessary.

I think the other thing I would point out is that if you have a highly tumorigenic cell, it doesn't make any difference what the host you use. If you have a cell line that has a TPD_{50} of 10^1 or 10^2 and a syngeneic adult animal, the TPD_{50} of that cell line in a newborn mouse, a nude mouse or a newborn nude mouse is going to be 10^1 or 10^2. It doesn't seem to be impacted by the level of the immunocompetence of the host as compared to the weakly tumorigenic cell line, which certainly is affected by the immune system.

CHAIR OVERTURF: Dr. Minor?

DR. MINOR: Two things. You said the HeLa cell DNA was oncogenic on one of your slides. Can you amplify that a little bit and say what genes are actually found in the tumors that were formed. And the second question was to do with the 293 tumorigenicity assay, where it looked as though there was a three week latent period before there were any tumors formed at all. Was that because the tumors were slow growing or was it because there was something changing going on in the cells that were injected?

DR. LEWIS: If I had a slide that said HeLa cell DNA was tumorigenic, something is wrong. I think that -- I do not have a slide. There is no evidence that I'm aware of that says HeLa cell DNA will form tumors in animals. To get at your other question, the latency period of the 293 cell, I can't explain. These are just the characteristics of that type of cell. And whether it is due to an immunological reaction to the host, I can't say. We just don't know.

But certainly, those cell lines seem to be weakly tumorigenic and I will go back to the original to that table I showed on the SV40, Me-1 and Me-2 cells how long it took them to make tumors. Those cell lines are weakly tumorigenic. But the point I would make is if you look, if you plot using the TPD_{50} evolution curves, if you plot cell lines, you can differentiate between latency of the different cell lines.

The area under the curve actually represents the average latency survival and there are significant differences in latency survivals among

various types of cell lines. Now, how those latency survival curves differ in highly tumorigenic cells among highly tumorigenic cells, I don't know. But among weakly tumorigenic cells, there are dramatic differences.

CHAIR OVERTURF: Dr. Farley?

MEMBER FARLEY: I have some questions specifically about the MDCK cells. It was mentioned earlier that there may be variability even within that single cell line and that it certainly has the capacity to be highly tumorigenic. Have you all studied the cell line? And if the testing is done with a particular representative of the cell line and its characteristics are defined as far as tumorigenicity, is that frozen in time in the storage process and in the manufacture process or is that subject to change over time?

DR. LEWIS: We have not studied MDCK cells. I think our corporate sponsors will have a great deal to say about their data on the tumorigenicity of these cell lines. But I think concerning your question about changes over time,

certainly it is widely recognized that as you take -- if you have a normal cell growing in tissue culture, especially a non-human cell line, and you pass it over time, these cells become immortal and they become tumorigenic sequentially.

The longer you pass them, the more likely they will be to be tumorigenic. And I think the vero cell perhaps is the best example of that, which is a cell line that we have had some experience with. Those cell lines are, basically, non-tumorigenic after 140 passages in tissue culture. They are immortal. They will grow ad nauseam if you just keep feeding them. But after 250 or so passages in tissue culture, those cell lines become, frankly, tumorigenic. They will make tumors in mice.

Some of the cell lines will make tumors in mice before that. And it seems to be determined on how you actually pass them. But the cells will change. Now, once you get a culture established and from a regulatory cell substrate perspective, the cell substrate or master cell bank is fixed at one point in time. The cells are then passaged at least 10 to 15

times beyond the endpoint that is used for production and then they are retested. And generally, the numbers, at least the numbers that I'm aware of for the information that I have seen, don't change.

So by the cell banking procedure, you can fix the tissue that is being used, if that's a given point in time, and it does not change. If it did change, I think, we would have to worry about that and so would the folks who made the cell bank.

CHAIR OVERTURF: Dr. Karron?

MEMBER KARRON: So could you quantify weakly tumorigenic and highly tumorigenic in terms of --

DR. LEWIS: I'm sorry, Ruth, I can't hear you.

MEMBER KARRON: Could you quantify weakly tumorigenic and highly tumorigenic in terms of numbers of cells that would produce a tumor of our long assay period?

DR. LEWIS: I would say, based on the experience I have had, any cell line that has a TPD_{50} of 10^6 or greater would probably be considered to be

weakly tumorigenic. And under that, it is hard to know. There is a borderline there between 100,000 and a million, a million and a half cells where there is not a lot of data.

MEMBER KARRON: I mean, I guess my follow-up question is really should we be -- we will, obviously, hear data about MDCK cells with various levels of tumorigenicity. And my question is really are those differences important? Is 10^1 different from 10^4 or 10^5? Should we be considering those differently?

DR. LEWIS: Well, certainly we did, yes. I mean, I certainly -- we would take into consideration the level of tumorigenicity of a cell substrate. In other words, how few, how many cells it took to form tumors, yes.

CHAIR OVERTURF: Dr. Self?

MEMBER SELF: Yes, to follow-up on that, I can see how your latency curves, you know, provide some mechanism for kind of ordering things out and seeing how long you need to follow, but it does seem to me that it kind of misses the point. The point to

me is about dose not about latency. And you have summarized the dose relationship by this TPD_{50} value, but that must be based on some sort of dose response curve.

And so I wonder if you could elaborate a little bit on what sort of dose response curve assumptions or models you are thinking of and whether you have used that to try and estimate the probability of tumor formation for the number of cells that would be roughly comparable to a vaccine, what would be exposed in a vaccine dose. I mean, that's ultimately the tie, the dots that we are trying to connect. So I wonder if you could talk a little more about the dose response relationship in your assay.

DR. LEWIS: Well, I'm not quite sure exactly what your point is. The dose -- obviously, there is a relationship between the number of cells that you inject in the animal and whether he has a capacity to form a tumor or not, and there is also a relationship between the number of cells you inject in the animal and the time that the tumor appears. The curves are obvious on that point.

I think other than using -- what we were basically trying to do with these numbers is to convert the incidence into a mathematic, a numerical value that we could use to examine the dynamics that evolves over the course of this very complex process.

Now, the one thing when we're doing these things we discovered is that if you -- animals inoculated with the same cell line at 10^5, you may get one or two animals that don't form tumors at 10^5. When you do the same thing at 10^7, you will also get an animal or two that doesn't form a tumor. Whereas, if you put 10^8 in, 100 percent of the animals form tumors.

So you have got this huge range of values that require some means of averaging them down and coming up with a 50 percent endpoint estimate, provided us with a way of looking at and meaning those values over a course of different assays, more than one, provided the opportunity to get all these things represented in one way. And I don't know how to explain it any more than that.

What determines a TPD_{50} value, we don't know. A number of things can influence it, but what--

actually, the molecular mechanisms involved in establishing the number of cells that are required to form a tumor is unknown.

MEMBER SELF: So I guess my point is that I'm not interested in how the tumors unfold over time. I would really be interested in very long-term follow-up, sort of the longest term follow-up, what is the probability of tumor formation as a function of dose, and I would be interested in the whole curve, what the probability of tumor formation is at fairly low doses, doses that are reflective of the dose that would be achieved after all of the purification process in the vaccine.

And so I'm interested in that low dose end of that curve and that is what I'm not getting by having you summarize that entire dose curve by a single TPD_{50} value.

DR. LEWIS: What I can say is this, that although a lot of these assays were stopped at what looks like 12 weeks, they didn't stop there. That was the end of the time in which the data basically achieved a plateau and it was two or three months

beyond the end of the last tumor.

A lot of these assays ran for a year and especially the tumors. You can see assays that we have done in vero cells and nude mice. We looked at these animals for a year and nothing changes after four or five months, and I think that is why we have been comfortable with that.

Now, in terms of extremely low doses, we have not tried to go below zero which is basically one cell, 10^{0}. We have not tried carrying it down to 10^{-1}, 10^{-2}. We have not tried that.

MEMBER SELF: Well, I wouldn't expect that you would, but there is an extrapolation problem --

DR. LEWIS: Yes.

MEMBER SELF: -- that is relevant there. The other thing that I notice is that nowhere are sort of statistical uncertainties represented in your graphs, and I don't have a sense from the design of your --

DR. LEWIS: Yes.

MEMBER SELF: -- studies what the precision of those are, but in translating those into

some threshold of risk, I would hope that you would incorporate that aspect as well.

DR. LEWIS: Yes. That is a fair question and the standard deviations on those numbers were left off just for the simplicity of presenting the data. In the assays that we have standard deviations, the standard deviations are mostly based on work we did on an adeno-12 transformed balancing mouse embryo cell.

We had 10 replicates of these assays over about a three year or five year period of time and the standard deviation of those values was plus or minus $10^{0.4}$ and I think that's about as good as we can do. I mean, doing one of these assays takes, as I said, at least three months and to do 10 of them, that's a lot of time and I think that probably represents as good a mean and average that we could probably get.

CHAIR OVERTURF: In the interest of saving time, I will have Dr. Markovitz ask the last question.

MEMBER MARKOVITZ: Yes. I would like to follow-up a little bit on what Dr. Self was asking and expand the question also to a more broad sort of policy-based issue. So I understand that there has

been historical concern about oncogenesis and tumorigenicity when you're using transformed cell lines, and I can appreciate that these data are interesting from a cancer point of view.

But what I'm not getting is why this is going to be relevant in the picture of, you know, real vaccine development, because with the vaccines that we're going to be dealing with, we're talking about highly purified proteins that have also undergone all sorts of, you know, DNA treatments and things like that.

So the issue really would seem to be when you have such a vaccine, do you actually have any cells left and if you have cells, I think what Dr. Self was saying, at the very low end do you actually have any concerns?

So I would like to know, because ultimately we're going to be charged to give opinions on something, you know, on things that have very large public health risks, i.e., influenza vaccine development. So I would like to understand how this is really going to impact on the real decision.

DR. LEWIS: I'm having a little bit of -- I had a little bit of trouble hearing your question because of the air conditioner, but I think I will try to answer.

I think the thing that this assay allows us to do is two things, well, three things. First, it allows us to determine where to place our greatest level of concern. If we have a cell line -- and this gets into the business of weakly tumorigenic versus highly tumorigenic.

If we have a cell line that is transformed by a known oncogene that requires a million, a million and a half cells, to produce tumors and all the testing is done carefully on that cell line, we feel like that represents less risk than compared to a cell line that would take many fewer cells to form a tumor. So I think that would be the first thing.

And from a regulatory perspective, we would be much more concerned about adventitious agent testing by looking at the level of residual DNA, by looking at different components about whether that cell line was going to be used in an activated or a

live virus vaccine, for example, with a cell line that requires very few cells compared to a cell line that requires a large number of cells. So that's the first thing.

The second thing is a little more subtle. The biggest problem with looking at neoplastic cell substrates, especially highly tumorigenic neoplastic cell substrates, are the possible presence of things that you don't know about. The reason why SV40 was a major problem in the polio vaccine was there was no way of identifying that virus in the cell substrate.

Now, as it turns out there was a way. Dr. Bernice Eddy in the FDA did a simple thing. She took the supernate and fluids off of that culture, inoculated it in hamsters and got some tumors. What that data represented turned out to be a very profound piece of information that was not acted on at the time.

We wouldn't like to miss something like that again. So the kind of information you can get out of this type of assay is, first, if you look at -- it goes back to that definition of tumorigenicity

versus oncogenicity.

If you found within the cells that you were inoculating, in this case dog cells, if you found mouse cells or a very high concentration of mouse cells in a tumor that was supposed to be induced by a dog cell line, you know, everybody's hair would stand on end. We would be very concerned about that and I think the sponsor would be very concerned about that and there would be a lot of worry as to what was going on in that cell line.

Now, that would also be true with the HeLa cell, for example, and some people are interested in using HeLa cells. In fact, a company has published a paper on using HeLa cells for adeno-associated virus vaccines in vaccines in this year.

And if we had a HeLa cell, for example, and that induced a tumor in the nude mouse and we looked at the DNA from that tumor and, in fact, found that there were, for example, papillomavirus Type 18 or some other type of human agent or some other type of DNA in that cell that appeared to come from the HeLa cell, we would have a major problem with that.

So I think that would be a second example of how this type of data would be important. Now, if you didn't do the titration, you might not be concerned about the level or looking at those substrates or those tumors for oncogenic activity.

The third example would be if you had a cell line that produced a tumor or produced tumors at 10^2, 10^3 cells per animal, but you have got up to 100 cells or 1,000 doses of that cell line and you have got tumors in only half the animals, then you have to worry that there is something in that cell line or something in that assay that has caused a problem. You have an aberration and then that would make us focus more carefully on that particular cell line.

So I think there are at least three ways in which these types of assays can provide information that we couldn't get otherwise. The biggest problem you have in looking for unknown things is how do you research when you do have an endpoint. You have nothing to look for, so you have to try to use the information that you can generate as an indirect indication that something not proper is going on.

MEMBER MARKOVITZ: But isn't in the end what's important what's actually in the vaccine, you know, in other words, after it has gone through all its multiple purification and perhaps inactivation and DNA steps and things like that? I mean, how do you tease apart the difference between what you see in these studies versus what you will actually see in a vaccine? That is what I'm not understanding yet.

DR. LEWIS: Well, I think folks who are interested, I mean, the vaccine will be evaluated in terms of the overall characteristic of the cell substrate. You have got the vaccine seed that goes into manufacturing the product and then the product will then eventually be tested.

But I think one of the basic perceptions that we have is if a substrate is clean and the seed is clean, unless there is some interaction between the seed and the substrate that is not recognizable, the product should be reasonably safe.

Now, the level of concern that we have about the substrate and possibly the level of concern you would have about the seed would then determine the

level of concern and probably the amount of testing that would go into the final product, that testing would be requested to go into the final product to make sure that it is as safe as it can possibly be.

But I certainly think if there is enhanced concern about the substrate and especially if the seed itself is made in that substrate, then there is going to be enhanced concern about the product and that is going to be reflected in both what we recommend of the sponsor and I'm sure it's going to be reflected in the sponsor's concern that we're testing their product to be sure it's safe.

Now, once you get into the business of inactivation, I think the manufacturers today are going to go into great detail to provide you with information about the type of inactivation procedures they use and the care in which they have gone into assessing the effect of these inactivating procedures to eliminate any possible adventitious agent or any possible activity.

And I think perhaps the answer to your question will come out as the session evolves if I

haven't addressed it adequately.

MEMBER MARKOVITZ: So, essentially, if you have something that is highly -- you know, it causes tumors in these assays, then that raises the bar is what you're saying?

DR. LEWIS: Absolutely.

CHAIR OVERTURF: We'll adjourn the meeting for a break for a short period of time and reconvene at 10:45.

(Whereupon, at 10:32 a.m. a recess until 10:52 a.m.)

CHAIR OVERTURF: We are ready to begin the second half of this morning's session. Please, take your seats. The second half will begin with a presentation by Dr. Khan on adventitious agents testing of novel cell substrates for vaccine manufacture. Dr. Khan?

MS. WALSH: Just a note to the Committee Members before we start. Dr. Khan updated her presentation so she was kind enough to provide updated copies of the slides. So the correct slide in your packet for her handout is the one without the

handwriting on the right hand side, upper right hand corner. That is the correct one.

DR. KHAN: Okay?

CHAIR OVERTURF: Okay.

DR. KHAN: All right. Thank you. I will continue the presentations with a discussion of the adventitious agent testing of novel cell substrates for vaccine manufacture. Oops. Why can't I move this? Sorry.

I will initially describe the various cell substrates that have thus far been used in U.S.-licensed viral vaccines and then I will present some of the safety concerns and challenges for testing novel cell substrates, especially tumorigenic cells, and also mention the FDA experience with tumorigenic cell substrates. And, finally, I will present OVRR's testing recommendations for novel and tumorigenic cell substrates such as MDCK cells that we are here to discuss today.

As you have heard earlier from Dr. Krause, thus far the current U.S.-licensed viral vaccines have been manufactured in primary cells or tissues, in

diploid cells and in a continuous cell line which is non-tumorigenic.

In this slide I have just indicated the various viral vaccines and the cell substrates that have been used for primary cell, vaccines prepared in primary cells. As you can see, there is a number of live viral vaccines and some inactivated vaccines that have been produced in the different cell substrates that are indicated here.

With the introduction of diploid cells for vaccines, the next generation of vaccines were manufactured in diploid cells, either in FRhL cells from fetal rhesus lung or from the two well-known human fetal lung diploid cells, WI-38 and MRC-5. And it should be noted that all of the live viral vaccines to date have been produced in either the primary cells or tissues or in diploid cells.

One continuous cell line has been used for the manufacture of a U.S.-licensed viral vaccine, the vero cells as you have heard, and in the U.S. it has been used for inactivated poliovirus, whereas in Europe it has also been used for live viral vaccine.

And it should also be mentioned that the use of the vero cells is restricted so far to low passage, because these cells become tumorigenic upon high passage. Okay.

The transition to novel cell substrates continues with the need to develop new vaccines. Additionally, guidance documents also evolve and get updated to assure that there is relevant testing being performed to maintain product safety. And today we will be discussing the use of the novel cell line, MDCK cells.

And, as in the past, we are here to have rigorous discussions on the use of this novel cell substrate in order to identify any potential safety concerns and address them to assure product safety. And in the case of MDCK cells, we have the additional responsibility to address any potential tumorigenicity concerns.

In order to assure the production of a safe product using a novel cell substrate, we need to develop a comprehensive testing regime, regimen, and the following factors are taking into consideration,

such as the health of the tissue donor, the viruses that can naturally occur in the donor species or that might be in the donor species due to any external exposure.

In addition, the cell growth properties of the particular cell substrate needs to be considered since it can increase susceptibility for virus infection and replication, as well as provide a broader host range to different viruses. And, very importantly, the passage history of the cells need to be considered in developing relevant testing for the cells, such as propagation in different labs, the biological reagents that may have been used through the passage history of the cells, including sera, trypsin and others.

Also, any other cell line that could potentially have been grown at the same time during the passage history of the cells or any other viruses that may have been grown, as well as the facilities or the lab conditions that the cells may have been passaged through.

And I should mention, as many of you may

know, that each of these points have relevance because there are examples when there have been contaminations related to any of these points here. And, of course, the cell phenotype is very important, as you have heard earlier, with regard to whether the cells are non-tumorigenic or tumorigenic and in terms of tumorigenic cells, you have additional concerns related to the oncogenic virus testing as well as DNA testing that you will hear later from Dr. Peden. Okay.

I just want to mention briefly that the FDA does have experience with tumorigenic cells. It started as early as the mid-1970s with the Namalwa cells being used for interferon and there are additional rodent cell lines that have been used as well as the 293 cells that have been used for therapeutic products.

It should be mentioned that all of these are known to contain viral sequences or actively produced viruses. However, it's noted that all of these products are highly purified and there are steps that address clearance and removal of all the

potential agents of concern. For inactivated vaccines, CHO cells have been used for investigational protein vaccines, which also are in the category of highly purified products. Okay.

Now, the use of such cells has been regulated as follows. The advantage of using the cell line outweighs the tumorigenicity concerns in certain situations, especially for therapeutics. There is an extensive testing regimen for testing different stages of production, the cell banks, the raw materials, the lots and I will address that later in my talk also.

Also, with the specific concerns have been the development of specific assays to address the concerns. For example, in the case of MVMV, a specific assay, infectivity assay, was developed that was highly sensitive for detection of this contaminant especially in rodent cell substrates that require large scale production, and the PERT assay was developed for retrovirus detection. This actually initially was developed for specific concerns in some chicken cell produced vaccines.

And, very importantly, when there are

concerns related to product safety, the incorporation of viral validation studies have been very important to evaluate the effectiveness of the manufacturing process in clearing virus that may potentially be present in the Master Cell Bank. Okay.

Now, my talk will focus specifically on adventitious virus testing of MDCK cells. Okay. I think it's blocked. I can't move it. Oh, okay. As I have mentioned, that for any novel cell substrate you need to develop a comprehensive testing regimen for detection of known and unknown adventitious viruses that should be designed to minimize the risk of virus contamination in the vaccines and, thereby, assuring product safety.

And this can be achieved by following these general approaches for viral safety, which include qualification of the cell banks, virus seed and biological raw materials, and I will provide further details in the next few slides about this, in-process testing to evaluate the bulk or the production lots for known and novel viruses, and a process validation which is designed to determine the

effectiveness of avoiding the risk of contamination or elimination to remove potential viruses or inactive potentially contaminating viruses. Okay.

Now, we already have a lot of testing guidelines and guidances in place that have been used successfully for generating and use of safe vaccines. So, of course, these must also be incorporated in the testing scheme and these include general testing, which is in vitro cell culture tests which involves the inoculation of cells from the same species, human diploid cells and monkey kidney cells.

It includes in vivo assays such as adult mice, suckling mice, embryonated hens' eggs, in some cases guinea pigs or rabbits. It includes evaluation of the cell substrate by transmission electron microscopy and testing for retroviruses by the PERT assay.

Now, these assays and tests are designed to detect a broad range of viruses. These are general assays that can help to evaluate the presence of a wide variety of different families of viruses. In addition, there are species-specific tests that must

be incorporated into the testing scheme and this is based upon the -- it may be product specific and in cases where you have animal reagents, derived reagents used in your production, such as serum and trypsin, then you need to evaluate for animal viruses according to the 9 CFR.

In cases of exposure to rodent, any cells or viruses, then you need to do testing specifically for mouse or rat or hamster viruses by antibody production assays. And also, for any known viruses, you need to use a variety of different sensitive assays, such as PCR infectivity assays or even Western Blot or ELISA or IFA, whichever can help evaluate the presence of any viruses in the most sensitive manner.

Now, in terms of the MDCK cells, this is a dog cell line, you can use specific assays for evaluating any naturally occurring viruses of concern which are listed here, the different families of viruses that can infect dogs. As noted, there are two families of oncogenic viruses, papillomavirus and some retroviruses here.

Additionally, you want to develop assays

or you want to use assays for any viruses that could potentially be present in the cell due to cell susceptibility and a list of different viruses are indicated here, and some of these are persistent viruses and can infect the cell without any indication of infection. So you really need to rigorously look for these viruses of concern.

Now, because the MDCK cells are a novel cell line and a tumorigenic cell line, we recommend additional assays that can broadly detect other viruses of potential concern, and these include endogenous retroviruses and latent DNA viruses and oncogenic viruses. And I will be discussing in more detail the various assays that may be used for detection of such viruses.

And additionally, because of the concern of the tumorigenicity of the cells that could be possibly an unknown agent, then you also want to do viral clearance studies for potential unknown agents using model viruses. And in this case that can include viruses that are resistant to the inactivated agent as well as oncogenic viruses, again to address

any potential concerns of any possible agents that might be there. Okay.

I will next describe some strategies for virus induction. This strategy is classical. It has been known historically that various chemical inducers can activate endogenous or latent viruses, and I have listed some inducers here, IUdR, AzaC, sodium butyrate and TPA. The first two inducers are known to activate endogenous retroviruses. The second two can activate latent DNA viruses. And the strategy here is to use inducers with different mechanisms of action to broadly activate any potential viruses that could be present in the cell.

I should also mention that, of course, the detection of the viruses resides heavily on the use of broadly detecting, as well as highly sensitive and detection assays after the induction, such as TEM, PERT for retroviruses, generic PCR assays for DNA viruses and infectivity coculture for either. And it should also be mentioned that the use of chemical inducers, especially IUd, has led historically to the discovery of many novel retroviruses from different

species. Okay.

As I have mentioned, the IUd and the AzaC are known inducers of endogenous retroviruses from a variety of different species including mammalian and avian species. And I just also want to note here that this strategy has also been useful to demonstrate the activation of viruses from tumorous cells even in the absence of activation of viruses from normal cells from the same species. And TPA and sodium butyrate are known inducers for a variety of latent DNA viruses such as herpesvirus, as well as some retroviruses like HIV. Okay.

I'm just going to present two results from ongoing work in my laboratory related to development, establishment and optimization of induction assays using different cell lines. These are results from a mouse cell line, K-BALB, which shows that treatment of the mouse cells with a combination of IUd and AzaC is successful in the production of endogenous mouse retroviruses, Type C retroviruses shown here. And then the activation or the production of these viruses was detected using a highly sensitive PERT assay and

this is showing supernatant tested daily and the peak activity here indicates the peak of virus production.

In terms of DNA virus we have used TPA to show activation of herpesvirus-8 from a human B cell line. And, again, this was used to establish the conditions in the lab and it's expected that this inducer can activate this virus from this particular cell line. And then we have used PCR for detection of the HHV-8 sequences. In this case it's showing that we get high activation after 72 hours of treatment and there is less at 24 hours. Whereas, without the TPA treatment, you have very low detection.

Next, I wanted to describe some of the cell lysate testing in vivo assays that we are recommending and this is for detection of oncogenic viruses. We are recommending inoculation of cell lysates and DNA, which you will hear from Dr. Peden in the next talk, from cells equivalent to 10^7 into less than 4 day-old animals, and here we have recommended newborn hamster, newborn nude mice and newborn rats and the assay is up to five months.

And this is based upon demonstration

historically that cell lysates or extracts from tissues can lead to the discovery or detection of viruses in the extract. The first avian retrovirus was discovered by Rous using filtered extract injecting into chickens.

Subsequently, many murine leukemia viruses have been discovered using extracts from mouse tissue, mouse tumor tissues, and also polyomavirus was discovered by Gross using similar tissues. And also in terms of cell culture fluids, you have heard Dr. Lewis mention that this was useful in demonstrating the presence of SV40 from primary rhesus monkey kidney cells.

Now, the use of the three species is supported by the results that are shown in this table which are a collection from published literature. And this shows that you can have situations with the same virus family in which you can -- that you need all the three species to enable the detection of the different virus types that might be present. Okay.

Next, I wanted to mention or discuss virus clearance studies in a little bit of detail because,

in general, in vaccines, viral clearance studies are not used because in most cases we're dealing with live viral vaccines and up to now we have been dealing with, you know, non-tumorigenic cells and mostly primary or diploid cells, as you have heard.

So when there is a specific concern, then you want to incorporate additional steps that will demonstrate that the potential agents of concern have been eliminated and this is where viral clearance studies come into play and this has been used in therapeutics, you know, regularly.

And the influence of viral clearance studies in vaccine manufacture is to evaluate the manufacturing processes for their ability to clear viruses that are known to be present in the cell substrate and, in this particular case, it is to estimate the robustness of the process for clearance of potential unknown viruses by using model viruses and these studies assist in the quantification of the risk, but they do not by themselves prove the absence of the risk. And details of performing viral clearance studies are in the 1998 ICH document, Q5A.

Okay.

I'm just going to discuss just some of the points that are critical for the viral clearance studies and the details can be found in the guidance document. The selection of the model virus, of course, is very critical. When you have a known virus or you know what to expect, you can use a specific model virus or a relevant virus.

However, in the case when you are dealing with the unknown, then you have to use nonspecific model viruses that can best represent the properties of the unknown viruses that you are concerned about in terms of the physical properties, the biological properties, as well as you want to include viruses that have a significant resistance to the inactivating agent, because you want to demonstrate that you have addressed any possible concerns related to the potential viruses. Okay.

Now, again, when you have expected or known viruses, then the number of viral particles in the starting material can be estimated and a specific clearance value may be used to calculate a specific

safety risk and this is what is routinely done especially in terms of rodent cells that produce noninfectious virus particles.

A 6 $\log_{10}$ reduction of virus above the starting value is generally recommended. However, in the case of unknown potential contaminants, the goal should be to provide sufficient virus clearance that can assure that the product is free of virus contamination.

Now, I just wanted to mention some of the limitations of the study that needs to be considered in evaluating the results, and this is that accurate determination of the virus reduction factors requires use of orthogonal clearance steps. It requires use of a relevant model virus and reduction values which are greater than 1 $\log_{10}$ for each individual step, because the total reduction factor actually is the sum of the individual steps.

And reduction factors are normally expressed on a logarithmic scale which implies that residual virus infectivity will never be reduced to zero, which means that the absolute absence of a virus

can never be statistically proven. However, the risk can be greatly reduced.

And it should also be noted that the behavior of the tissue culture grown model viruses used in the virus clearance studies may be different from that of the native virus that might be present in the cell substrate and, in the case of unknown viruses, the model viruses are selected just based upon the best representation in terms of the various properties that I just mentioned. Okay.

With that, I would like to conclude with OVRR's recommendation for adventitious virus testing of novel cell substrates and tumorigenic cell substrates, specifically MDCK cells for inactivated flu vaccine that is being discussed today.

This includes extensive testing of the cell bank for species-specific viruses or other viruses based upon susceptibility of the cells, for rodent viruses due to extensive and unknown passage history of the cells in different laboratories, for bovine, equine and porcine viruses based upon the raw materials used in the history of propagation due to

the serum and the trypsin, and also to test for unknown potential viruses of concern like DNA viruses and retroviruses by using in vitro induction assays and to evaluate for the presence of potential oncogenic agents due to the tumorigenicity of the cells by using the in vivo cell lysate assays with the three species.

And, additionally, the testing of the virus seed and all biological raw materials for the presence of any potential viruses need to be done and the viral clearance studies need to be done to demonstrate the evaluation of inactivation using different viruses, to evaluate virus removal during the manufacturing process and to estimate virus reduction using appropriate model viruses and spiking studies.

And with that, I will leave you with the multi-step testing scheme that is, I guess, recommended for assuring safety of products.

(Applause)

CHAIR OVERTURF: Any questions for Dr. Khan? Yes?

MEMBER LaRUSSA: Could you say something about what you think the relevance of the in vitro induction assays are to what we know about the in vivo mechanisms of reactivation of the viruses you're looking for?

DR. KHAN: Using what we know about the in vivo mechanisms for reactivation?

MEMBER LaRUSSA: Well, you're using chemical inducers.

DR. KHAN: Right.

MEMBER LaRUSSA: To do an in vitro induction to find these viruses.

DR. KHAN: Right.

MEMBER LaRUSSA: How relevant is that to--

DR. KHAN: Oh, okay.

MEMBER LaRUSSA: -- what we know about the in vivo, how the viruses naturally reactivate?

DR. KHAN: Okay. In vivo viruses. And I guess the best example I can discuss is the mouse system, because that has been very well worked out. Rodents are known to contain endogenous viruses, so in vivo it's known that viruses, endogenous murine

retroviruses, can be activated with age. So you're talking about maybe two years.

So the chemical induction in vitro shortens that process and in vivo, there may be, I guess, different factors that might induce it and, in certain cases, you know, you -- those factors are not under control. So in vitro, if there is an endogenous virus that can come out, you are creating a situation that you are enhancing the production of that virus. So you are testing the cell substrate early on to see whether any virus can be activated.

And I guess, again, this is to characterize the cell substrate. It's to know what are we starting with and, therefore, what should we test for during production?

MEMBER LaRUSSA: So can you just give an example of what the sensitivity might be if you compared in vitro induction to just letting the mice live out their lives? What percentage?

DR. KHAN: Well, I think the most relevant example I can give is with the mouse cells and in vivo in mice. Like I said, in vivo there are only certain

strains of mice in which you can get virus easily out, you know, with age and in some cases there are viruses that exist but cannot be detected, because they will not replicate in the mouse. These are not ecotropic viruses.

Whereas, in vitro you can activate both of these type of viruses in a very short assay. This is a 24 hour culture and then you do it for five days, you get the peak. So you can detect both the ecotropic viruses and the xenotropic viruses as well as any defective viruses in vitro.

Whereas, in vivo, first of all, you have a very long period of time before a virus will spontaneously come out and also, you will only pick up the virus that is replicating in the mouse, which is one of the different classes of endogenous murine retroviruses.

Now, in the case of a tumor, of a spontaneous tumor, you know, then of course you can detect the virus in the tumor. But in mice tumors spontaneously occur also only in certain strains of mice between maybe 6 months to 12 months of age also.

So it's the early detection in the in vitro system that gives you an indication of what to look out for.

CHAIR OVERTURF: Dr. Farley?

MEMBER FARLEY: You mentioned that we have used some tumorigenic viruses in the past or cell lines, sorry, in the past for production of some therapeutic products and in inactivated protein vaccine, but you pointed out that they were highly purified products.

How would you compare the level of purification that goes into those products to the inactivated influenza vaccine process?

DR. KHAN: Well, you have to remember that when your product is a protein, you can achieve high levels of purification using very potent reagents. You can do low pH. You can do, you know, very strong detergents. So there the level of purity I think, of course, may not be achievable for vaccines in general.

Now, having said that, in the case of the -- so I guess I just want to add to that. In the case of vaccines, in general, you have to maintain the integrity of your vaccine, you know, which in this

case is an enveloped vaccine. You have to maintain the immunogenicity, you know, and the antigenicity of the envelope to actually make a successful vaccine.

So I think in terms of vaccines in general, you will hear the sponsors, you know, discuss about their product and what they have done in terms of achieving, you know, a level of purification of the product and I think then, you know, you can sort of evaluate it, you know, based on the data.

But clearly in this case, you know, there is inactivation. There are other additional steps, you know, that have been incorporated, I guess, you know, to achieve the balance between purity and, you know, reactivity of the vaccine virus.

CHAIR OVERTURF: Yes, Dr. Cook?

DR. COOK: It seems like you could better leverage your use of animals instead of restricting it to the use of newborn animals in which you inoculate your lysates or your induced cells perhaps where you could expect either a fatal outcome or maybe, if you waited long enough, some kind of a tumor to form.

It seems like if you used immunocompetent

animals to inoculate these lysates and if you're looking for unknown agents, you could ask those animals to respond in a way to that unknown agent that you could detect, whether it's an antibody production if you happen to have an antigen or whether it's a cytokine response or something to give you an indication that that lysate contains something that is being reacted to because, again, you're looking for something that you don't know what it is and your in vitro molecular assays are obviously constrained by the probes that you have.

DR. KHAN: That's a good idea. Thank you.

CHAIR OVERTURF: Any further questions? Thank you, Dr. Khan. We'll proceed to the last presentation of the morning, which is by Keith Peden on the issues associated with residual cell substrate DNA.

DR. PEDEN: Thank you. My name is Keith Peden and I'm going to address what you have all been waiting to hear from some of your questions. Why we can't take a cell substrate off the shelf, due to two things. What you have heard before is, first of all,

the adventitious agent question and the second question is DNA. And my charge is to discuss why anybody would be concerned with DNA.

So today I'm going to discuss some of the history of cell substrate DNA and biological products, just mention some methods used to quantify DNA since there is still, in fact, some controversial thoughts about it, which method to use, perceived safety issues associated with DNA, so this will give an outline of what issues we are concerned about, review the assays in published data on the biological activity of DNA, go on to discuss some of our work on the development of quantitative assays to assess risk and, from those experiments, extrapolate from data to assist in the regulatory process and give an example of how such data can be used to assess safety and, finally, a summary and what we recommend now.

As Dr. Lewis and Dr. Krause talked about, 1954 was a banner year for cell substrates when this group of people discussed what cells should be used and normal cells should only be used. The ramifications of that we're still suffering from.

In 1986 the WHO established a DNA limit of vaccines manufactured in cell lines at less than or equal to 100 picograms per dose, and in 1996 several groups discussed whether that could be raised and the DNA limit was raised to less than or equal to 10 nanograms per dose for those vaccines grown in cell lines.

So viral vaccines and biological products contain residual DNA. You cannot remove all of the DNA and the amount of that DNA in the vaccine will depend somewhat on the vaccine. For example, a protein or subunit vaccine is going to have less DNA than probably an inactivated viral vaccine, such as IPV or influenza, which will probably have less DNA than the live attenuated viruses such as MMR and varicella. So each vaccine has DNA but it depends on the vaccine how much.

So the cell substrates and the WHO-recommended DNA limits for parenterally administered vaccines, these are what is currently recommended from the WHO, and they specifically exclude oral, vaccines given via oral routes. So primary cells, they decided

there should be no limits and that's true for diploid cell strains such as MRC-5 and WI-38 and in cell lines, continuous cell lines, and they didn't differentiate between whether it was tumorigenic or not, use less than or equal to 10 nanograms per dose.

So how do you determine how much DNA? Well, historically spectrophotometry was used but that, as you see, is very insensitive and over the years we have moved from hybridization through immunological methods and to PCR methods, which are generally used now.

And if you use PCR methods with unique sequence DNA, you can detect down to the centigram range and even if you use highly repeated DNA such as small interspersed nuclear elements or the Alu sequences, you can get down to the attogram range. So this is extremely sensitive assays for detection of DNA. And now with the use of quantitative PCR, you can get pretty good numbers about how much DNA is, in fact, present.

So here is the age old question. Is DNA a risk? Well, if you read what has been discussed over

the last 40 years on this, DNA assessments of risk vary from DNA is an "impurity" or even a contaminant whose amount needs to be measured, but is not a safety concern to DNA is a biologically active molecule whose activities pose a significant risk to vaccinees. Thus, the amount of the DNA needs to be limited and its activities reduced.

So how does DNA get into the cell? Well, there is a whole series of steps. First of all, of course, the binding of the DNA to the cells, the uptake of the DNA, the transfer of the DNA to the nucleus since DNA has to be expressed in the nucleus, the expression of that DNA and, in many cases, the integration of that DNA.

So all these steps, as people have studied over the years, are low efficiency events. DNA itself is not directed to get into cells or to get into the nucleus and be expressed. So these are all very inefficient events. And when people have looked at the efficiency of all these events, numbers of probabilities vary extensively but also, they are not much use.

So the activities associated with residual DNA, DNA has two activities. It can have an oncogenic activity or an infectivity activity, and an oncogenic activity can either be due to the induction of a dominant oncogene, such as the activated ras oncogene or a viral oncogene, or DNA can have oncogenic activity through the consequences of integration.

So the integration of the DNA can cause the disruption of tumor-suppressor gene, such as p53, Rb, etcetera, or if it sits down in close proximity to a dominant proto-oncogene, a cellular oncogene, then it can cause activation of that gene and ectopic expression which could also lead to oncogenesis.

So the infectivity activity is the capacity to generate an infectious agent. So, in other words, if the DNA of the cell contains a DNA viral genome or a retroviral or proviral copy of the DNA in the genome, then if you inoculate that DNA into the cell, into the vaccine recipient, that DNA could produce the virus and that virus then could become an adventitious agent in that host and have pathogenic consequences. So this is a possibility and, of

course, this does work in vitro. So this is not a theoretical risk, at least in vitro.

So I want to turn first of all to the oncogenic activity and discuss why integration has been considered a low risk. So let me just tell you what I mean by integration. The integration could be of any DNA. This is not an oncogenic DNA specifically or oncogene-encoding DNA. This could be any DNA.

So when you estimate, get estimates of the probability of integration of a DNA molecule to induce an oncogenic event, they vary from, I guess, 10^{-9} up to 10^{-23} and this, again, becomes what I have just mentioned a couple of slides ago, is the efficiency of all the events leading up from the DNA binding to getting into the nucleus and then integrating that DNA in the nucleus are extremely low. And when you consider you have to inactivate two copies of a tumor-suppressor gene to be active, that's where these very high or in the case of very low probability events occur.

Regulatory agencies have looked at this and decided that very high DNA of primary cells or

diploid cell strains, there is no limits of the DNA. And also, the levels of plasmid DNA vaccines up to several milligrams now per dose have been permitted by OVRR. So if you take that into account, I think it's difficult to imagine mechanisms by which some types of DNA or plasmid DNA pose a higher integration risk than others.

So it's hard to imagine that any DNA is different from another DNA. Maybe we can discuss that, but it's hard to imagine that. So I think these are the reasons why oncogenic activity is now limited really to the introduction of a dominant oncogene and, again, the infectivity activity. So these are the two major risks of DNA that we have to deal with.

So oncogenic activity is measured in vitro by transformation assays and these are immortalization, loss of contact inhibition and acquisition of an anchorage independent phenotype. And in vivo oncogenic activity is measured by tumor induction and infectivity activity can be measured both in vivo and in vitro and, again, it's the establishment of a virus infection. So these are the

outcomes of DNA given to cells.

So why can't we just give DNA of the cell substrate to animals? Well, we can and we do, but there are complications in testing cellular DNA and this is because of the dilution factor of a gene or virus because of genome sizes. So a haploid mammalian genome contains 3×10^9 base pairs. The single copy gene or virus varies from, say, 3,000 to 30,000 base pairs in size.

Just by the arithmetic here, a single copy gene or virus is 10^5 or 10^6-fold less abundant for equivalent amounts of cellular DNA or as compared with the plasmid DNA containing the same gene or virus. So in other words, if one microgram of a cloned gene or virus has a biological effect, just translating that to how much cellular DNA you need is 10^5, 10^6 micrograms, which turns out to be .1 gram to 1 gram of DNA. Now, I don't know if anybody has made DNA, but making a gram of DNA is not that easy.

Secondly, there is no validated assay for these type of experiments. So that is the complication of just measuring DNA itself.

So now, I want to just review some of the published literature on this with both viral oncogenes and cellular oncogenes and there aren't that many data on this, in fact, v-src in chickens and polyomavirus DNA in rodents and H-ras in mice. So the oncogenicity of src DNA was shown in Hsing-Jien Kung's lab in 1983 and cloned viral src DNA, 2 micrograms induced tumors in about 70 percent of the animals inoculated subcutaneously in their wing-web.

Also by Halpern in 1990 who also looked at v-src DNA. In this case 20 micrograms induced tumors in about 80 percent of the animals inoculated in their wing-web and 22 percent if you inoculated by IV. So what we like to use is the most sensitive assay here and, therefore, we say that 2 micrograms of cloned v-src is oncogenic in chickens, and this corresponds to about 2.5×10^{11} molecules just to give you some idea of the inefficiency of the process.

So with polyoma DNA, these were safety studies done. In fact, over the years, first of all, in Wally Rowe, Malcolm Martin and Mark Israel's lab's, and they showed that if you inoculated polyomavirus

DNA, .5 micrograms of DNA, whether it's supercoiled or linear, can cause tumors in newborn hamsters.

They looked at cloned polyomavirus DNA and, again, these DNAs were also oncogenic and induced tumors in various efficiencies. And so if you look at the minimum amount of DNA required to be oncogenic, it's .2 micrograms of polyomavirus DNA is oncogenic in newborn hamsters. That correspondents to about 4 x 10^{10} molecules.

And just parenthetically, if you look at the slides and you look at your notes, some of the numbers have changed and that is because I used a calculator instead of my brain and so there a couple of minor differences.

So the only study that is on oncogenicity of a cellular gene is this study by Burns and colleagues in 1991, again a safety study as it turned out, looked at the activated H-ras, Harvey-ras, from the T24 bladder carcinoma. 10 micrograms were inoculated by scarification of mouse skin and lymphangiosarcomas developed in almost all of the mice and usually within 12 weeks, but certainly after 12

months. Normal ras failed to do this.

So this is the first study and, in fact, the only study that has shown that ras itself is oncogenic in animals. And so 10 micrograms corresponds to about 10^{12} molecules of inactivated ras. Again, a very inefficient process.

So what do we know about DNA infectivity? Well, I can give you a lot of studies, but this is the summary of looking at retroviral DNA and polyoma viral DNA and between 15 and 500 micrograms intramuscular injection of retroviral DNA can establish an infection in an animal and that is about 10^{12} to about 2×10^{13} molecules. With the polyoma viral DNA, 5×10^{-5} micrograms or 50 picograms can cause an infection in mice and that is about 10^{7} molecules. That's where one of the differences, I think, is.

And so we can conclude, first of all, that infectivity of different retroviruses is similar. So these may be mouse retroviruses or simian immunodeficiency virus, but they all fall into this range and depending on the route of inoculation, 15

micrograms can be infectious and the infectivity of polyomavirus DNA is high and, approximately, 50 picograms of the polyoma viral DNA is infectious in mice.

So when you compare oncogenicity and infectivity in animals, as I just said, the .2 micrograms is oncogenic of polyoma viral DNA but, in fact, the ID_{50}, which is a little higher, is 1.3×10^{-4} micrograms of polyoma viral DNA corresponding to about 2×10^{7} genomes. And for retroviruses, infectivity is 15 to 30 micrograms in most cases.

And so if you compare this value and this value, it turns out to be about 1,000-fold difference. So, therefore, the DNA infectivity assay is about 1,000-fold more sensitive an assay than DNA oncogenicity and that's important because if, therefore, as I'm going to tell you, you remove the DNA infectivity activity, you almost certainly have removed the DNA oncogenicity activity.

So what are our operating principles for assessing the decisions on cell substrate DNA? So we need to, as Phil Krause mentioned in the Defined Risks

Approach, we like to base our estimates on quantitative experimental data on the biological activity of DNA. As long-term human safety data are usually unattainable, it is prudent to make estimates based on the most sensitive and model systems.

So we prefer to use the most sensitive rather than the least sensitive. And as more data are obtained, risk estimates may change and recommendations may be revised. When Andrew Lewis presented that table with pluses and minuses saying that that was our estimate now, he said that those pluses may disappear over time and I think that's what we all think, that as more data are accumulated, we may well have different risk estimates based on the different factors.

And in fact, parenthetically, I think that's where Phil Minor's comment was that HeLa DNA was in that table, which is, in fact, an assumption as opposed to demonstration. HeLa cell DNA is oncogenic in vitro, but has never been shown to be oncogenic in vivo. So what do we do about this? Well, we tried to develop quantitative assays and with the help of

several people we got these studies started. From the Office of the Commission initially with the pilot grant and now NIAD has considered these sufficiently important questions to answer.

So we have developed an assay and we chose oncogenes that have been shown to transform efficiently primary cells in culture, so we wanted to choose the best system that we could imagine that could work and express these oncogenes under promoters known to function efficiently and for prolonged periods in mice. Many promoters in mice get shut down over time, so we don't want to use those promoters.

So without giving you any great details, these are the two plasmas we have investigated. We derived the expression of the H, Harvey, activated ras in red under the 5 prime LTR murine sarcoma virus and we have the analogous plasmid over here with the murine c-myc. So the red oncogene and the yellow oncogene are what we are using here. One is H-ras and one is c-myc.

We inoculated these plasmids into mice, newborn and adults, and assessed the oncogenicity of

those. And they turned out to be oncogenic and this is one of our volunteers, and as you can see, the tumor rises within about eight weeks, a tumor rises. That, the pathologists tell us, is an undifferentiated sarcoma. And we establish cell lines from these and this is a cell line that came from this tumor and we have shown without going into the data that dominant oncogenes can induce tumors in normal mice, both ras and myc are acquired. We were not able to find tumors either with ras or myc alone. And the newborn animal is more sensitive than adults. So these are our conclusions. And, therefore, models to evaluate DNA oncogenicity are being established.

We can go into the sensitivity, if you would like. So the other thing we are doing is to develop an in vitro assay to assess infectivity. Now, why do we care about DNA infectivity? Well, when the VRBPAC, your predecessors, discussed this many years ago, in fact, the infectivity risk of DNA may be higher than oncogenicity, and I have just showed you before in vivo experiments that have been done by others, there is about 1,000-fold difference. So they

were rather perspicacious, one assumes.

So DNA infectivity has been incompletely studied. We don't know the specific infectivity of different viral genomes. And what I mentioned before, clearance of DNA infectivity will also clear DNA oncogenicity since this is a more sensitive assay. And also, this assay will allow other aspects of DNA activity to be studied.

So I'm not going to show you any data. I have slides if you would like to see some. But what we found was that one picogram of a retroviral DNA can be detected. This corresponds to 1×10^5 molecules. This is an extremely sensitive assay. So this is a transfection coculture assay in vitro with HIV as our initial viral genome. And we can also find that one microgram of cellular DNA from an HIV-infected cell is infectious.

And again, rather interestingly, this is a million-fold difference in sensitivity between a microgram and a picogram, again suggesting that the arithmetic is valid that the concentration of the DNA in a plasmid is a million-fold higher than the

concentration of this. And so it corresponds quite nicely.

So now, we're going to use this assay to look at the various things. For example, DNA inactivation methods. In the live viral vaccines, nuclease digestion frequently by Benzonase is used to reduce the biological activity of DNA. In activated viral vaccines, chemicals are often used, such as beta-propiolactone or formaldehyde. So we have done experiments so far with nuclease digestion and propiolactone treatment, and I just want to show you one example of DNA experiment in this gel here.

This is untreated and then the following lanes is one minute all the way up 15 minute treatments with the nuclease, the Benzonase, and as you can see, the DNA is degraded rather rapidly and it gets very small about here. What we have done is looked at the infectivity of these fractions along here and just summarized the infectivity of the parent all the way to this point. But after that, no infectivity could be found.

And if you look at the mean size of this,

it's around 300 base pairs, so that's roughly -- that's where we can draw the signature between infectivity and lack of infectivity. So before you can make calculations, we have to know something about assumptions. So for a given DNA, the level of the response of a cell to that DNA is proportional to the amount of DNA. I think that's pretty straightforward.

The activity of a gene/viral genome integrated in the chromosomal DNA or as part of a plasmid DNA is equivalent. So the amount of uptake and expression of a gene/viral genome virus cell is related to the concentration of the genome virus in the DNA. Again, that's the arithmetic that I mentioned earlier on. And the activity of a gene/viral genome inoculated as chromatin is the same as when the same gene/viral genome is inoculated as free DNA.

Now, this is an assumption and we are going to test this with this infectivity assay. As you may, obviously, be aware that the DNA, cellular DNA in residual cell substrate or the cell substrate in vaccines is not free DNA. It's part of a nuclear

histocomplex and so that is what we really should be assaying. But it's not that easy to do that.

So then I'm going to go on and mention to you what the definition of safety factor is. This is the factor by which the biological activity of DNA is reduced. And the reduction can occur by lowering the amount of DNA or by inactivating the DNA. And thus, it's analogous to clearance of adventitious agents that Arifa Khan just mentioned to you. And we would like to think the safety factors of 10^{7} or more would provide the substantial safety margin here.

All right. So here's some more numbers. What we found was from our experiments, just bear with me, the digestion of DNA to mean size of 300 base pairs resulted in the loss of biological activity in this case of .15 micrograms of cloned viral DNA. So based on the proportion of the retroviral genome in the cell, which is 1.67×10^{-6}, 150 nanograms of viral DNA corresponds to 90 milligrams of DNA.

So, therefore, if you wanted to get the same effect, you would have to use 90 milligrams. So relative to the theoretical risk of infectivity of 10

nanograms of DNA, so now we're stipulating that we need to get down to 10 nanograms of DNA, then cellular DNA with a single provirus, the safety factor is 9 x 10^6, close to 10^7. All right. So that's just based on those numbers, which are based on the experimental data.

And now, what we have done now is just based on those numbers for 10 nanograms of cellular DNA, then the safety factor just using cloned DNA, the safety factor is only 60. So 1 picogram of HIV DNA is infectious. We have shown that in vitro. Based on the proportion of the genome, 10 nanograms of DNA will only give you a safety factor somewhat surprisingly of only 60. From the BPL treatment, the safety factor is 3 x 10^7. And from the Benzonase digestion, the safety factor is 9 x 10^6. So there are our calculations based on our experimental data.

And for the oncogenicity it's more complicated, but we know that 10 micrograms of the two plasmids induce a tumor. It turned out to be 12.5, but we'll just go down to 10 micrograms. The oncogene represents 10^{-5} to 10^{-6} of the remaining genome. That

is 10^6 or 10^7 micrograms of cellular DNA would be required to induce an oncogenic event, based on the 10 micrograms, and therefore for 10 nanograms the safety factor is 10^8 to 10^9.

This is really demonstrating why oncogenicity, even in introducing a duller oncogene, we consider is very improbable. And that factor, in fact, excludes the fact that these two oncogenes are necessary in the same cell to induce the effect. And again, in cellular DNA, of course, these are unlinked oncogenes and therefore that probability is extremely remote. An additional safety factor is from the size of reduction of the DNA, and I'm not even concluding that here, and that you get another, approximately, 10^5 for safety factor based on the reduction of DNA that I showed you based on the infectivity assay.

So how can we use this in the regulatory process? Now, what I'm going to give here is a hypothetical example. So here are the facts of the case. A tumorigenic cell substrate is proposed for the manufacture of an inactivated vaccine. The manufacturing process reduces the amount of that DNA

to less than 2 nanograms per dose. Less than or equal to 2 nanograms per dose. An the inactivation procedure reduces the size of that DNA to below 200 base pairs.

So what can we do? So with an oncogenic risk, first of all, from a consideration of the DNA quantities alone, our current data suggests that the safety factor for an oncogenic risk from 2 nanograms of DNA is 5×10^{8} to 5×10^{9}. That's just based on how much DNA those plasmids cause the tumor. So again, just without doing anything to the DNA, it is 5×10^{8} and 5×10^{9}.

Now, this number excludes the additional safety factor derived from the size reduction. And if you factor in that 1.5×10^{5}, now, you're getting another 7.5, 10^{13} to 10^{14} safety factors. However, there could, of course, be a number of oncogenes and Robert Hess has estimated there is at least 200 dominant oncogenes in the human genome or murine genome, but still, that 200 whole factor is not going to change these numbers very much.

So the infectivity risk, which is, as I

say, the more important risk, from a consideration of DNA quantities alone, our current data suggests that safety factor with 2 nanograms of DNA is 300. That's just 60 x 5. So 300, if you did nothing to the DNA, you would get that number. However, because the manufacturer reduced the size of that DNA to below 300 base pairs, in that case below 200, then we can use our 9×10^6 factor for 10 nanograms of DNA. So this value becomes greater or equal to 4.5×10^7 for 2 nanograms of DNA.

So from that we can conclude that for this inactivated vaccine, the manufacturing process adequately deals with the safety issues with respect to residual cell substrate DNA.

There are additional considerations, as Andrew Lewis mentioned, about the multi-stage nature of human carcinogenicity for the oncogenic activity and so it's unlikely that a single dominant oncogene will induce cancer. However, the possibility of initiating a cell remains a potential concern, but because there is no known assays to assess this, we can't yet deal with that. But I think again, because

one event is not sufficient, I think people think the oncogenic activity that causes an initiation event is a much lower concern.

So what do we know about can we change these numbers from DNA infectivity studies? Well, the amounts of viral DNA to establish an infection in vivo, based on the polyoma viral DNA at 50 picograms, which is 9 x 10^6 genomes, and 50 to 30 micrograms of retroviral DNA is this number, so if you base it on the polyoma viral DNA, you can increase that number that we have already come up with by 50-fold for polyomavirus DNA and up to about 10^7-fold for retroviral DNA. So again, if we just use the 50-fold factor from an in vivo study, so again, we're increasing the safety factor.

Okay. So we can conclude by development of quantitative in vivo oncogenicity assays and in vitro infectivity assays are feasible, because these assays are highly sensitive, they represent the worst case. And data from these assays will assist in resolving safety concerns associated with residual cell substrate DNA and permit the introduction of new

cell substrates.

Some issues are remaining to be addressed. The biological activity of chromatin, we need to know whether it is more or less active than free DNA. We need to know the routes of inoculation delay affect. One group has determined that, in fact, the uptake of DNA orally is about 10,000-fold less efficient than IM route for DNA update and the nasal, the efficiency of uptake of nasal through the nasal route is unknown.

And again, where the DNA can induce an initiation event is not known. Now, whether hereditable epigenetic effects can induce oncogenic events in vaccine recipients and whether these have a safety concern is not known.

So what are we recommending for our sponsors? Well, now, with tumorigenic cells, that is MDCK cells, we are recommending a clearance of DNA. That could be reducing the amount of DNA less than or equal to 10 nanograms per dose and reducing the size of the DNA to below about 200 base pairs. And this, as I have explained from the experimental data, will provide a greater than or equal to 10-fold safety

factor, 10^7-fold safety factor.

We are also inoculating, asking inoculation of cell DNA into animals analogous to what Dr. Khan talked about with the cell lysate and about 100 micrograms of cell substrate DNA has been recommended into newborn hamsters, newborn rats and newborn nude mice. And the animals are monitored for five months or so for tumor formation and general health, and again, as Dr. Lewis mentioned, determining the species of the tumors that arise.

However, these assays are not validated and have undefined sensitivity. However, as I have mentioned before, these assays to work in vitro, so you can inoculate DNA in vitro and detect viral genomes in mammalian DNA. So there is some advantage in that. And I'll stop there.

(Applause)

CHAIR OVERTURF: Are there questions? Yes, Dr. Minor?

DR. MINOR: How I got this right, Keith, that the reason why cellular DNA doesn't cause oncogenesis is just because you can't get enough in

there? Is that the conclusion?

DR. PEDEN: The conclusion for the integration aspect that the probability is extreme. You know, in fact, even in highly sensitive in vitro systems, integration has not caused activation even in the most sensitive systems, such as NIH-3T3. So integration through -- oncogenesis through integration we don't consider.

To answer the other part of the question, that's right. It seems that you cannot get enough DNA into the cell and you need probably multiple genes to cause a tumor in a human, and at least two in a mouse. So I think that the efficiency of that process is so small that that's the reason.

DR. MINOR: Is that affected by the model that you are using, do you think? Do you think there are other models which may be more sensitive in that?

DR. PEDEN: We hope so. I mean --

DR. MINOR: No, like vaccinated humans is what I'm thinking of.

DR. PEDEN: I agree. I mean, people ask us, we are trying to look at different models of mice,

immunosuppressed, p53, heterozygous, various animal models to look at that. We have looked at one already expressing rats and, in fact, that made no difference. In fact, we got no tumors at all. And our reason being is, I think what you are getting at, that humans are out-bred and there are many humans who have maybe different genetic diseases.

So it may be more important in some humans, you know, the DNA repair defects may be. So DNA may be an issue of that. So that's really why we want to test that in as many models as we can. Also, we would like to know the answer whether DNA from a cell can be oncogenic. I mean, to answer that question. At the moment, these assays that we have, the model systems we have, are not sensitive enough to detect that. But if we can get a more sensitive model, then we may be able to answer that question.

And again, getting to what Dr. Lewis was talking about earlier, a highly tumorigenic cell versus a weakly tumorigenic cell, if we take DNA from that and we can find a difference than in an in vivo assay, that would be extremely important. So, yes, I

mean, we are still working it out, but mainly it's because humans are out-bred, we hope.

DR. MINOR: Can I have another go actually? The safety factors, like you say, for example, you require a certain amount of DNA to go in to go get a tumorigenic dose. Okay. Is that quintal? I mean, for example, if you give us a thousandth of a tumorigenic dose to 1,000 animals, are you going to get one tumor or do you get no tumors?

DR. PEDEN: Yes, I mean, that's a good question. I mean, that's what the WHO Committee in, I think, '86/87 talked about that issue. First of all, you can't do that experiment. And it's possible that there is a threshold, so I think when you get down to those levels, I don't think we can answer that question unfortunately. But that's the assumption. And based on that assumption, that's where those extrapolations came from.

CHAIR OVERTURF: Are there other questions from the Committee? Yes, Dr. Robinson?

DR. ROBINSON: Yes, do you see, given your results with Benzonase and BPL treatment, are they

cumulative or do you see a synergistic effect?

DR. PEDEN: I think I heard that. So if you have -- you do two processes, right? Well, it's hard to imagine once you get below about 200 base pairs that the DNA is -- you're going to measure activity. I think they could be cumulative, because BPL, as you know, is an allocating agent and that affects not just the size of the DNA through cleavage, but also it's immunogen. You know, it's involved in GC, AT transitions and also a purinic site. So you can -- and it cross links. So it does many more things than just get the DNA smaller. So, yes, the answer is I think it can be. It certainly is additive, but it may not be necessary.

CHAIR OVERTURF: Yes, Dr. Royal?

MEMBER ROYAL: Just going back and looking at your, I guess, tumorigenicity assay, your in vivo assay, your ras assay. Isn't what you really want is some sort of way of detecting the development of tumorigenicity in real time?

DR. PEDEN: Could you say that a little louder?

MEMBER ROYAL: Right.

DR. PEDEN: I'm not quite sure what you are getting at.

MEMBER ROYAL: So in going back and looking at your in vivo assay, your tumorigenicity assay, your oncogenicity assay, sorry.

DR. PEDEN: Yes.

MEMBER ROYAL: Isn't what you want is to be able to detect the occurrence of oncogenicity when it occurs?

DR. PEDEN: Yes.

MEMBER ROYAL: As opposed to sort of looking back and sampling to see if after getting your product now that happened in a tumorigenic environment or oncogenic environment.

DR. PEDEN: I mean, the assay is an endpoint assay, as Dr. Lewis mentioned. I mean, so we inoculate the animals and within about eight weeks we see these large tumors on the animal. Are you asking whether you could see it earlier than that? So a pre-malignant state, is that what you are asking?

MEMBER ROYAL: Well, the problem that I

have with a lot of these assays is that what you do is you sort of take your cells and before you use them as your actual substrate, you look at how tumorigenic they are or whether some oncogenic effect has occurred. Then you go ahead and get your product. Who is to say that during the process of your synthesizing your vaccine or whatever the case may be those cells don't become tumorigenic? In which case, you have already concluded that your product is safe.

DR. PEDEN: So these are -- we're trying to develop an assay, so we can determine whether DNA can ever be oncogenic, can ever form tumors in animals. So this is quite apart from the tumorigenicity of the cell. As Dr. Lewis mentioned, if a cell is highly tumorigenic, what many people would believe is that the cells are that way because of the number of activated oncogenes they express. That may not be true, but at least that's, to a first approximation, what we want to believe.

But we can't ever test that, because their assays are not sensitive enough to detect the oncogenic activity even, we think, of a highly

tumorigenic cell. We want to answer that question. So they are related, but they are different in that sense that we can't measure the oncogenic activity of a highly tumorigenic cell. So we cannot directly answer that question.

We're getting at the issue by understanding the biological activity of DNA through its infectivity activity, which is far more sensitive an assay than an in vivo oncogenesis assay, oncogenicity assay. I have trouble with those words, too. And as we show, we can detect 1 picogram of DNA, which I didn't think that we could ever do, all the retroviral DNA, and we can clear that by about 10^7-fold with various chemical and antiemetic treatments. We will, obviously, then have cleared any oncogenic activity that is present in that DNA.

So we are reaching around answering that question, at this stage, because we cannot answer it directly. Does that help?

CHAIR OVERTURF: Yes, Dr. Markovitz?

MEMBER MARKOVITZ: Yeah, Keith, where did that original 10 nanogram figure come from?

DR. PEDEN: It came from the Committee that looked at these things. Are you asking --

MEMBER MARKOVITZ: No, I mean, data-wise. I mean, how did they arrive at that conclusion?

DR. PEDEN: Oh, okay. So the 100 picogram came from the infect -- the oncogenicity of polyomavirus DNA in 1986 when, you know, Malcolm Martin and Doug Lowy, I mean, I can't remember all their names who are on that Committee. People who looked at that and they extrapolated that 100 picograms would represent, I think, it's 10^{-6} or something of a tumor producing dose based on those results.

So that's where the 100 picogram -- now, are you asking why it was suddenly raised to 10 nanograms? Well, it was raised to 10 nanograms, first of all, considering loss of information that had existed, not a lot of information, some information in those intervening 10 years had surfaced. One is the John Petricciani experiment of injecting animals with milligram quantities of DNA in monkeys and after 10 years nothing happened. You know, that's one piece of

evidence they cite in the discussion.

I mean, if you read the discussion, we don't think it's a risk anyway and the people before made it too stringent an assessment. So I think as Phil Krause mentioned a few years ago at one of these Committee meetings, that since those were based on polyomavirus DNA, which is a highly oncogenic and infectious agent, and if that sort of virus had existed, does exist in say MDCK cells, we would have found it, because it is highly infective, highly oncogenic.

So I think all of that and the numbers considerations that I always go through in this, I think that's the reason why. And the other reason that nobody likes to mention is, in fact, cost. I mean, the manufacturers, it costs a lot of money to try to engineer a vaccine that only has 100 picograms and a 100-fold difference apparently makes a big difference. So that was also one of the considerations this group did discuss and consider.

CHAIR OVERTURF: Yes, Dr. Cook?

DR. COOK: I would like to take a spin at

-- I'm not sure this is what Dr. Royal was talking about, but something I was thinking about and we will see. You are measuring the risk of a substrate in its native form before it is used in vaccine preparation. So how might that relate to that cell when it is infected? Would it change? Would infection with say influenza or something else be likely to do something like activate endogenous oncogenes or latent retroviruses or other things that you can't measure in the absence of the stimulation of the cell during viral infection? And is that worth considering?

DR. PEDEN: Yes, everything is worth considering, I think. But, I think, we are asking people to look at lysates in DNA. If we ask them to look at DNA, the reason why a lot of endogenous viruses are suppressed, some of them is due to the chromatin. Now, if give them free DNA, that is gone. Some of them are due to methylation, which we can't deal with. So that's one aspect.

Should we be looking at it after infection? That's a possibility, but since we can't, we haven't got an assay for cell substrate DNA anyway.

I don't know how we can look at it yet. And again, your influenza is a side of plasmid virus, but that's again a silly argument, I agree, because it could have consequences on the cell as well. So I think that is something to consider, but I'm not sure yet we can address it experimentally.

CHAIR OVERTURF: Dr. Minor?

DR. MINOR: How is random integration affected by the size of the DNA, Keith? Does it have to be a large DNA to be randomly integrated? What I'm thinking was if you go and treat with Benzonase and you get under 200, are you increasing the frequency of random integration?

DR. PEDEN: Yes, that's a good question. Not much is known about the size, because it's not so easy to measure integration of small pieces, but that's always a concern. Now, of course, you have generated far more ends and if it's just end dependent, then it may well be you have, in fact, increased the oncogenic risk. But again, I come back to in vitro. Nobody has ever seen any oncogenic activity through integration in a cell system that

needs one hit, which is NIH-3T3. So I think yes, that may be an issue, but I don't know if there are any data that address it, except for those in vitro experiments.

CHAIR OVERTURF: If there are no further questions, we will adjourn for the morning and reconvene at 1:00. Thank you.

(Whereupon, the hearing was recessed at 12:13 p.m. to reconvene at 1:18 p.m. this same day.)

A-F-T-E-R-N-O-O-N S-E-S-S-I-O-N

1:18 p.m.

CHAIR OVERTURF: I would like to call the meeting to order for the afternoon and the first thing on the agenda is the open public hearing, and I'll ask Christine Walsh if there is any open public hearing applicants.

MS. WALSH: Good afternoon. As part of the FDA Advisory Committee meeting procedure, we are required to hold an open public hearing for those members of the public who are not on the agenda and would like to make a statement concerning matters pending before the Committee. I have not received any requests, at this time.

Is there anyone in the room who would like to address the Committee, at this time? Dr. Overturf, I see no response and I turn the meeting back over to you.

CHAIR OVERTURF: I would like then to begin the afternoon session with the first manufacturer's presentation, which will be by Chiron Corporation, Rina Rappuoli.

DR. RAPPUOLI: Well, good afternoon. I sam pleased to be here today as Chiron Scientific Officer to present the next generation safe culture-based influenza vaccine that we have developed to meet unmet public health needs. I will show you today after the very good interaction of the morning why we have selected the MDCK cell line and why we believe it is safe to use it for large scale manufacturing of influenza vaccines.

Each year globally influenza viruses circulate and are the cause of significant illness and mortality. Influenza also causes significant economic losses. The influenza viruses continue to circulate each year because of introduction ways into the population, the waning of immunity in those previously exposed or immunized and the change in presentation of viral antigens because of genetic mutations.

Unexpectedly, but periodically, through the massive genetic changes and essentially new influenza virus begins to circulate to which the overwhelming majority of population is naive. And then a pandemic begins, as happened in 1918 and more

recently in 1957 and 1968.

The keystone of public health response to counter influenza morbidity and mortality is immunization. At present in the United States, influenza vaccines is routinely recommended by the Center for Disease Control for, approximately, 185 million people. The manufacturing capacity based on the production of vaccine in embryonated eggs to meet this recommendation, however, does not exist.

Similarly, the capacity to meet and extended universal recommendation does not exist. And the capacity to respond to demand fluctuations does not exist. Although, so far I will just focus on the United States needs, we must keep in mind that we are a world community of nearly 6.5 billion people. Globally, there is nowhere near the manufacturing capability or flexibility to meet routine vaccine needs and there is certainly no capacity to meet pandemic needs.

In the face of an influenza pandemic, the rapid production of a vaccine for nearly 300 million people in the U.S. alone would be needed. Moreover,

this need for vaccine against the pandemic could erupt in the middle of normal influenza season. In fact, the H5n1 could be a problem this influenza season. We don't know yet. It is clear that when egg-based production process is unlikely to be very effective to respond to an influenza pandemic.

The twin concerns of surge capacity and the potential lethal avian pandemic influenza strain such as H5n1 are illustrated in this slide. The present paradigm is essentially one egg, one vaccine dose. But if there are not eggs because they have been already used, then the ability to respond to an increased demand is gone. If there are no chickens, because of a lethal avian influenza strain, then again there is no vaccine. In summary, no chickens, no eggs, no vaccine.

Do you understand the consequence to public health can be enormous. It is primarily for this reason that the U.S. Department of Health and Human Services has emphasized the need for research culture vaccine production. To address the need/ research capacity in the event of a shortage or

pandemic and to provide security against risk associated with egg-based production.

These themes were echoed by the President during his visit to the National Institute of Health to discuss the U.S. Pandemic Preparedness Plan. The President Bush also emphasized the need for a cell culture-based manufacturing process for which development he has requested $2.8 billion. For various reasons, we and others have opted to use continuous cell lines.

As far as Chiron, it was of particular importance to have a scalable, flexible, high volume manufacturing process that was free from animal-derived components and one that could not be limited by long lead times. I will be more specific about our choice of a cell line in the next slide. At this moment, I want simply to acknowledge that while there are many advantages to continuous cell lines, there are also potential risks. However, I must also stress that continuous cell lines have been routinely used for the production of numerous biological products for nearly 20 years and with a remarkable record of

safety.

Let me now be more specific about Chiron choice of a cell substrate for influenza vaccine production. We have chosen the MDCK cell line because it is well-established in the scientific community as one of the best cell lines for the replication of the influenza virus and also, because it is highly permissive for a wide variety of influenza strains. Indeed, in our hands for the growth of influenza strains, MDCK cells were superior to other cell lines that we had tested.

We also chose MDCK cells because they are relatively resistant to the growth of non-influenza human pathogens. This is a safety feature that we wanted. Having chosen MDCK cells for these reasons, then we really worked to have, to adopt them, to grow in suspension, to provide a high-yield, high volume production process that will provide an affordable vaccine to meet public health needs.

Growth in suspension also provides the means to address fluctuating demands. We have also adapted the cell line, so it can grow in a very -- in

a chemically very well-defined medium. This eliminates the adventitious agents that would be necessary to accomplish animal-derived medium.

At this juncture, it may work well for me to say a few words about cell substrates in general. These cell substrates for the production of biological products has evolved from the exclusive use of primary cells in 1950s to the addition of diploid cells in 1970s to the addition of continuous cell lines in 1980s. In large part, this progression has been driven by safety issues, particularly, those associated with adventitious agents.

Primary cells are taken directly from an animal and used with minimal processing. Although, safeguards were and are in place, primary cells cannot be totally characterized and tested each time they are isolated to insure the absence of adventitious agents. Primary cells also require complex animal-derived medium for growth, another potential source of adventitious agents.

Diploid cells in contrast can be well-characterized with regard to adventitious agents an

banked for subsequent use. However, they do suffer from the requirement of complex media for their growth and, therefore, have a risk for adventitious agents. This risk can be avoided through the use of continuous cell lines. They can be well-characterized, banked, grown in chemically defined media, free of animal-derived materials.

Moreover, continuous cell lines can be adapted to grow in suspension providing cost and scalability advantages. Although there are clear advantages to the use of continuous cell lines, the multiple passages needed to obtain the desired properties renders them tumorigenic or better potentially tumorigenic. Not unexpectedly, the Chiron MDCK cells are tumorigenic, at least in the immunocompromised animal.

This is an issue that must be dealt with and we have dealt with, and I will explain how. In addition to being tumorigenic, continuous cell lines may be oncogenic. That is they may contain agents that are able to transform host cells. Oncogenicity could arise from three sources. The cells, the cell

DNA or sequestered viruses.

As mentioned, a number of biological products have produced in tumorigenic continuous cell lines. Regulatory approaches through the use of such cell lines have been developed and used successfully. Recently, CBER has addressed the potential need for the use of continuous cell lines for vaccine production and developed an approach to evaluating the risk and eliminating the risk.

This approach has been formalized by CBER in their Defined Risks Approach Algorithm. At Chiron we have followed this approach as well other pertinent regulatory guidelines and advice.

Because MDCK cells have been shown to be tumorigenic, there is the fear that if they are present in the vaccine they might propagate in the recipient causing a tumor. The solution to this is to ensure that intact cells are completely removed from the product. There is the additional concern that the continuous cell lines contain an oncogenic agent, DNA or a virus, that is able to transform the cells of the recipient host.

The solution to the former concern is to reduce the levels of DNA in the product and to degrade and inactivate the residual DNA, that means to make it nonfunctional. The latter concern can be addressed in two ways. First, through a combination of rigorous testing on known classes of oncogenic viruses to demonstrate their absence and, second, by having in place a manufacturing process that removes or inactivates potential occult viruses.

Let me now expand on these themes. Let us now look at the tumorigenicity of the MDCK cell line and the manufacturing process which removes them. As you can see in this slide, in immunocompromised mice the MDCK cells were notably tumorigenic. As few as 10 cells were able to form tumors. Therefore, removal of cells during manufacturing process is our primary concern.

Usually, one deals with the user tumorigenic cells in the manufacturing process by ensuring that the cells are eliminated from the product. We have a manufacturing process that contains steps that are introduced specifically to

remove the MDCK cells and steps that, although incorporated into the manufacturing process for other reasons, will also affect the removal of any intact cells that might remain.

These cell removal steps are based on different chemical and physical principles and are multiply redundant. There are physical removal steps such as centrifugation and filtration and chemically disruptive and inactivating steps. We should also bear in mind that most of the cells are simply lysed by the influenza virus itself at the end of the culture.

In this slide I will start to illustrate to you the capacity of the process to remove the cells both in terms of the individual steps and the steps in combination. The initial centrifugation steps already removes 99 percent of the cells. The centrifugation step found later in the process will, obviously, remove the additional cells. The centrifugation, however, was not validated for cell removal, so we do not attach a clear factor to this step either here or in subsequent calculations.

Filtration steps are extremely effective in removing cells. There are four filtrations in the manufacturing process. Depending on the effective pore size, these filtrations can reduce the cell numbers by, approximately, 6 to 11 orders of magnitude.

To help understand why filtration works so well, we should look at the electron micrograph on the right. The micrograph shows an MDCK cell, which has a diameter of 15 microns. Positioned next to this MDCK cell is a circle of 0.2 micro in diameter. As you can well imagine, it's difficult for these cells to go through that 0.2 micron pore.

In addition to the physical removal, the MDCK cells are also inactivated by detergent, by the BPL that is used to inactivate the influenza virus and by the viral splitting process. Treatment of the cells with the detergent that is used to split the virus kills the cells within a few minutes. Much longer detergent contact times are used during manufacture.

This cytotoxic effect is illustrated in

the two photos on the right hand side of the slide. After adding and subsequently removing the splitting agent, we are unable to observe any live cells. All the cells are stained, in fact, by Trypan blue indicating that they are dead. We are also unable to observe any cell growth after incubation up to three days in fresh medium as you can see on the right hand image.

This slide illustrates the cumulative cell removal potential of the manufacturing process, the centrifugations, the filtrations and the chemical steps. When combined, the process is such that there is a cell removal capacity in excess of 41 orders of magnitude. This means, for example, that if 10 million cells are needed for one dose of vaccine, then intact cells are removed to the point where it will be fewer than one cell in 10^{34} doses. This is an incredibly small probability.

I will try to illustrate what it means in practical terms in the next slide and, really, have you ever thought what one in 10^{34} means? As an example, it means that if we were to vaccinate all the

people who ever lived plus all the people that will live before the sun burns out, and we vaccinate each of them 100 times, we already applied universal vaccination and a very long life span, 100 years, then the possibility that even one of them will get one cell is still less than one in a trillion.

The basis for this statement is provided at the bottom of this slide. Hopefully, this example provides some perspective on the capacity of the process we have developed to eliminate cells from the vaccine and eliminate the residual risk.

Having dealt with cell removal, we know that MDCK cells, while tumorigenic, were not observed to be oncogenic in all our experiments. As shown by histopathology and on a subset of tumors by PCR analysis, only canine-derived tumors were observed in the studied animals. Also, neither MDCK cell lysate nor purified DNA from the MDCK cells were observed to be oncogenic. No tumors were observed from the administration of these materials.

Let me now expand on this issue of oncogenicity by the cells, the DNA or oncogenic

viruses. As shown on this slide, up to 10 million cells were injected into adult nude mice and no murine tumors were observed.

Lysates prepared from MDCK cells, both influenza-infected and noninfected, were injected into neonatal nude mice, rats and hamsters. Neither lysate was observed to be oncogenic. Finally, using purified high molecular weight DNA at nearly 3,000 times the final product specification of 10 nanograms, no oncogenicity was observed.

Although the DNA was not observed to be oncogenic, a validated manufacturing process that eliminates DNA and degrades or inactivates any form of DNA was developed to ensure maximum safety. First, we introduced a set of manufacturing steps to reduce DNA labels to less than 10 nanograms per dose.

In addition and more importantly, the remaining DNA is chemically inactivated and reduced to a size that is nonfunctional. The residual DNA is less than 200 base pairs in length and is alkylated. As a test of DNA degradation, we'll look for functional genes by PCR and we are not able to detect

them.

Now, we need to turn our attention to the issue of potential presence of oncogenic viruses. Let me remind you that these studies with the cell lysates and with the DNA were negative. Neither these studies nor the tumorigenicity test indicated the presence of an oncogenic virus. The only tumors that we observed were of canine origin deriving from the proliferation of the injected cells. They were not murine which would have been indicative of a transforming agent.

All cell substrates pose a risk from viral adventitious agents, pathogenic or oncogenic. They could be introduced from many sources. They could be present in the original isolated cell line. They could be introduced into the cell line from the complex media that is being used to propagate them or they could be introduced by accidental human or laboratory contamination.

There are two basic ways to address concerns related to virus in cell substrates. The first is extensive testing for possible viruses. We have tested the MDCK cell for viruses and I'll be more

specific about this testing in a moment. However, I want to emphasize now that this testing has been redundant. We have tested MDCK cells at the three cell bank stages, the Master Cell Bank stage, the Working Cell Bank stage and at end of production.

We use various methods to screen for potential viruses, such as PCR for a particular virus, or broadly screening methods, such as electron microscopy or use of indicator cell lines. At the end of all these studies nothing was found. The literature supports our findings.

Redundant PCR testing has also been performed on the MDCK cells looking for herpesviruses and polyomaviruses. None were found. Induction assays to search for latent viruses are in development right now.

Although extensive testing found no viruses, we have addressed the potential presence of adventitious viral agents by a manufacturing process that will remove or inactivate them. As with the cell removal, there are a variety of steps that inactivate or remove viruses and here I would like to stress that

these processes remove a variety of viruses, enveloped viruses, non-enveloped viruses, etcetera.

The manufacturing steps that affect viral removal are illustrated in this slide. Potential viruses are inactivated by beta-propiolactone, by the viral splitting agent, by ultracentrifugation and by adsorption into chromatographic media.

The next slide illustrates the effectiveness of these steps with three model viruses. In addition to influenza virus, which must be inactivated by the process, three model viruses chosen for their characteristic properties are shown. The three viruses are herpes simplex virus, reovirus and murine retrovirus.

After evaluation of many viruses, three were chosen because they are less sensitive to BPL inactivation and are representative of a range of viral classes. As shown, the manufacturing steps are effective in eliminating or inactivating these viruses by 9 to 12 orders of magnitude.

Well, let me now summarize. Experimentally, we have noted that the MDCK cells are

tumorigenic. We have evaluated the manufacturing process for cell removal and have shown that the intact MDCK cells are effectively removed. We did not observe the MDCK cells to be oncogenic. However, DNA is removed and degraded to a nonfunctional state.

Additionally, although we did not detect any viral agent in the MDCK cells, and we did try, a manufacturing process is in place that will effectively remove contaminating undetected viruses. In essence, we have demonstrated that MDCK cells can be safely used for influenza vaccine production.

Well, now let me briefly mention where we are with the clinical development of an influenza vaccine based on MDCK cells. Phase 1, 2 and 3 studies are being carried out in Europe and they are continuing. Today more than 3,000 subjects have received the vaccine and its safety and potency, specifically immunogenicity, was shown to be comparable to licensed products. In the United States, a Phase 1 study has recently begun. Enrollment of 600 people/volunteers has been completed and the study is still underway.

Conclusion. There is an unmet public need for a readily available and reliable supply of influenza vaccine. Chiron has developed a robust, scalable and safe manufacturing process, which utilizes MDCK cells to meet these needs. And with that I will stop there. I will be happy to take questions. Thank you.

(Applause)

CHAIR OVERTURF: I have a couple of specific questions. One was you mentioned the immunogenicity of the vaccine in some 3,000 individuals. Do you know what the actual chemical effects are on the neuraminidase and the hemagglutinin with your processing? Has that been looked at in any way?

DR. RAPPUOLI: Is the question whether the process is going to change the immunogenicity of the vaccine? Well, I think in one of the slides we showed that the process we are introducing is changing only half of the manufacturing process to make a vaccine.

The inactivation of the virus and the purification and the manufacturing of the vaccine

remains the same as in the egg-based vaccine, but it changes the way we produce the virus which is produced in the cell line instead of being produced in eggs. So the manufacturing process and the final vaccine is more or less identical to the one produced in eggs.

CHAIR OVERTURF: The other thing was when you mentioned the analysis for DNA removal, you provided figures for less than 10 nanograms and less than 200 base pairs. My question was have you carried it further to actually know what the actual limits of that are? I mean, do we really know how many nanograms of DNA, you know, actual amount, not just less than 10, but do you know the absolute number?

DR. RAPPUOLI: We do, we do. It's like 10-fold less, in the range of 10-fold less than the 10 nanograms, and so it's well within the specs. But what I wanted to emphasize is that it's important to be below 10 nanograms, as we have heard this morning, but the actual importance of making sure that the amount of DNA which is left is actually degraded to a size where we cannot call it a gene and since you treat it with BPL, you actually isolate and modify the

basis in such a way there can never be a substrate for anything.

CHAIR OVERTURF: Yes, doctor?

MEMBER FARLEY: I realize that this is an advantage over having to have the egg supply available, but I'm curious whether it changes the time that it takes to actually produce the vaccine. Once you have a seed vaccine, seed virus, using the cell line versus using the eggs, is the manufacturing process about the same time table?

DR. RAPPUOLI: I will try to answer in a couple of ways. Overall, the time from the day you inoculate the egg or the day you inoculate the fermenter to the time you have the first batch of vaccine out, that time doesn't change too much. The virus has to grow in the process of activation and purification as to the change.

Where the time is very different is the lead time. If I need to manufacture an egg-based vaccine today, I can only do that if a year ago or 10 months ago I placed a contract with a manufacturer that will raise the chicken who will make the eggs and

now it will be enough chickens to make enough eggs to make the vaccine.

If I forgot to do that, there is no way I can start today to manufacture the vaccine. If I did it but I miscalculated, I did not take into account a pandemic need and I need 10 times more vaccine, it's too late. The order should have been placed 10 months ago.

On the other hand, with the same culture, what I need to do is to go to the freezer, take the cells, put them into fermenters. So the lead time goes from, I mean, 10 minutes or one day to 10 months to a year. So that is one advantage.

The other advantage is that we are talking about pandemic influenza. The avian virus kills the eggs so there is no way you can make a pandemic vaccine using the wild type virus. So if you wanted to have a rapid response now with the egg-based manufacturing, you have to take the wild type virus, go to the laboratory, make reverse geneity, generate a new virus, do all the controls and then give that to the manufacturer so they can now start manufacturing.

This is a period that more or less takes three months. With the cells which are not killed by the wild type virus, you can start manufacturing the next day. So that's another flexibility that you have.

CHAIR OVERTURF: How many strains of virus? Just as a follow-up to that question, you find no variation in viral strains from year to year that have the same growth rates?

DR. RAPPUOLI: I mean, all the vaccine manufacturers they know that in -- with eggs you get 20, 30 percent variation from strain to strain and the manufacturing processes are designed to cope with that variability every year. We have been using this cell line from 1996, using basically all the viruses that are being used for vaccine production since then, and we have not seen a variation. We have seen a variation but it's not greater than the one you observe in eggs. So it will not change the things.

CHAIR OVERTURF: Yes, Dr. Minor?

DR. MINOR: I have got two questions. One is I noticed that when you were doing the DNA

oncogenicity type assays, you were putting in of the order of 10^7 cells equivalent, which as I understand it is about the number of cells you need to make one dose.

I mean, is it possible to put in a lot more than that? I mean, how much DNA can you put into a mouse before you actually have a genuine toxic effect because of the DNA? Could you put in 10^{10} cells worth, for example? And if you can, why haven't you?

DR. RAPPUOLI: I think I can be more specific. My impression was that we used more than the equivalent 10^{10} cell. The 10^{10} cells was for the lysate where physically you cannot put more than that, but for DNA we did use more.

DR. MINOR: Okay. Okay. And the second question was to do with pandemic vaccines. I think you or Karen have done some trials at least, which at least suggested that a subunit vaccine without an adjuvant is not terribly immunogenic when you start looking at a new strain as other people have shown as well. And one possibility that people have proposed is to use a whole virus instead of a subunit vaccine

in which case, if that's what you would do, your process would change and it might very well affect all the clearance.

Would you intend to be using a subunit vaccine or would you use a whole virus vaccine and, if so, are there consequences to that?

DR. RAPPUOLI: Well, I think we switched from whole viruses some years ago and usually I don't like to go back with technology, but so our strategy is to go with subunit vaccines because with the adjuvant we have shown that we can meet the capacity and the safety that is necessary.

There are others. They feel that we should go with whole viruses, different opinions, different strategies. And I think, I mean, this process, the numbers would be slightly different from the one I showed but will not be dramatically different if you had to go with the whole virus, but that is not what I would suggest to do.

CHAIR OVERTURF: Yes, Phil?

MEMBER LaRUSSA: I was curious if you knew what the growth characteristics of the original MDCK

cells were. Did they grow in monolayers and is the ability to grow in suspension a process, a result of the adaptation process? And I guess the second part of that question, if those two statements are true, does that correlate with change in tumorigenicity?

DR. RAPPUOLI: That is a very good question. We discussed a lot about that. The MDCK cells are cells which are polarized. They grow in addition and they form a monolayer when you grow them in the lab. Actually, it's one of the cell lines which is mostly used all over the world for research purposes and it's a monolayer.

So most -- I would say all these MDCK cells with the exception of the one I showed you are cells that grow in addition. That means that, I mean, when you need to turn into high scale manufacturing, that's a limit at least in our hands. So we have been working hard to passage the cell line in well-controlled conditions in such a way that will lose the property to grow in addition and will be adapted to grow in suspension and that took a long time and many passages.

In doing this, yes, the tumorigenicity of the canine cells in nude mice increased slightly, but the advantage that we see in the manufacturing process to be able to scale, industrialize, really to meet the demand that we are talking about is enormous. And we felt there was absolutely no risk, because you have seen the numbers which are there for cell removal.

CHAIR OVERTURF: Yes, Steve?

MEMBER SELF: Yes. I have a question about the oncogenicity assays. As I understood it earlier, the safety factor that you are shooting for is 10^{-6}, 10^{-7}, something like that, and even though the results that you show particularly for the lysates and the cellular DNA are impressive, zero out of 139 animals and 204, that still only bounds the probability of an oncogenic event at about 10^{-4}, so that actually leaves a gap in terms of the evidence that these data provide in getting to that safety margin.

So what are your thoughts about that gap or are you thinking of expanding these data to numbers of animals that would close that gap or are there

other ways that you would sort of ameliorate that?

DR. RAPPUOLI: I think during the morning you heard how the regulatory agency is approaching these things. The way we are approaching that is we have continuous discussions with them and we try to do all the work which is necessary to answer those questions. Obviously, some of them are difficult technically to answer.

I mean, now modern technology has allowed to do a lot of things that we are doing and we are planning to do, so this allowed would be further characterized. But our approach is that we will discuss with the regulatory agency and we'll do all the tests which are necessary to make sure that the product is finally safe, secure and there is no problem.

CHAIR OVERTURF: Dr. Farley?

MEMBER FARLEY: You mentioned or you presented information that you do the viral testing at various points in the production from the pre-cell bank, Master Cell Bank and then the post-production.

Is there any reason or have you done or

considered doing the tumorigenicity assays at the post-production phase or have there been enough passages for there to be concern that they may have changed in any way in terms of the numbers of cells required, that sort of thing, in that stage?

DR. RAPPUOLI: I'm not sure if you're asking tumorigenicity or oncogenicity. I mean, the tumorigenicity at the end of the process is difficult because the cells have been lysed by the virus. So the oncogenicity, yes, has been done at the end because, as has been shown this morning, you want to make sure that the viral infection has not triggered an unknown agent into cells, so that has been tested.

CHAIR OVERTURF: Dr. Cook?

DR. COOK: Your downstream processing is really impressive. I just have a technical question about the tumorigenicity testing that you showed in this. At least in the handout it's on slide 13.

Was this done with bioreactor cells or what kinds of cells were used for these nude mouse tumor studies where you did the dose ranging 10^1, 10^3, 10^5, 10^7 challenges?

DR. RAPPUOLI: They were the cells that are used in the production, yes.

DR. COOK: Okay. So then just from a technical perspective, what I don't understand about these data is how you can dilute the cells essentially a million-fold and go from a tumor instance of 11 out of 24 and, after a million-fold dilution, you have a tumor instance of three out of 24.

What do you think about that in terms of what it says about the characteristics of these cells that you're using for the challenge?

DR. RAPPUOLI: Well, it's a good question that we have been discussing and, at this point, we do not have an explanation for that. You understand that this, I mean, the animal numbers are usually limited and those are the data that we are dealing with.

I mean, the way we dealt with is that the safety margins to remove any chance that any cell is going to be there is so big that eventually that is not an issue. I mean, it's a good scientific question but it's not a safety issue from our point of view.

DR. COOK: Well, I guess, my point is

there are other data that suggest that when such tumor titrations are done, when you get below 10^5 cells there were no tumors formed. And so the question is the nature of the starting material not -- as I say, the downstream processing is pretty impressive and the question is what are you trying to protect against and you don't know.

But if you have two different kinds of cells that have two different characteristics and that is considered to be a parameter that is important, it would just be interesting to understand what the differences are that cause this difference in tumorigenicity at limiting cell numbers.

DR. RAPPUOLI: Well, I understand the question and, as I said, we have been discussing that. The answer is always the same. I mean, in the absence of cells there are no tumors, whatever the scientific rationale is behind that. So what we wanted to do is to make sure that cells were not there and the clearance factors that I showed you is compelling.

Now, yes, I think that's the answer. No

cells, no tumors, so that is all we need to do. It's important, obviously, that we address the scientific questions and I think those are the things that we will continue to do.

CHAIR OVERTURF: Dr. Hetherington?

DR. HETHERINGTON: You mentioned that you plan on doing studies looking at the activation of latent virus. Could you discuss briefly at what point in the manufacturing process you think latent virus might be activated and comment on whether or not your current processes of viral reduction would address any of those risks.

DR. RAPPUOLI: Are you referring to the induction studies that were --

DR. HETHERINGTON: Yes.

DR. RAPPUOLI: Yes. Those are the things that I think is being well-addressed in the morning and what we are doing now is we are, I mean, discussing how those studies should be done and will be done. So I think, again, it's through the interaction with the regulatory agencies that will define the right protocol to do those studies.

DR. HETHERINGTON: But just in follow-up, theoretically where in your processing would latent virus become a potential problem? That's really the question, not so much what are you doing.

DR. RAPPUOLI: You are asking to a non-expert this but let me try to answer and then we'll have a lot of experts around.

If I had to guess one place where there is a risk for activating something would be during the influenza infection of the cells, because the influenza infection changes all the gene regulations, all the -- a lot of genes go up, others go down. The cell is completely disregulated. So that is the way I will expect something to come out and that is very early in the process, and so I think all the rest of the studies, the process will take care of that.

CHAIR OVERTURF: Dr. Robinson?

DR. ROBINSON: Is there any difference in the tumorigenicity profile of your cell line at the Master Cell Bank stage versus the production stage before you infect and at the commercial scale in your facility?

DR. RAPPUOLI: I guess the answer was given this morning that the number of passages between those two things is so small that usually there is not a difference.

CHAIR OVERTURF: What are the number of passages?

DR. RAPPUOLI: I need some help from -- yes.

MR. VALLEY: For the tumorigenicity studies we performed, we used end of production cells. That means we took the cells from the end of the process and also the DNA, which was isolated after infection with the influenza virus, came from end of production, were sets from the passage number of the end of production cells.

DR. RAPPUOLI: And the passages you said are, approximately, 20. Is that correct?

MR. VALLEY: Yes. We put a small safety number on that.

DR. RAPPUOLI: Yes.

MS. WALSH: Excuse me. Can you just identify yourself for the record, please?

MR. VALLEY: Ulrech Valley, Chiron.

MS. WALSH: Thank you.

CHAIR OVERTURF: Yes, Dr. Robinson?

DR. ROBINSON: Just a follow-up to that. And how many cell generations would you say that would be, because it may not be the exact same passage or split ratio between each passage?

DR. RAPPUOLI: Are you asking how many passages from the beginning of the process to the end?

DR. ROBINSON: Passages or cell divisions, either one.

DR. RAPPUOLI: Well, I think it was 20. Yes, that was the answer, approximately, 20.

CHAIR OVERTURF: Were there other questions? Well, thank you very much. I think we'll go ahead and proceed to the second presentation, which is by Solvay Pharmaceuticals Incorporated. Dr. Medema?

MR. MEDEMA: Good afternoon, ladies and gentlemen. My name is Jeroen Medema and I am a senior scientist for vaccines at Solvay Pharmaceuticals. I would like to thank CBER for the invitation to present

to this Advisory Committee our MDCK-based project and I am delighted to be here today to continue our ongoing dialogue we have with the Agency on the use of MDCK as a substrate for the production of an inactivated influenza vaccine.

What I would like to do in the next 30 minutes is to give a background of our company and its role in influenza control, a background on the MDCK cell line that we use and the vaccine that we produce on that cell line and how we came to choosing that cell line as a substrate for influenza vaccine production.

And, most important to today's meeting, I would like to share with you data-based safety analysis on the MDCK cell line and the vaccine that we produce on that cell line. And based on that safety analysis, I will come to the conclusion why we are confident that, indeed, MDCK is a safe substrate for the production of influenza vaccines.

First, allow me a moment to introduce Solvay Pharmaceuticals to you. It is the Pharmaceutical Division of the Solvay Group, which is

also active in chemicals, biochemicals and plastics, and the Pharmaceutical Division is a global company belonging to the top 40 pharma. We have major R&D sites in Marietta, Georgia, in France, Germany and the Netherlands.

With respect to influenza vaccines, we were the first in Europe to introduce an egg-based influenza vaccine in 1950 and we have a track record of uninterrupted supply since then. During that period, over 250 million doses of egg-based vaccine were administered to humans and currently we are the fourth supplier worldwide and we distribute to over 50 countries in the world.

On this map you can see in which countries the egg-based vaccine is licensed and this vaccine is produced in production facilities in the Netherlands. Well, just like Solvay, the vaccine industry has used eggs for the production of influenza vaccines with a good track record, a good safety record for over 50 years so why would we decide to go for a cell-based vaccine project?

Well, as we have heard from our colleagues

of Chiron, eggs are an open production system which make them prone to contamination from the outside. And, secondly, which might turn out to be one of the major drawbacks of the world relying on eggs for the production of influenza vaccines, the availability of eggs is certainly not a given during an outbreak of disease in poultry like, for example, the current outbreak of avian influenza in Asia and Eastern Europe.

As an example, we were confronted with an outbreak of avian influenza in the Netherlands in 2003, so two years ago, and indeed the supply of vaccine eggs was severely compromised during that period. So these were the two main reasons why Solvay decided to embark on a project to develop a cell culture-based vaccine for the production of influenza vaccines.

Well, you could use different continuous cell lines, also primary cells, of course, and so why did we select MDCK? Well, MDCK is known for its broad susceptibility to influenza viruses and also for its good growth characteristics for influenza viruses.

Over the decades there has been substantial experience with MDCK both in influenza research and surveillance and it is the most commonly used continuous cell line in the World Health Organization Global Influenza Surveillance Network.

The good growth characteristics of MDCK for influenza viruses render high virus yields which means that, indeed, MDCK is an economically feasible substrate for the production of an influenza vaccine, but also these high virus yields mean that to produce a certain amount of virus, we need fewer cells and, therefore, there is less to remove.

So with respect to our MDCK-based vaccine project, we initiated these projects in the early 1990s, which gives us more than 10 years of experience with this cell line, and we have developed a production system which uses microcarriers, so we have retained the adherence, the original adherence growth characteristics of MDCK cell line, and we have developed serum-free conditions which diminishes any risks of contaminants from animal sera.

With that production system we have

performed a preclinical and clinical development program, which was mainly directed to support licensure in the European Union, and we were the first to be granted a license for a cell culture-based influenza vaccine ever in the Netherlands, and we were the first to be granted a license ever for a product for human use that uses MDCK as a cell substrate.

This license was based on a product on pilot scale and we are currently in the final stages of validation of a commercial scale facility and with products coming from that facility, we will update our current marketing authorization and we will follow that by applying for licenses throughout countries in the world, including the United States.

These are pictures of our new purpose-built, dedicated production facility for the MDCK-based influenza vaccine. It is an inactivated subunit influenza vaccine and this system allows us to grow MDCK cells in closed bioreactors and also so this is less prone to contamination compared to eggs, and it also uses fully closed waste treatment systems which is important when we want to produce highly pathogenic

pandemic-like influenza viruses.

So we not only protect the product from the outside, but we also protect the outside from highly pathogenic influenza viruses. This facility has been designed to operate under Biosafety Level 3 conditions, which allows the production of highly pathogenic influenza viruses like the current H5n1.

With this introduction, I would like to turn to what is most important to today's meeting, the safety assessment of our MDCK cell line and, well, the Defined Risks Approach as designed by CBER has been extensively discussed this morning.

We follow this approach for our MDCK cell line and this contained three steps. We first characterized the cell line that we used. We then assessed our downstream processing, so that is the vaccine purification process to eliminate any potential risks that may exist with our cell line. And, finally, we performed a preclinical and clinical development program so that also gives us experience on the safety of the final product.

I would like to go through each of these

three one-by-one starting with cell line characterization and, again, this was threefold. We did an audit trail on the passage history of our cell line. We tested for absence of adventitious agents and we assessed the tumorigenicity.

Well, as presented by Dr. Krause this morning, the MDCK cell line was isolated from the kidney of a healthy female cocker spaniel in 1958 by Drs. Madin and Darby and it was subsequently deposited by Drs. Madin and Darby in 1964 at the American Type Culture Collection or ATCC.

The ATCC only started again in 1991 with this official deposit to prepare a larger working stock and Solvay acquired cells from this working stock in 1992. This gap is quite important. This means that between 1964 and 1991 there has been no manipulations with the MDCK cell line and, therefore, there is no risk of introduction, no concerns of introduction of any bovine spongiform encephalopathy-like agents. Solvay acquired files from the ATCC cell stock in 1992 to produce a Master Cell Bank and Working Cell Bank.

If you look at passage level, the MDCK cell line that was deposited by Drs. Madin and Darby at the ATCC was at passage level 49 and ATCC performed three subsequent passages to prepare the larger working cell stock, so at passage level 52. We acquired passage level 52 from the ATCC to prepare a Master Cell Bank at passage level 56 and a Working Cell Bank at passage level 57.

However, in order to study the cells that we are going to use for vaccine production, so that will be cells from the Working Cell Bank, in order to study the passages that we use for vaccine production are stable and safe, we also prepared what we call an Extended Cell Bank at passage level 97.

So passage levels between 57 and 97 will be used for vaccine production and we have used passage levels at 97 or above to assess the safety and with that assessment, we can indeed extrapolate the safety of the passage levels used for vaccine production.

The second part of cell characterization is the testing for presence of any adventitious agents

and, as presented by Dr. Khan this morning, there are some general tests that indeed assess for adventitious agents and there are some more specific tests.

This is the testing that Solvay performs, so these are the more aspecific tests that Solvay performed on its cell banks, so both on the Master Cell Bank and on the Extended Cell Bank, and this included indeed, for example, the inducer assays and also the PERT assays for retrovirus testing. All the tests were negative, so we did not find any evidence for presence of adventitious agents in our cell banks.

Next to this more general test we also assessed the potential presence of adventitious agents that could originate from the cocker spaniel from which MDCK was isolated and also, we assessed the susceptibility of the MDCK cell line for specific viruses, because there are the adventitious agents that might be of concern.

So we did specific testing for viruses that might naturally occur in dogs and we performed specific tests for viruses for which MDCK is susceptible, and this includes viruses that were

presented by Dr. Khan this morning and also some more. Again, all tests were negative so, again, we did not find any evidence for presence of adventitious agents in our cell banks.

Then I would like to turn to the third part of cell characterization which is the assessment of the tumorigenicity. Again, tumorigenicity is a phenotypic characteristic of a continuous cell line and it means that the cell line can lead to the development of tumors in certain animal models. And, of course, there is a concern of exposing a vaccine recipient to any tumorigenic component of that continuous cell line.

So in consultation with CBER, we performed a program to assess both the tumorigenicity and the oncogenicity of the MDCK cell line, and I would like to compliment CBER with the Defined Risks Approach because by using this approach in practice we, indeed, see that this is a very practical approach to assess the safety of continuous cell lines, tumorigenic cell lines, for vaccine manufacture.

We first studied the tumorigenic potential

of intact cells and we performed two studies, one of four week duration and one of six months duration, both in adult immune-deficient nude mice. We assessed the tumorigenic potential of cell lysates to assess the potential presence of any oncogenic viruses and this was again in a study of six months duration in adult immune-deficient nude mice, but we also added a larger panel of animals, also the newborn nude mice, the newborn hamsters and the newborn rats, and we assessed the oncogenic potential of DNA by inoculating, again in a study of six month duration, the same panel of susceptible animals.

To start with the study with intact cells, we performed a study of six months duration in the adult immune-deficient nude mice and we inoculated these mice with different levels of MDCK cells, so 10^7, 10^5, 10^3 and 10^1 cells. Next to that we also acquired the lowest passage level from ATCC that is currently available to make a comparison between the cell line that we use at our passage level, so at a high passage level to the passage levels currently available from ATCC, so let's call it the parent cell line and we

included negative and positive controls.

Well, if you first look at what happens at the site of inoculation of these mice, there are some important observations to be made here which are important to assess the safety of the cell line. First of all, this cell line does not lead to nodule development at the site of inoculation at low dose levels, so we do not see any nodules when exposing the mouse to 10 or 1,000 cells. But we do see development of nodules at the site of inoculation when exposing them to higher dose levels.

From this we can, indeed, calculate a tumor producing dose at 50 percent of animals, so TPD_{50}, and this is just below 5. Therefore, this cell line should not be considered highly tumorigenic. If we look at the sizes of nodules at the site of inoculation, you see here that by exposing them to lower dose levels the nodules also are smaller, but at the higher dose levels, indeed, the nodules are larger.

But when you compare the nodules to the positive control animals that were inoculated with

HeLa cells, then you again clearly see a difference. These animals already display aggressively growing tumors after day 40 and they were humanely killed at day 40 to prevent any suffering.

If we compare the MDCK cell line at passage level 98 to the passage level 56 of the parent cell line, we again see a difference. The nodules are clearly smaller, so this shows that the MDCK cell line at higher passage level has, indeed, an increased tumorigenic potential and this is likely caused by the fact that we have adapted the parent cell line to grow under serum-free conditions, which means that we have adapted it to grow under more difficult circumstances such as the immune-deficient nude mice.

We also examined regression of tumors throughout the observation period of six months and we, indeed, see that the majority of tumors that were inoculated with both 10^5 and 10^7 cells partially regressed and we even see complete regression after six months in five animals in the 10^5 group and four animals in the 10^7 group.

After six months we sacrificed the animals

to do a characterization of any tumors that we have observed throughout the study and this was first done by histopathology. If we again look at the nodules that develop at the site of inoculation, we could confirm the presence of tumors by histopathology in six animals that were inoculated with 10^5 cells and in 16 that were inoculated with 10^7 cells.

So, again, we could not confirm tumors in all animals that did show a nodule throughout the six month study and this is another sign that, indeed, nodules regress throughout the six month observation period.

We also assessed other tissues for presence of any neoplastic growth and we, indeed, found three tumors. We found a tumor in the spleen of one mouse that was exposed to 10 cells and we observed a tumor in the lung of another animal that was exposed to 10^7 cells at passage level 98 and in the lung of a mouse that was exposed to 10^7 cells at passage level 56.

Also, these tumors were characterized by histopathology and if we talk first about the tumor in

the spleen, this was characterized as a histiocytic tumor, a murine histiocytic tumor which is not uncommon in these types of animals, and it was confirmed by PCR to be of murine origin. So this is a murine tumor that spontaneously developed in this animal and is not related to the exposure to the intact cells.

All the tumors that we found at the site of inoculation were characterized again by PCR to be of canine origin, so these are MDCK cells that, indeed, can grow in the immune-deficient nude mice.

If we look at the two tumors in the lungs, these were characterized by histopathology to be murine adenomas and also here we performed PCR analysis to characterize, identify the species of origin, and here we found a very low level of canine DNA in the canine PCR just above background level, which is several magnitudes of order below the signal that we find for these tumors. So, again, we believe that these are, indeed, spontaneous tumors in these types of animals, which is not uncommon in these types of animals.

We also looked at lysates at the dose level of 10^7 cells, so this is a dose level at which, indeed, the intact cells do lead to nodule development and we observed again in a six month study what happens in both adult and newborn nude mice, in newborn hamsters and in newborn rats.

And here we do not see any nodules, not at the site of inoculation nor in other tumors. So we did not observe any oncogenic potential or tumorigenic potential of the lysate of cells at a dose level at which the intact cells do lead to nodule development in the nude mice.

If we look at the study where we assess the oncogenic potential of MDCK-DNA, again a study of six months duration using the same panel of animals as in the lysate study, and here we exposed these animals to at least 100 micrograms of purified, but still intact, cellular DNA. Just as in the lysate study, we do not see any nodule development at the site of inoculation and we do not see any signs of neoplastic growth in any other tissues, except in two mice that were inoculated with the DNA.

And, of course, we were somewhat concerned about these tumors and we further assessed these two tumors. We characterized them by histopathology. So one mouse displayed a tumor in the liver which was characterized as a histiocytic tumor, again not uncommon in these types of animals, and another mouse displayed a tumor in the liver which was confirmed to be a lymphoma.

These are like the spontaneous tumors that you would expect in these types of animals, but we did not observe them in our negative control group. We only observed them in our test article group. Therefore, we have initiated follow-up studies to further assess the incidence rate of spontaneous tumors in these types of animals, because one of the drawbacks of these test systems is that there is not a lot of information available about incidence rates of spontaneous tumors.

So we will perform a study comparable to the one I have just presented to you, but using larger group sizes to, indeed, generate more data on the incidence rate of spontaneous tumors in these types of

animals and we will also perform a fetal and neonatal safety study in rats where we will include at least again 100 micrograms of MDCK-DNA as one of the test articles. With this we believe we will, indeed, generate every data that we can to show that there is no evidence for any oncogenic potential of MDCK-DNA.

So to summarize the tumorigenicity we show a moderate tumorigenic potential in our cell line in immune-deficient animals. The majority of the nodules that we have observed in these animals partially regress or sometimes completely regress, and the tumorigenic potential indeed increases with passage level, which is likely caused by the fact that we have adapted it to serum-free conditions. All the histopathology observations that we made at the high passage level were in line with what you expect for an MDCK cell line in accordance with literature.

We also performed other studies, so these were not tumorigenicity studies, but where we indeed exposed immune-competent animals to intact cells and also to cell lysates and there we have never observed any tumorigenic potential of intact cells. So we only

observe a tumorigenic potential in the immune-deficient nude mice.

The lysates of MDCK cells at a high dose level, we do not find any evidence for presence of a tumorigenic potential with these lysates and we consider that we did not find any oncogenic potential of MDCK-DNA, but we will initiate follow-up studies to further confirm this.

Well, as largely discussed this morning, it is not only about what is present in your original cell line, but it is also important to assess what is present in your final vaccine. So we also assessed our production process to eliminate any potential concerns that might be associated with the MDCK cell line and we looked at elimination of intact cells and elimination of cellular DNA.

Here is an overview of our vaccine production process. This was largely based on the egg-based subunit vaccine production process, but of course using MDCK cells rather than eggs for virus production. And we have added several specific steps to eliminate cellular components like host cell

proteins and host cell DNA.

First, the elimination of intact cells. The cells will already be lysed by the infection of virus and we have several steps already very early in the process to eliminate intact cells. So we know that by homogenization and centrifugation we already get rid of practically all cells. Subsequent to these early process steps, we have several very efficient steps to remove intact cells, which will indeed give a redundant removal of intact cells from the final product.

We indeed validated the elimination of intact cells at pilot scale and we assessed several of the steps that I just showed you, centrifugation, detergent treatment, ultracentrifugation and the .22 micron filtration and our three subsequent steps at the end of the process, and here we find that indeed we have a safety factor or a clearance factor of at least 10 to the order of 21.

In our current validation package that is ongoing for our new facility, we have included the same validation on large scale, so we will generate

also validation data on large scale to show that we indeed redundantly remove intact cells.

Next to the actual assessment of removal of intact cells, we also assess if we removed the tumorigenic potential early on in the process and there were some questions this morning about potential activation of any tumorigenic components by the vaccine production process.

So we, indeed, inoculated MDCK cells at this step so after they were processed until this step at a dose level of 10^7 and also after this step, and we did not observe any tumorigenic potential already early in the process. So we know that we do not only remove intact cells, but we also remove the tumorigenic potential already early in the process.

If we look at elimination of DNA, there are some other specific steps that are designed to physically remove DNA or digest DNA into nonfunctional fragments and we use two steps with Benzonase, which was shown in the presentation by Dr. Peden to be very efficient in digesting DNA into nonfunctional fragments, and we also thereby lose the infectivity of

the residual DNA. The total process time that we'll use Benzonase is at least 24 hours, so that puts it a bit in perspective with the data that Dr. Peden presented with the four minutes.

Next to these steps early in the process we also have several steps that specifically or physically remove DNA, any residual DNA, and, therefore, that will fully eliminate or that will efficiently ensure that the DNA levels in the final product will be below acceptable levels.

Again, we assess this on pilot scale and we validated this on pilot scale and you see here a clearance factor of at least 760,000, and we have also included this in the currently ongoing validation on commercial scale where we will not only assess the content, so the residual content and also the clearance factor for DNA content in our final product, but we will also assess the size of any residual DNA in our final product.

We are confident based on the data we have obtained on pilot scale that our production scale indeed will render a final product that will meet the

specifications of below 10 nanograms per dose and also below any length that might be specified by regulatory authorities.

So a summary of the downstream processing, adequate purification and testing will warrant vaccine safety and this is independent of any potential concerns that might be associated with the original cell substrate. This is an ongoing process and we are committed to follow-up this process in accordance with the latest scientific insights and also with regulatory guidance.

Well, as I explained earlier, we have generated a body of evidence both on the final product, on the safety of the final product, and I would like to present some data of that.

We did several preclinical studies where we showed indeed, for example, local tolerance, systemic toxicity, pyrogenicity, the mutagenic potential and active and passive anaphylaxis of our final product, so the MDCK-based subunit vaccine, and we used several species, several administration routes and several doses, various doses, to assess the safety

of the final product.

And the results are that we do not observe any local irritation, no adverse effects with regard to systemic toxicity, no distinct increase in body temperature, so no evidence for pyrogenicity. We did not observe any increase in number of micronuclei in the test for mutagenic potential and there is no active anaphylaxis associated with our MDCK-based vaccine, and we observed by passive anaphylaxis that the MDCK-based vaccine is favorable to the egg-based vaccine.

With respect to clinical experience, we have performed 14 studies including in total just over 1,000 subjects that were administered with the MDCK-based subunit vaccine. This was in different populations, so both in healthy adults and in elderly up to the high 80s and early 90s, and also included patients at risk for complications with influenza. And the major objectives of these studies were to show comparable immunogenicity or non-inferior immunogenicity and comparable safety with, as a comparator, an egg-based influenza vaccine.

With respect to safety, we observed that the local and systemic reactogenicity profile is comparable to the egg-based vaccine. All reactions that were observed with the MDCK vaccine were minor and short-lived and we did not observe any unexpected safety findings. With respect to immunogenicity, we demonstrated with these studies that the MDCK-based vaccine is not inferior to the egg-based vaccine.

So to summarize, what we demonstrated with this clinical development program, that the MDCK-based vaccine has a comparable safety and immunogenicity profile as the egg-based vaccine and this was also the basis for granting the license in one of the European Union member states.

Well, to conclude this presentation, Solvay is confident that MDCK is a safe substrate for the production of an inactivated influenza vaccine and we believe that we, indeed, have generated the data to show this. The use of MDCK will improve the reliability of influenza vaccine supply not only for seasonal influenza vaccines, but it will greatly enhance and will play an important role in improving

current pandemic preparedness plans, and we will apply for licenses in countries throughout the world, including the U.S.

We are committed to assist public health initiatives to fight the burden of influenza and to maintain our front runner position in this field. With me are several colleagues of Solvay and also some external experts that are happy to address any questions you might have. Thank you very much.

(Applause)

CHAIR OVERTURF: Yes, Dr. Minor?

DR. MINOR: Can you say some more about these tumors that you are seeing, which weren't at the site of inoculation? If I heard you right, you characterized some of them by PCR and shown they were murine, but didn't you say that you had also looked by canine PCR and there was a low signal or did I mishear that?

MR. MEDEMA: You missed me there, because we performed indeed the characterization by PCR of the tumors at the site of inoculation both by murine, for murine DNA and for canine DNA, and they were all shown

to be canine DNA. They were not murine.

DR. MINOR: I was talking about the tumors which were away from the site of inoculation.

MR. MEDEMA: Okay.

DR. MINOR: We heard about four or five animals had a tumor away from the site of inoculation, didn't they?

MR. MEDEMA: Yes.

DR. MINOR: And were they characterized by PCR as well?

MR. MEDEMA: The tumor in the spleen was characterized to be murine, not canine, so not of canine origin. We had the two tumors in the lungs in the intact cell study and there we found a very low signal for canine DNA. We found a very high signal for murine DNA. And so there is a discrepancy between the PCR results and the histopathology results.

DR. MINOR: Right. So can you say a bit more about your canine PCR? I mean, if you are looking at oncogenicity of DNA, for example, as opposed to tumorigenicity of the cells, you might expect perhaps to see just a small piece of dog DNA

put into the mouse cell, so predominantly the tumor will be murine, but you would have a small canine signal perhaps.

Can you say something about the canine PCR that you're using here?

MR. MEDEMA: Yes. We used the SINE sequences, so the short -- well, I don't know exactly what the abbreviation stands for, the SINE sequences, so repetitive elements to assess more in general canine DNA.

DR. MINOR: And do you still have the tumors and are you transplanting them and carrying them on and establishing cell lines from them and so on, because I think you should actually.

MR. MEDEMA: Well, these tumors were all wax-embedded to perform histopathology and that gives you some complications first to extract any nucleic acids, and so it's quite difficult to perform PCRs on these tumors, and it will certainly give you some complications in establishing any cell lines from them.

CHAIR OVERTURF: Dr. LaRussa?

MEMBER LaRUSSA: Two questions. If I heard you correctly, I think you said that you stuck with the adherence cell system for the MDCK, and if I heard that right, I'm curious why you decided to do that and not adapt to cell suspension.

And the second question is, and I may have missed this, in the tumors that developed in the mice after injection of DNA, did you perform PCR on those for canine DNA?

MR. MEDEMA: Well, first to address your first question, we indeed did not adapt the MDCK, so the original MDCK cell line, to growth in suspension. And the main reason for that is that we prefer to maintain its original growth characteristics and also to maintain its polarized character, because for correct processing of influenza viruses you need polarized cells for correct processing and released budding and release into the supernatant. So that is the main reason why we decided not to go for adaptation into suspension.

To address your second question about the two tumors that we observed in the DNA study, in

consultation with CBER we attempted to assess any presence of murine retroviral sequences in there, because you would not expect any canine DNA to be present there.

You would more expect that if there is an oncogenic potential in the canine DNA, you would expect that the murine tissue would be transformed into a tumor. So we assessed the presence of murine retroviruses to support information that these are indeed murine retrovirus-associated lymphomas.

MEMBER LaRUSSA: Wouldn't it also be possible that some of the canine DNA had been incorporated?

MR. MEDEMA: Yes, that would be a possibility. However, you would still expect that the vast majority of cells would be of murine origin.

CHAIR OVERTURF: Yes, Dr. Cook?

DR. COOK: Your observations in the nude mice with the tumors at remote sites raise an interesting question to me, and that is it seems like the thing we're all struggling with is what is the safety of the vaccine when it all gets made?

And so an initial question that I can follow is is there any toxicity of the vaccine itself in mice? Is there an LD50? Can you inoculate influenza vaccine into nude mice? Do you know?

MR. MEDEMA: Well, we haven't observed it. I can imagine that you might even expect an LD50, when you have to inject so much volume that you might expect an LD50 from the volume that you have to inject into the mice, but --

DR. COOK: But it's an inactivated virus, theoretically.

MR. MEDEMA: Yes.

DR. COOK: So you're injecting antigen.

MR. MEDEMA: Yes.

DR. COOK: So an interesting experiment to me, whether this has relevance, but is if you were to inoculate newborn or weanling nude mice with the final product and say, okay, I want to observe a large cohort of these animals over the course of their, you know, admittedly short lifetime, say three years, and a control cohort to answer your question about spontaneous tumor formation rates in control and

treated animals.

Then you would have some way to look at a population, admittedly not human, of control and vaccinated or animals exposed to this putative risk and ask whether there is any difference. And then you can go off and say well, so there are some spontaneous lymphomas and there are some other things that occur in this cohort of a few hundred nude mice and what happens to those that we have inoculated with like one tenth of an LD50 of the vaccine? Is there any difference?

And then go off and sort out those tumors to see what happens. Otherwise, you're spending a lot of time trying to check it along the way, but you don't really ever ask the final question that we're all interested in, which is you give this all to kids and they live 100 years, what happens?

MR. MEDEMA: Well, it's an interesting suggestion and we will certainly take it into consideration, yes.

CHAIR OVERTURF: Dr. Minor?

DR. MINOR: This is the same question I

asked the previous speaker. If you get to the stage of wanting to make a pandemic influenza vaccine, bearing in mind that currently your product is a non-adjuvanted, split subunit, highly purified preparation and jolly good and so on and so forth, it may be that if you're going to a pandemic influenza vaccine, you would want to use a whole virus, right, to make it more immunogenic.

I mean, if that is the case, how does that impact on the clearance of your DNA, for example, throughout your process?

MR. MEDEMA: Well, our current approach for pandemic influenza vaccines indeed is to pursue subunit or split-like vaccines and if we for some reason will be unsuccessful in developing an effective vaccine, we will consider developing a whole virus vaccine and then we will certainly need to revisit all our clearance data that we have obtained for intact cells, for DNA and for viruses.

CHAIR OVERTURF: Other questions? Okay. I think with that we'll plan to take a break and we're scheduled to reconvene at 3:15. Thank you.

(Whereupon, at 2:53 p.m. a recess until 3:23 p.m.)

CHAIR OVERTURF: I would like to open the remaining sessions and before we start, Dr. Krause is going to provide some guidance in providing goals for what this discussion should be.

DR. KRAUSE: Yes. So do I need to turn this on? Oh, it's okay. Function F8. Okay. Right. So this is just the last slide from the talk that I gave. And so what I did was I put into a file here the concluding slides, each of the talks, for Dr. Lewis, Dr. Khan, Dr. Peden just to remind you of what the OVRR recommendations are.

But, obviously, the goals for the meeting are the discussion of the use of MDCK cells, including those that are highly tumorigenic, in manufacture of inactivated influenza vaccines, a discussion of the OVRR approach to evaluating the safety of tumorigenic cells for use in vaccine production, and then discussion of any additional steps CBER should take to address issues associated with the use of neoplastic cell substrates.

And just to remind you then, Dr. Lewis in his talk went through some specific recommendations for how tumorigenicity testing of tumorigenic neoplastic cell substrates could be done, including the duration of testing and the doses that should be tested, determination of the species of origin, necropsies and evaluation of spontaneous tumors that develop for evidence of DNA from the cell substrate.

Dr. Khan described the cell bank testing that generally is recommended, including the same kinds of testing that are done for any cell bank with a specific focus, because of the tumorigenicity, on in this vitro induction assay for unknown retroviruses and DNA viruses with subsequent generic detection assays, as well as the in vivo cell lysate assay for unknown oncogenic viruses. She also went through in-process testing and described viral clearance studies and how those might most appropriately be done.

And then Dr. Peden described the concepts of clearing the amount of the DNA both by reducing its amount and reducing its size to below 200 base pairs, talked about the safety factors that can be obtained

by doing that and then described also an animal inoculation assay that can be done to provide further assurance about the safety of residual cell substrate DNA.

So that is the OVRR approach that we would like you to discuss in the context of this second question, and so I will sit down now and allow you to begin this discussion unless you have further questions for me.

CHAIR OVERTURF: Any questions for Dr. Krause?

DR. MINOR: When you say in your second bullet point vaccine production, you mean any vaccine. Is that right?

DR. KRAUSE: So --

DR. MINOR: So we're talking like live measles and things like that, are we?

DR. KRAUSE: So if you have a comment that you think is relevant to the use of tumorigenic cells for vaccines --

DR. MINOR: Yes, I do.

DR. KRAUSE: -- other than MDCK cells, we

would welcome that comment. But, of course, what we really need to get out of the meeting today is an understanding of how you feel about the use of these particular cells in the context of the inactivated influenza vaccines.

CHAIR OVERTURF: I think as I see it, I really think the role of the Committee is to, first of all, evaluate the process that we have used since 1998 and have tried to develop with repeated presentations to VRBPAC to see whether that process has worked and whether we feel that process for evaluating these kinds of vaccines, cell lines, have been sufficient and whether additional strategies need to be considered. And then, lastly, whether we are at the point perhaps where the process could be used to develop cell lines specifically for vaccines either in the future or some vaccines which are currently needed like the inactivated influenza vaccine.

So with that, I will open up the discussion and see. This is a free, open discussion. Forthrightness is appreciated and we'll go from there. Any comments? Dr. Karron.

MEMBER KARRON: I actually just had a question for both of the manufacturers and this really had to do with the issue of elimination of intact cells, and I think both of you clearly showed a great reduction in terms of the potential for introduction of intact cells.

But I was really curious to see that it seems to me that your processes are really quite analogous, but your estimates are very different. So, for example, for filtration, you know, one estimate was 3.6 logs and one was 8.8 and it does give me some concern about the robustness of your calculations, and I was just wondering if you could each comment on that.

DR. RAPPUOLI: You're right. You will see for a similar process like filtration, you are seeing different numbers. Now, the numbers you are seeing are the numbers for which the process has been validated for. So a .2 micron filter has the potential up to 10^{11}.

But if you validate during your process for 10^{8}, that is where you put your number or

calculation. So it's actually the real validation which is put in those numbers not the potential of the filter, so that is what the process is guaranteed for.

CHAIR OVERTURF: For the transcript, that was Dr. Rappuoli from Chiron. Would the speakers, please, when they approach the microphone, identify themselves. Thank you.

MR. MEDEMA: Jeroen Medema from Solvay. The numbers that we have shown, for example for sterile filtration or .22 micron filtration of at least 3.6, all these assays depend on the level that you can start with and the sensitivity of what you can still detect after you have performed, for example, this filtration.

And, well, I would like to discuss with our colleagues at Chiron how they did this, because we would love to get these numbers at 8 logs, but I am confident that these are robust processes and, indeed, sterile filtration is quite an absolute way to remove intact cells.

CHAIR OVERTURF: Dr. Self?

MEMBER SELF: Just to follow-up on that,

one of the morning presentations by someone from the FDA referred to this process and I think there was a part of the slide that said that often when you add these clearance factors across different steps, it somewhat overestimates the total clearance when viewed, you know, from beginning to end.

And I wonder if you could comment on that and just how much of a fudge factor should be accounted for by this kind of phenomena.

MR. MEDEMA: Well, if you look, for example, at the clearance of adventitious viruses, it would -- well, indeed, you should not use the same steps to add up to a total clearance factor, because while a virus will escape a sterile filtration, it will also escape the second sterile filtration.

But if you look at removal of intact cells with, for example, a .22 micron filtration, this is such an absolute physical removal that, indeed, you can add up these types of processes for your total clearance factor, but you have to ensure that you have different, independent processes that indeed -- multiple processes that will ensure efficient removal

of, well, any component that might be associated with any risk.

MEMBER SELF: So is there any overall assessment from beginning to end that could be applied and then compared to this summation or is this just more of a qualitative point?

MR. MEDEMA: Well, you always have to perform spiking experiments because it is impossible to start with very high levels, for example, of intact cells. If you have higher concentrations than 10^8 then, well, that's physically impossible. So if you would start with 10^8 and then assess what you end up with, you will end up with less than 1, but that is the limitation of these types of studies. So you will have to perform spiking in different steps to come up with this more comprehensive assessment of your process.

CHAIR OVERTURF: Dr. Royal?

MEMBER ROYAL: Thank you. I actually had the same question as Dr. Karron, as well as an additional question, but to get to the first one, the fact that using the same procedure to take cellular

material out of the vaccine product has given you sort of different calculations at the end, it makes me think that maybe the procedures aren't really the same and whether or not there may be a role for CBER in providing some oversight as to exactly what is being done as the purification process is being performed.

The other question that I had had to do with the fact that once you have got your viral product, there is the six month add-on associated with inoculating the 4 week-old rats and observing them.

Is that done in parallel with sort of quality control type procedures to look at your vaccine product or does that wait until the six months is over? Because one of the reasons for arguing for pursuing the MDCK cell line approach is that you save 10 months on the back end, but if you lose another six doing that post-production check, every step is a positive step, but it would make it seem as though it's smaller than what it might otherwise seem.

MR. VALLEY: Ulrech Valley, Chiron. I wanted to follow-up the answer to the first question because of the uncertainty of the ability of the

filters to remove cells or not, and I just wanted to say that we use a validated system for that. And what we did was we did a filter validation with model organisms.

That means we used yeast cells and also the organisms which are used to ensure filter integrity for sterile filtration, and this is how we put the load of organisms and, therefore, you can calculate these high numbers.

This allows you to increase the sensitivity of the test system and because this micron is much smaller, more than 25 times smaller than the cell, you get up with this high numbers and this was developed together with the filter manufacturers, this system, so we are very confident with these numbers.

And just to -- we didn't mention that we have one more filtration step and we mentioned that there is also an ultra filtration step. So I think the numbers we get for the total removal of cells are still an underestimation.

MEMBER ROYAL: Just to follow-up, I'm not trying to express doubt in the quality of your

purification process. It's just that the fact is that it's not the same outcome in both environments, so there is probably something. It might be proprietary, but there may be something, probably something going on that is different than one versus the other and it may be that that's where the quality control has to be extended so that the outcome is the same.

MR. VALLEY: A short answer to this. It just depends on the spike level you can apply to the filters. If you work with cells, the problem is that you cannot detect and you can only apply with a special amount or a maximum amount of cells until the filter will block and you can increase the number or you can do it with other microorganisms. You can detect better and you can apply higher challenge numbers, so you get higher reduction values.

DR. KRAUSE: So I think what he is saying is that the tests were different using different kinds of challenge cells.

MEMBER ROYAL: Or reagents.

DR. KRAUSE: So they were able to prove things differently. Your other question though I was

just going to comment on. One of the advantages of using these kinds of cells that can be banked is you can do these tests once on a cell bank and there are those cells you know are going to be good and will have passed those tests as long as you keep going back to that Master Cell Bank.

And so while there is -- for these particular tests there may be a six month lead time. Once those six months are over, then you have your bank and then you can use that to rapidly manufacture a vaccine, whereas -- so, in fact, one does put a little bit of extra work in up front, but then that saves you time at the end.

CHAIR OVERTURF: Dr. Markovitz?

MEMBER MARKOVITZ: Yes. I would like to-- we were talking about this in the Committee during the break and a very interesting question was raised that I certainly don't know the answer. If I could ask both manufacturers.

Once you actually -- in the final product, besides the hemagglutinin and neuraminidase, what else is there at the end of the day as long as that's not

violating proprietary questions?

MR. MEDEMA: Jeroen Medema of Solvay. We have several tests for which we test each lot of the final products. One of them is indeed the amount of hemagglutinin and the amount of neuraminidase present and we, indeed, end up with a highly purified vaccine. Next to hemagglutinin and neuraminidase there will be some viral phospholipids present and we know that there is some non-antigenic hemagglutinin present, so that has probably -- throughout downstream processing has been disrupted or the confirmation has changed and, therefore, it is no longer antigenic.

There is some residual DNA present, as we have shown. There might be some residual host cell proteins present, but in principle it is a highly purified vaccine.

MR. VALLEY: Ulrech Valley, Chiron. Yes, I can confirm this statement. So mainly we found hemagglutinin and we also have inactivated hemagglutinin. This is quite common for split processes, so that it produces sort of a fine part of the hemagglutinin inactivated for splitting procedure.

The other protein we have is M1 protein which is also part of the virus and we also have host cell proteins, but this is below 5 percent.

CHAIR OVERTURF: Yes, Dr. Cook?

DR. COOK: One thing I would just like to raise for a discussion, maybe for consideration by the FDA, is that the way this is being sort of discussed is as if MDCK cells are the same. There is this one thing that is being used for creation of vaccine by two different companies and then there is a processing step and then out comes the virus or the proteins used for the vaccination.

But it sounds to me like these are quite different cell populations being used. They both came from the same cocker spaniel, but then they went through very different courses to end up in these two companies to be created as a source, a substrate, for vaccines.

In one case they have been adapted through what somewhat sounds like heroic efforts to become suspension culture growing cells that can be used in these biofermenters. In another case they are growing

on microspheres and adherent substrate. It sounds to me like they even came from different sources in the first place.

So I don't think it's fair to assume that all MDCK cells should be considered equal when trying to make these judgments, and I'm not sure exactly what to do about that, but it's probably a good thing to discuss.

CHAIR OVERTURF: Well, I think it also gets to the larger question of whether the process for evaluating the safety of these vaccines, particularly in terms of their oncogenic capabilities or tumorigenicity capabilities or both, are adequately defined over the last seven years and whether, during the time of either ramping up to Phase 3 trials with these vaccines, whether they are going to provide adequate guidance from the FDA for the licensure or approval of these vaccines.

Dr. Minor, you may have a thought.

DR. MINOR: Well, it seems to me that certainly one of the slides at least, there were three specific issues that were raised. Okay. One was

adventitious agents. One was the tumorigenicity of the cell and the other was the oncogenicity of whatever you mean by that, okay, it seemed to me.

It seems to me that the adventitious agent issue, I mean, I just say this because this is my personal opinion, okay, you can deal with that by the procedures which are already in place. It's not necessarily an easy thing to do, but I think the procedures are fairly clear what you have to do or what you should be trying to do, and I don't think that these kind of cell substrates raise issues over and above any other kind of cell substrate from that point of view. That's not to say it's not an issue, because I think it's a major issue, but they are not issues which are unique to this kind of cell substrate.

With respect to the tumorigenicity of the cell line, this has always been a big discussion in these kind of meetings about does a highly tumorigenic cell line matter more than a low tumorigenicity cell line.

I think the chances of having a viable

cell in the end product are vanishingly small and I really don't think that that, quite honestly, matters simply because provided the process is appropriately validated, provided you have got all this treating with whatever you treat with, I think the chance of a live cell coming out of the end of it is not very high at all.

And that to me leaves just the oncogenicity issue and it's not clear to me whether the high tumorigenicity cell lines are actually associated with more oncogenicity than the low tumorigenicity cell lines or, indeed, whether any of them are associated with tumorigenicity or oncogenicity at all. And to me that is the outstanding issue, I think, which I have, you know, some brooding about. All right. Okay.

MR. ONIONS: I wonder, Chairman, if I can make a comment on Phil Minor's position. I am David Onions. I am Chief Medical Officer of Invitrogen Corporation, a consultant to Chiron and a former consultant to Solvay.

I think it's important to understand what

genetic differences might account between the low tumorigenicity cell lines and the high tumorigenicity cells lines. In fact, there is quite a lot of published data on the MDCK cells.

If you look at the papers from Rindler and from Taub in 1978 and 1981, they looked at first of all cell lines that were regarded as low tumorigenic MDCK cells. In fact, they didn't cause tumors at the 2×10^6 level. Now, if you take a single oncogene, the ras oncogene, transfect it into those cells, those cells now are highly tumorigenic defined by them as causing tumors at 2×10^6 and are also metastatic.

So if we take the case that was presented earlier by Keith Peden that probably any tumorigenic cell line has probably four to six genetic hits, then the addition of a single genetic hit can radically transform the tumorigenicity of that cell line.

So I think when you think of it in those terms and then look at the consequences of that in terms of the kinds of inactivation steps that are taken for the DNA, it's 2 to less than 200 base pairs and alkylated or it's treated with Benzonase in the

case of the Solvay process. Both of these really -- I think the additional genetic changes are really insignificant in comparison to those processes that occur in manufacturing.

CHAIR OVERTURF: Yes, Dr. Hetherington?

DR. HETHERINGTON: Well, with respect to the question about general use of tumorigenic cells or oncogenic cells in production of vaccines, the cellular removal and the DNA inactivation steps seem to be quite robust and quite rigorous. The step though that -- and it relates to Dr. Minor's comments earlier about can you make whole virus vaccines out of these processes.

If I understand what I have heard today about the manufacturing processes, you would lose the viral reduction processes in the preparation of the whole cell virus or live virus vaccine based on the MDCK description today. And, in fact, I guess the question is it may not even be achievable to get appropriate reduction in adventitious particles using these processes if you're going after a live virus vaccine.

I just want to see if that understanding is correct and I assume then that for any additional proposal to use a cell line such as this or another for a different type of virus, you would really have to rediscuss the whole aspect of what is the viral safety that you can achieve.

CHAIR OVERTURF: No, I think that's a good point. I think what is being discussed actually in terms of specific safety is this specific vaccine, which is a split viral vaccine, and it would seem to me that what you're suggesting is if you consider a vaccine that is a host cell vaccine, it would have to go under -- a whole new process would have to be considered. Yes, Dr. Krause? Oh, I keep doing that. Dr. LaRussa. I'm sorry.

MEMBER LaRUSSA: Not to belabor the point, but I think this, the whole issue that Dr. Minor brought up, is a very important one and I think if you can't make an immunogenic vaccine for H5n1 and you have to go back to this approach of making a whole virion, I'm not really sure what we're talking about here, because the point of doing all this was really

to respond to pandemic flu.

I mean, everybody would like to have a better process for making seasonal flu vaccine, but we can sort of live with that while we transition to a better process. So if we're doing this first and foremost for pandemic flu, are we premature in talking about this now?

DR. RAPPUOLI: Rina Rappuoli from Chiron. Well, the answer to that is that, I think I said before, we have no intention to make a whole virus vaccine for a pandemic flu. The reason is that there are published and unpublished data that using an adjuvant called MF59, we can get very immunogenic response, protective responses with pandemic influenza using as low as 3.75 micrograms of antigen. These studies are being conducted for us by the NIH. They are being written right now. They will be published at some point.

So I think the solution to pandemic influenza not necessarily needs to go back and go to the old fashioned vaccines. We can go one step forward and use the mother technologies, well-known

adjuvants to answer those questions.

MEMBER LaRUSSA: Just to follow-up on that. With the use of an adjuvant, would that be a one dose or a two dose regimen?

DR. RAPPUOLI: Well, I think we are doing studies to address those things. The preliminary answer is that so far we have done two studies, one which was published in the Lancet in 2001 and the one which was being just finished. And, as I said, there has been -- the data has been reported at the WHO meeting in Geneva by our clinical investigators, by the NIH clinical investigators.

And so what we can see is that one dose with the adjuvant, you reach what in Europe we would say the borderline protective levels. That means you meet one of the three CPMP criteria which are used to determine protective levels, so with one.

The answer is preliminary, because it needs to be confirmed by further studies that one dose even with 3.75, you get at the level of antibody levels which are borderline with protection, so already protected. After the second dose you exceed

by a long, I mean, largely exceed the protective levels. So these are the data we have right now.

CHAIR OVERTURF: I think the answer was two doses.

MEMBER LaRUSSA: Yes. I guess maybe this is not the right place to discuss this, but I am wondering whether, you know, giving two doses in a pandemic situation is a reasonable thing to undertake.

DR. RAPPUOLI: Well, that's why when I said we need more studies is that my feeling is that one dose, you will reach a protective level that will not last for long and if you want a long-lasting immunity, you will need two.

I will assume that under pandemic, one dose will be good enough, but if you want to be really relaxed afterwards, you will give another one. But, as I said, these discussions will not be different from using a known adjuvanted vaccine, whole virus vaccine. Those questions will be exactly the same.

CHAIR OVERTURF: Dr. Karron first.

MEMBER KARRON: Actually just to bring the discussion back to the cell substrate and the vaccines

that we're considering. I was actually wondering about whether any of the manufactured vaccine had been, for example, put into nude mice and whether people have looked for tumorigenicity of the finished product in nude mice. We heard a lot about cell substrates, cell lysates and so forth, but just wondering that.

MR. MEDEMA: Jeroen Medema of Solvay. What we did is we already assessed steps early in the process to see if that was still tumorigenic or not, so already early in the process, for example, an inactivated virus concentrate, so this is a whole virus, whole virion concentrated virus and this did not show, did not lead to nodule development in immune-deficient nude mice or in newborn hamsters or in newborn rats. So, well, this probably will not be the case with the final product either.

I think to come back to the issue if we're doing this for a pandemic vaccine or for a seasonal vaccine and for a subunit vaccine or for a whole virion virus, I think the issue on the table is that we are discussing the use of a weakly or highly

tumorigenic cell line to produce a vaccine with acceptable safety and I can envisage that we can develop manufacturing processes for whole virion vaccine that, indeed, will result in the same safety margins.

So I don't think the discussion is really between a subunit or a whole virus vaccine. We will need to revisit our processes if we were to produce a whole virus vaccine.

CHAIR OVERTURF: Dr. Minor?

DR. MINOR: Yes. I mean, I agree with that but, I mean, I think the preceding discussion was a little bit about are we saying that a tumorigenic cell line is okay for anything or whatever, and I think the answer is we're not saying that at all. I think at least I'm not saying that at all.

I don't know about anybody else, but I think what we're considering is a very, very specific vaccine produced by a very, very specific process and if you need to go to a whole virus vaccine, which I believe you don't, okay, but if you did, I think you would have to reevaluate the process and then

reconsider the safety issues related to the cell substrate again. So I think the discussion is very specific, I think, about the two kinds of vaccines that we're actually hearing about.

PARTICIPANT: I agree with you.

DR. MINOR: And I think it's probably appropriate actually.

CHAIR OVERTURF: Dr. Farley? Yes?

MEMBER FARLEY: I just wanted to very briefly revisit the finding of the distant site tumors in the second product that was discussed, and I wonder if Dr. Lewis or someone from FDA might comment on, you know, the tumors that were seen in the lung or elsewhere and not in control groups.

I mean, is this likely to be, as was thought, a spontaneous occurrence and how best can we assure ourselves that that's the case? Is there something that needs to be standardized in the assay, in the assessment, the length, the number of animals, the control group in particular, that sort of thing that might sort of just set that whole issue aside?

DR. LEWIS: Yes. Andrew Lewis, CBER, FDA.

Based on the experience that we have had with the newborn and adult nude mouse model, we have similar experiences in our vero cells. We had an incidence of spontaneous lymphomas and, in fact, one case of a pulmonary adenoma.

In I think 350 animals, our experience was about 2 to 3 percent of these animals had these types of tumors. They developed usually, and fortunately for us, in situations where the animals were not inoculated with vero cells, but some of the tumors did involve animals that had been inoculated and that did not -- and didn't develop tumors at the injection site.

And we did not look at every tumor for evidence of vero cell DNA, but of the tumors that we did look at, they were all of murine origin. And if you look at the literature, as the manufacturers have quoted, there is a definitive incidence of these types of tumors that have been reported in at least one or two studies in nude mice. So I think that our feeling is that these probably do represent spontaneous tumors.

Now, concerning the information that was presented by Solvay about finding a low level of canine DNA in, I believe it was, either a histiocytic lymphoma of the spleen or perhaps a lymphoma that was present in the liver, that is not within our experience. But I happened to run across a discussion of histopathology on cell-induced tumors just recently in the past few days in reviewing for the meeting and they, in fact, pointed out that these tumors that develop in nude mice are encapsulated by murine mouse cells.

They have a fibrous capsule around them. They can, in fact, be invaded, from their perspective, by murine inflammatory cells. So the possibility that a low level of mouse DNA could be present in a tumor cell line that is composed mostly of dog cells is possible.

The converse of that I'm not so sure about, but I think, for an overall perspective, I think finding spontaneous tumors in these animals is the norm rather than the exception to the norm. The worry is that when you find them in animals that are

inoculated and that you're looking for evidence for oncogenic activity from the substrate, then it becomes a problem for all of us and exactly how we have to deal with that, I think it's not quite clear at this point in time.

But I think at least I'm pleased that the Solvay folks are looking at that problem. They are continuing to look at it and I think that's about the only thing we can do. These systems are not perfect and we have to try to work as best we can with the imperfections that we're given in these models.

CHAIR OVERTURF: Dr. Hetherington?

DR. HETHERINGTON: A tremendous amount of preclinical in vivo work has been done. Nobody has made claim that these are going to be validated or predictive one way or the other on the complete safety profile of a final vaccine product, so this next question is for the FDA or for the sponsors.

What thoughts or what talk has gone on relevant to potential long-term follow-up once a vaccine is available through this technology to look at the long-term safety of these products in humans?

CHAIR OVERTURF: Well, for one, it sounds like you're making a recommendation that there should be long-term follow-up in Phase 3 or Phase 4 recipients of the vaccine, at least in subsets.

DR. HETHERINGTON: I mean, it could be something as simple as looking at databases at a national or, in the U.S., at an HMO or large health database for what happens to folks that get vaccines in the future. And I just want to know if anybody has even started wading into those waters as yet or what.

DR. PEDEN: Could I just come back to the question there that Dr. Lewis answered? My name is Keith Peden, FDA. I am curious about the PCR you did on those spontaneous tumors because I think you said, and I think Phil Minor was trying to get at this earlier on, that there was a background level of repeated sequence DNA.

Is that what you said when you did the PCR analysis?

MR. MEDEMA: Jeroen Medema of Solvay. All these assays are not very well-validated. That is the problem with these assays. So we included negative

control tissue, so murine tissue to see what the signal was for canine PCR, canine DNA PCR in negative control tissue, and the signal that we obtained with these two tumors was just above this background signal that we obtained in the negative control.

If you compare that to what we observed as a signal for the nodules that grow at the site of inoculation, this was really five, six magnitudes of order above that. Next to that we are a bit concerned that this, indeed, was a false positive, because, well, as you probably know with using PCR, it's a highly sensitive method and if you cross-contaminate samples, and that can happen if you are processing tissues from animals all at the same time, this might be one of the problems.

The PCR data were not in line with the histopathology data, so we are -- well, we think it was indeed a false positive.

DR. PEDEN: Yes. I think I agree with that and I just want to say since you are using the PCR to the sign, is what you said, right, which is a small interspersed nuclear element. And if you

remember one of my slides, if you do PCR on those, it's down to the attogram level which is at the single molecule level.

So I'm not surprised that you had contamination with that. I think if it were a tumor induced by oncogenic activity of the cell substrate material, it would be clonal and you would see a lot more of the DNA in it. So I think that's a correct interpretation. The worry is that this is exactly what we ask you to do, is to determine the sequence of spontaneous tumors and now, you know, we're not really very helpful about what we do with that information. But I think it is spontaneous.

The other question, what nude mouse strain do you use?

DR. KERSTEN: Alex Kersten, Solvay. We used athymic nude mice with a CD-1 strain.

DR. PEDEN: So it's not the BALB/c?

DR. KERSTEN: No, it's not.

DR. PEDEN: Okay. Thank you.

MR. ONIONS: Chairman, could I just maybe add a comment to Dr. Peden's comment? David Onions.

I really concur with Dr. Peden's comments. If you look in canine tumors in nude mice, you certainly do see a high copy number of SINE elements of murine sequences because, of course, there are infiltrating murine cells in those tumors. That is clearly established and you said even histopathologically.

If we were expecting to see, and I'm not commenting on Solvay's data, I'm making a more general comment about the assay system, if you were to look for SINE elements, looking for a single canine oncogene that had integrated into a murine tumor, then you would probably expect to find at least one link SINE animate to that. That has generally been shown from NIH-3T3 transfection studies.

That would give a signal that is significantly above background and you would see a signal that is several orders of magnitude below the signal from pure canine DNA, because you have multiple SINE elements but you nevertheless see a very significant signal, and I suspect that is not what is being talked about from my colleagues from Solvay.

And so I think you do have a mechanism for

distinguishing between true background canine DNA and an integrated single element, but then you would have to go and demonstrate formally that's the case.

CHAIR OVERTURF: Yes, Dr. Minor?

DR. MINOR: This is on the same thing. Did anybody -- I mean, is it possible to sequence these things? When you get your canine SINE element signal coming in, can you not determine the sequence and decide whether it's a real canine SINE element or a mistake and was that done? I mean, I feel that there is actually an issue here that needs a little bit of further effort, I think.

MR. MEDEMA: Jeroen Medema of Solvay. It was not done so the sequences were not -- the genetics were not -- the genomes were not sequenced and I am not certain that we are technically able to do so, especially when you talk about wax-embedded tissues and already have difficulties in extracting nucleic acids.

DR. MINOR: But if you can get a signal, surely you can get a sequence, can't you? I mean, it's not difficult I don't think, is it?

MR. MEDEMA: Yes. That is true, but we already get a signal with negative control tissue, so this is -- indeed, if you look for a specific sequence with a highly sensitive PCR, that is different from sequencing the whole genome.

DR. MINOR: Yes, but your murine SINE element that you have amplified would have a different sequence from your canine SINE element that you amplify, right, if it's an artifact because your PCR is being oversensitive and it has gone funny. You would determine that by the sequence, right, wouldn't you? And if it was cross-reactivity between the murine sequences and the canine sequences, you show that you get a murine sequence amplified.

I mean, it seems to me that it would actually tell you something to actually get a sequence on whatever signal you could get at, and if you couldn't get a sequence then I think that would also be informative, because it would mean that you got so little there that you can't actually pick it up, you see? I mean, never mind.

MR. MEDEMA: Well, what we did is we

performed both a PCR for repetitive murine sequences and for repetitive canine sequences and we tried to normalize the results to indeed give a statement of the amount of canine DNA present as a ratio to the murine DNA. And there this was, well, we believe indeed comparable to the negative control tissue.

CHAIR OVERTURF: Dr. Royal?

MEMBER ROYAL: I guess I have a question about the tumorigenicity assay. When you inoculate these animals to look for tumor induction, are you looking for just localized tumor or metastatic tumor and if you are looking for metastases, how rigorous is that done? Are you sampling and looking immunohistochemically or doing PCR on tissue samples?

MR. ONIONS: David Onions. Generally, in these procedures, there is a gross histopathological--sorry, a gross pathological examination of the mice at the point of postmortem. There is not a general PCR analysis of those tissues, but there is histopathological analysis of those tissues. If you're asking the specific question, could micrometastases be missed, I think the answer to that

question must be yes, but I don't think gross metastases would.

MEMBER ROYAL: Then that issue would be very important if you have a cell line that is no longer adherent but it's now a suspension cell. It's always possible that you might not get a localized tumor, but micromets elsewhere.

Is there known to be a difference in the metastatic potential of your suspension MDCK cells as opposed to the adherent cells?

MR. FINN: Peter Finn, toxicological pathologist for Solvay. In answer to the question, I think there is a difference between Chiron and Solvay in that we did look at a small range of tissues by microscopic histology to see if there were any metastases. I can reel off most of them, but they are the obvious ones.

If I could go back to your question even earlier, as I am a toxicological pathologist, I am therefore innumerate but there were some statisticians here. I believe that at the instance that these spontaneous tumors are seen, which is of the order of

1 to 4 percent or something like that in the group size that we had, one might predict that there would be none in some groups, and I think the only way you get around that is to just have the normal size of groups that are done in carcinogenic potential trials, which everybody is used to handling.

CHAIR OVERTURF: Dr. Royal?

MEMBER ROYAL: Just to go back to my last question, whether or not -- I guess it would need to be directed to Chiron, whether or not you have had a chance to determine whether the metastatic potential for your suspension MDCK cells is the same as the adherent originator cells.

MS. NOVICKI: I can't comment. Oh, Deborah Novicki, Chiron, toxicologist. I can't comment too specifically about specific differences between Solvay's and our cells in the tests that we have run, because we have done no work that does head-to-head comparisons.

But just in general, the biology that allows the growth of cells in suspension is absolutely -- some of those characteristics can be predisposing

toward metastases and we do see metastases in some of our animals that had injection site tumors, as well as some animals that did not have apparent nodules at their injection sites.

So we do see metastases in a small number of animals in our study, but I think it is something that one could expect and I think there is a lot of research that supports the fact that forcing cells to be able to be anchorage independent, grow without serum, and some of those attributes actually are associated with phenotypes consistent with metastases.

CHAIR OVERTURF: Dr. Markovitz?

MEMBER MARKOVITZ: Yes. I wanted to just follow-up so I can understand how much of Dr. Minor's concern I share. So my question is, I can't remember from the slide, but in those tumors that were, you know, distant tumors that you guys saw, how many cells had been injected to see those? So not the nodules, but the distant tumors.

MR. MEDEMA: We found three distant tumors in the intact cell study. We found one tumor that was both characterized by histopathology and by PCR to be

murine. That was in the 10^1 group. And we saw at both high passage level and in the parent cell line, so the ATCC cell line, at the 10^7. At those levels we saw a distant tumor in the lung, so it's at 10^7 level.

MEMBER MARKOVITZ: What was the 10^1 though?

MR. MEDEMA: That was a histiocytic tumor in the spleen of one of the mice, which was confirmed or characterized by histopathology and by PCR to be of murine origin. So that was not related to -- that was indeed a true spontaneous tumor and also characterized by both assays to be a spontaneous tumor.

CHAIR OVERTURF: I would like to refocus and what I would like to do is to read the three discussion points, but I will read them one at a time and then I would like to go around to the Committee Members to comment on each discussion point. Going to put you on the spot.

The issue was the discussion of the use of MDCK cells, including those that are highly tumorigenic, in manufacture of inactivated influenza vaccines. So the first question really is is there

general agreement that this issue of inactivated influenza vaccines should proceed in MDCK cells. Is there convincing evidence of safety, manufacturing stability and potential for use and, if so, whether that should be primarily directed for a pandemic vaccine?

So I started with Dr. Markovitz last time, so I will start with Dr. Self this time.

MEMBER SELF: Gee, thanks. So I guess I will maybe take a step or two back. I like the Defined Risks Approach. I think it addresses the issues in a very systematic way, but the devil is in the details.

I tend to agree that even though there are some questions about the details of the process for removing cells, that it seems to be very efficient and so, like the comment earlier by Dr. Minor, I don't -- I'm not terribly concerned about the tumorigenic aspect. However, the oncogenicity aspect seems to be where the action is.

There it seems to me that there is a gap between the empirical evidence for risk and the risk

threshold that the FDA was putting out. It seems to me that that would be well-addressed by larger animal model studies. The fact that there are spontaneous events suggest that they might be well-controlled studies.

Although, I think that if you try and statistically take care of the spontaneous events by means of a control group, the size of those studies would put them out of any feasible range. So my sense is that larger studies, but with a much more careful look at each event trying to determine whether it is spontaneous or related to the MDCK cells, that is the approach that makes the most sense to me.

The other point, I think, that I would like to make is that there are two steps in the DRA process outlined by the FDA. First is that both involve estimation. First, estimating the frequency of these events under experimental conditions, but the second is estimating frequency of the risk event per dose of vaccine.

And there has really been very little discussion so far about the connection between the

frequency of these events that might be defined in an animal model and what might be seen in humans. I know that's always a pretty tough topic to address, but it seems to me that there should be some explicit attempt to address that difference.

So having rambled on, I actually forget the three questions that you put to me, but I tried to summarize my thoughts.

CHAIR OVERTURF: I actually think you covered two of the questions, which was the MDCK cells and also the discussion of the OVRR's approach, and I think you actually talked about one of the additional steps that they should consider taking to address these issues, which were larger studies with more defined approaches to tumors.

Dr. Karron, did you want to comment?

MEMBER KARRON: I guess just to make a couple of comments. One is that I think I concur with Dr. Minor about the issue being this issue of oncogenicity. The other thing that I wanted to pick up on that Dr. Self mentioned, and this really will end up in the form of a question back to the FDA, risk

event per dose of vaccine.

And so one of the questions that I really have for the FDA is are we to be considering both use of this for a regular epidemic inactivated vaccine and pandemic vaccine or can these be considered separately?

You know, I'm thinking particularly at this point there are some unknowns. When we think about risk event per dose of vaccine, you know, are we thinking about if, in fact, we do move toward mandatory influenza immunizations starting at 6 months of age for young children, we're talking about many, many doses of vaccine over a lifetime.

Do we have enough information at this point about the use of MDCK cells to think in those terms? Are the questions different if we're thinking in terms of a pandemic vaccine and, certainly, a situation where risks and benefit assessment might be a bit different?

CHAIR OVERTURF: Dr. Krause?

DR. KRAUSE: Yes. So, of course, we don't want to make things too easy for you. I think it's

very easy that if a pandemic is sweeping the world to decide that one is willing to take on a little bit of additional risk to deal with that. But if, in fact, one wants the manufacturers to have the capacity to make vaccine to deal with these pandemic situations, they also need to have licensed processes in place and need to be capable of making these vaccines and be running these processes.

You know, I suspect if you were to ask them to get up and answer that question as well, they would say that they don't think they will be able to do this just for pandemic, because they wouldn't -- it would be a completely different facility. It would be completely different processes and everything else from what they routinely do, and so it would be very difficult to separate the two. I see nods over there anyway.

CHAIR OVERTURF: Dr. Karron?

MEMBER KARRON: Am I allowed to follow-up with a question? I know we were just supposed to comment, but is that okay?

CHAIR OVERTURF: I think there's probably

more questions than there are comments, so go right ahead.

MEMBER KARRON: Well, I guess my question for the manufacturers is really if over time this process were approved, would the goal then be to move totally to a cell-based manufacture for influenza vaccines? I mean, is your overall goal to completely dispense with egg-based manufacture?

DR. RAPPUOLI: The short answer is yes, long-term, things like that, but for those that have never seen how vaccines are made in eggs, I mean, I think you should see that and technology in 1950s. If you ask me what are the risks you are mentioning, I mean, I will feel there are more risks with that one than with any other cell lines.

So the way I see this is we are obviously very concerned. We are asking the risk questions, what the risk, things. My personal opinion is that this is a step forward towards having safer vaccines with lower risks. That's the way I see it, because the cell lines are characterized. The cells can be removed, all the tests we can do, more technology,

microrays.

We can ask and we'll address. A lot of the questions have been addressed. So these cell lines are the next step forward to have processes and vaccines which have lower risk than we had in the past.

CHAIR OVERTURF: Does Solvay want to comment? Okay. Dr. Minor?

DR. MINOR: Well, the first thing is that I think if mandatory vaccination against flu from the age of 6 months was introduced, I think you would have serious considerations about the egg-grown vaccine as well simply because it hasn't been used on that kind of scale before, so I think you would have the same kind of issues there.

Getting back to the point at issue, however, as I said earlier, I think that the cell contamination tumorigenicity issue is not an issue because there is not going to be a live cell left in the final product, in my opinion. Okay. I think the adventitious agent aspect of MDCK cells can be dealt with to varying degrees of efficiency, but it can be

dealt with. It's quite clear how you deal with that.

And that leaves the oncogenicity of the DNA over which I think there are still questions and I think, I suspect, that if the processes were not able to either remove or inactivate or destroy the DNA that was introduced at the beginning, I think maybe you would be a little more concerned about it than you are.

But I think as it removes DNA, as there is a beta-propiolactone treatment that is introduced to inactivate it and as it's also treated with Benzonase or whatever and it's reasonably well-purified, I mean, I think there is a great deal of safety and reassurance that comes from those particular steps in the process.

But I think if those steps were not there, then I think there might be some concerns about the oncogenicity of the DNA even now, although I accept again that there is no evidence that DNA from cells is oncogenic.

CHAIR OVERTURF: Dr. Cook?

DR. COOK: I think that the tumorigenicity

issue under question one has been addressed multiple times. I still think there are differences between these two groups in terms of the basic cell substrates that they are using that are worth considering. Why are they different in their tumorigenicities? Obviously, they have been evolved differently, but that should somehow or another be addressed just so that everybody is comfortable that they are not dealing with the same one cell population.

The only reason that's interesting at all, it seems to me, is what Dr. Minor just said and others have said, and that is what it might mean in terms of what it could convey in the context of the vaccine to the recipient and that has to be conveyed presumably through either an adventitious agent or some kind of contaminating thing that could cause illness. Whether it's tumorigenicity or something else, we don't know because it's an unknown thing.

The OVRR approach I think has been excellent. At least it has put some definition to things that otherwise were really nebulous and were sort of just anxiety. So I think it's good to have

specific things to test. I think it might be interesting to more effectively use, since you're looking for unknown things that are going to happen, the prospective studies about comparing larger groups of controls with animals that get vaccined to see what happens to those have been discussed already, and I think that would be very interesting.

And the spontaneous tumors will probably be much more interesting than the MDCK-induced tumors in terms of their frequency, which is difficult from a statistical point of view, but in terms of whether that might have been something that happens, every time you get vaccined, you get more spontaneous tumors and why is that?

The additional thing CBER could do, I suppose, would be to think about other ways to use animals in response to vaccines or substrate lysates to tell them whether there is anything there that isn't just tumor cell lysate, because right now it's all focusing on if these animals get tumors or not. It's all oncogenicity.

But there are things that contaminants and

other things could do to you that aren't just causing you to form a tumor that might be undesirable. So do these animals respond in a way that is unpredictable? Do they develop, you know, inflammatory reactions that would suggest autoimmune disease or whatever, using animal responses as an amplifier to tell us something about what these cells do?

And then I think the one thing that's missing from this whole discussion is the fact that the humans who are receiving these agents have host defenses. And I know that's not the purpose of this discussion, but if you're going to transfer something that is unknown into these humans, the question is if you want to have a defined risk assessment, you have to consider the person who is receiving the vaccine, the innate and adaptive immune responses they have to that vaccine that might not only induce an immune response, but also provide them with some protection against any of this stuff we're talking about that could be conveyed.

CHAIR OVERTURF: Dr. Word?

MEMBER WORD: It's funny, as you begin to

come down the line, your comments become somewhat similar. I guess, I think, as has been pointed out, when you talk about the tumorigenicity, as many of my colleagues have stated, I don't think that's as much a concern, but the oncogenicity might still be a question. I mean, as far as the approach with the discussion with the OVRR, I think, that has been adequate.

I think someone across on the other side, and I'm sorry I can't recall who it was, I think it was probably one of the pharmaceutical representatives when they talked about additional steps and one of the things I think you talked about was following some of the vaccine recipients long-term just to find out what has happened to them. And I think that would be something reasonable that should be done.

CHAIR OVERTURF: Dr. LaRussa?

MEMBER LaRUSSA: I don't have a lot to add to what has already been said. I think the approach is a really good one looking at the issues separately. I think if you asked me if I'm comfortable enough to say we're ready to use this approach for development

of all inactivated influenza vaccines, I don't feel comfortable enough yet to say that we're there. I think that's where we have to be and where we will be, because I think all the preliminary data that has been presented is very reassuring.

I actually would like to go back to the point of actually seeing what happens when you inject the final product into the animals and follow them in a control group and see what happens.

CHAIR OVERTURF: Yes, I would agree that an awful lot revolves along greater numbers, particularly both in the animal studies and I also think in the control groups. I just am not quite sure how you're going to resolve some of these issues without some sizeable control groups and I know it's expensive, but it seems the logical thing to do.

I thought the comments about what the human immune response will do in modifying some of this is very important. And I mean, I think, it comes back to the original question that Dr. Self mentioned which is very hard to resolve what happens in humans versus what happens in animals. Animals are the best

markers we have at the present time.

When we were discussing this earlier, I think there was some concern about whether this was the first step and I think the manufacturers answered that to an eventual production of vaccines, of all seasonal vaccines. And I think the information is convincing enough now that it is certainly a reasonable alternative in a pandemic setting. And many of the questions we are asking might get answered actually provided they were essentially set up as Phase 4 trials during that.

And I think maybe the risks would be acceptable during that time. But I think right now the discussion really still has to stay limited, primarily, to the pandemic vaccine. But there is the issue about whether the pandemic will come and whether it will come in six months or a year or two years or three years.

And I think there will be some point where if there was continual review of this process and the development of cell vaccines over the next two years, regardless of whether we use them for a pandemic

vaccine or not, that we might get enough information with periodic review that's brought back and forth to VRBPAC that we might build, then it might move to that.

So I think one of the recommendations has to be to keep this and perhaps increase the intensity and scrutiny with which it is looked at over the next few months actually. Dr. Robinson?

DR. ROBINSON: Thank you. I concur with Dr. Minor in most of his comments, but it seems to me that there is a balancing act here and that is they have shown that the DNA content is lower than less than 10 nanogram level, that it's alkylated in some cases, the DNA size with Benzonase treatment is smaller than 200 base pair and that it is cross-linked with beta-propiolactone.

I mean, you simply have a dead molecule there, as far as most biological systems, and the balancing act is how much more -- how many more animals do you have to actually inject to give you the level of comfort that you want or do you actually lower the limit of DNA there? And, I mean, you know,

you could do both, but to me there is a threshold amount of what should be required there.

Both of whether it is pandemic or seasonal, I mean, the thing about a pandemic is that if you are having 600 million doses made in the United States, that's going to be the equivalent of about, you know, 10 years or eight years worth of vaccine that would be given seasonally and given at one time. So, I mean, that gets -- the other thing is that the questions 2 and 3 is that, I think, there is some definite prelicensure and post-licensure homework assignments for both the manufacturers and the FDA. And I think they are being clearly eliminated here.

But also, one thing that is, there would be drug master files of over 4,000 individuals that have received these vaccines in Europe. So the master files will include that. And the follow-up on those individuals may gleam some information toward these data. Thank you.

CHAIR OVERTURF: Ms. Province?

MEMBER PROVINCE: Well, I concur with many of the remarks that have already been made. I think

there has been a lot of good work done already and I'm grateful for that. I agree with Dr. Self that the devil, however, is in the details. I'm not, like Dr. LaRussa, completely at my comfort level yet and I think we all need to remember, and I know everyone on this Committee does, that ultimately what we discuss here and decide here has to do with human safety and public confidence in vaccines in general.

And so having said that, I concur that I believe larger animal model studies are needed. There does need to be a more careful look at each of these events to, as the research is ongoing, see if these are, indeed, spontaneous events or if they are related to the intervention. And I also agree that there needs to be an explicit attempt to relate the animal models to human data as best we can, although, I know that's a problem, and also to follow vaccine recipients.

I think that since we do have available some data that we could access, that we definitely need to do that ongoing and that's going to help us decide or make decisions as we go into the future.

CHAIR OVERTURF: Dr. Farley?

MEMBER FARLEY: Yes, I think we're all evolving in similar ways. I guess I feel as if this direction is really relevant, if not more so, for the seasonal vaccine development rather than saying that it is a specific plan for pandemic flu, because I think that we do need to move on from the eggs as a regular process on a seasonal basis. In this case, we can use the concerns about an impending pandemic to kind of drive us forward perhaps.

One of the original thoughts I had was that there is a lot of work that has gone into looking at this cell line and a lot more yet perhaps that needs to be done, but in some ways it might seem to me, at least initially, to be more practical to have it sort of a centralized process of review and certification of a cell line that then is made available from a centralized place that is standardized and is available.

But I can see now from a manufacturer's point of view they clearly have taken two different directions in their process, in the manufacturing

process of suspension versus the polarized cells. And so that may not be practical. Although, then all of this invested large numbers of animals and such things could be done kind of one time and in numbers that are comforting.

So I'm not sure that that's very practical in the end, but if there were ways for the future of trying to come up with new cell lines that might be made available for manufacture of other vaccines, that it might have some relevance or part of the process. I do think that while -- because of the fact that we're so comforted by the end processing and how effective it is at clearing out every last cell, which somehow, you know, I see in labs all the time where we had incomplete digestions and incomplete -- things aren't always perfect.

Hopefully, it is as perfect as Dr. Minor is comforted by, but that the regulatory or monitoring of the end product seems very important to make sure there is no one cell left intact. And assuming that would be the case anyway. But the idea that we are asking the sponsors to monitor for these distant site

tumors is another thought that maybe some work on, you know, improving the guidance of what to do with those tumors, how to evaluate them when they arise, so that we can again be all comforted by the fact that they are not related in any way, shape or form to an oncogenic process.

And how best to put a handle on that seems to be another area for continuing thought and research and guidance then that can be produced by FDA and others for the sponsor. I think that's it. So I am in favor of this progress towards using this cell line for this specific use of the inactivated influenza virus.

CHAIR OVERTURF: Dr. Royal?

MEMBER ROYAL: Oh, thank you. I guess, I would like to start off the second bullet and really commend OVRR for bringing this whole issue to the table and developing the research in this area, monitoring it and really it has been very commendable. I would like to, and I guess moving on to the third bullet, see more of an effort at standardizing how some of these assays or some of these assessments are

done in promoting more sensitive tracking of tumors that might be induced in these animals being able to better see where they are using more sensitive techniques and being able to estimate the total tumor burden, which I think is important.

I mean, you're talking about the case of different cell lines, modifications of the same cell line being used, not necessarily getting the same effect in the tumorigenic studies which takes me to the first bullet. I agree that the issue is on oncogenicity, but it seems to me that it is hard to isolate the two, because products of a tumorigenic cell should greatly influence how oncogenic the cell-free products would be. So I think that if you not keep a tight handle on one, the other may start to be a problem at some point.

CHAIR OVERTURF: Dr. Hetherington?

DR. HETHERINGTON: I would like to just add my agreement on the whole approach to evaluation of the safety of tumorigenic cells for the use in vaccine production, the second bullet there. I think everybody has done a fine job. I think it is complete

to the level that I'm not certain that larger animal experiments will really manage probably the key point that I think was brought up in one of the earlier talks from the FDA, and that is how do you manage the perception of risk?

And it is in the context of that that I would like to just add the rest of my comments. Management perception of risk has -- there is nothing better than a long history of use of the product in real people and real data collected. We are always going to have the kernel of doubt until we have 5, 10, maybe longer years worth of data. So you're not going to get rid of that completely.

But I think what fuels that kernel of doubt is things that we don't understand at this point in time. For instance, what studies would be required before there is an approval of a vaccine made by this manufacturing process? Are you talking about immunogenicity studies? Are you talking about large Phase 3 studies for clinical efficacy or larger safety databases? None of that has been discussed today and I understand that is not within the framework of what

we were asked to do, but it addresses the whole issue of this kernel of doubt.

And I think along with that, the question that comes up is what's the anticipated time line for rolling out vaccines made under this manufacturing process? We have talked about well maybe we should just restrict it to the pandemic situation. And I disagree with that. I think you are going to have to fish or cut bait and go with vaccine use for all flu or none.

But how you roll that out, I think, is going to be important. It's not going to happen tomorrow, but is it going to happen over the next year, five years? Is it going to completely replace egg culture-based vaccine and over what time scale? And then I think the final point I would just like to reiterate is that there is no substitute for long-term safety data. Whether you start it during your Phase 3 or you do it as opposed to Phase 3 or Phase 4 commitment is up to the discussion between the manufacturer and the FDA.

But there should be methods by which you

can get at least basic long-term follow-up on your people who are participating in the trials or large populations receiving the vaccine. It doesn't help you today, but at some point in time you're going to want to answer that question. And you're going to have to start by collecting the data now.

CHAIR OVERTURF: Dr. Markovitz?

MEMBER MARKOVITZ: I would like to first thank Dr. Overturf and Dr. Self because it is much easier to speak last rather than first, so thank you, Steve. I think that I would like to comment on two aspects of this. First of all, the safety issue. I'm quite comfortable with what has been presented in terms of safety.

I think that the issue of adventitious agents is always a sticky issue, as Dr. Minor said, but I don't see any reason why adventitious agents will be any more of a problem with these vaccines than any of the others we've dealt with and, indeed, offer some advantages over eggs in terms of adventitious agents, particularly, if we include bacteria and things like that. So that's one thing.

I think in terms of the DNA, you know, it's chopped up, it's in minimal quantities, it's chopped up, it's not going to encode any oncogenes that could actually insert into a bad place, but that's I think a very minimal risk with such a small amount of DNA. Then in terms of the cells, they are gone, so they are not going to cause tumors. And even this distant oncogenesis, that should be gone, too, because the cells and the DNA are gone.

Anyway, it's a little hard, frankly, for me to understand the basis of a distal oncogenic event that would take place with one cell, so that's very hard to imagine in that setting. But be that as it may, I think, the safety issues are pretty clear. In addition to that, we have the benefit that our friends in Europe have already been taking this vaccine and so they have also done us a service. And so I think that, safety-wise, things look good.

Obviously, ongoing monitoring, I think, just as Seth and several others have emphasized, ongoing clinical monitoring is going to be hugely important and perhaps animal studies, although, I'm

not convinced that those were necessary. Those could be done to look for the distal oncogenic events if one must.

I think in terms of the other issue is, to switch to the second part of my comments, that I think we haven't really discussed the fact that this is a technologic advance that we really need. When we are talking about vaccines here, we're talking about risks that are very hypothetical so far, real but hypothetical, real in the sense that they are important, but hypothetical in the sense that we haven't seen problems yet with this vaccine and vaccines like it.

So there are real problems. I'm glad and I commend the FDA for addressing these directly, as well as the manufacturers for facing them, but I think that the issue of flu is a very, very real threat to all of us. And I think both, I would like to agree with Monica Farley about the idea that I think this isn't just for pandemic flu, but also for seasonal flu.

Two or three years ago, I can't remember,

Dr. Overturf, exactly when that was, but we had to pick the wrong antigen on this Committee, because we couldn't grow, no one could grow the virus in eggs. So that was a very real recent event where had we had better technology, we could have actually put the antigen into the vaccine that everybody acknowledged was the right one.

And then pandemic flu, of course, is an extremely scary proposition. And while this may or may not turn out to be the answer, it is certainly one very important possible element in the armamentarium. So I favor this advance. I think that the fact that the FDA has set the bar high and the manufacturers have had to rise to that bar has been very good. And I certainly would think that continuing close observation is good, but I'm very enthusiastic about this as a possible advance.

CHAIR OVERTURF: Anybody else want to comment? I think what I'm hearing is that I think there is general enthusiasm for tracking along this development of these vaccines. I think everybody is probably whetted to the idea that eventually this will

become probably a mode for seasonal vaccines. I think there is a question about the time line of that and exactly how that should happen. And part of it may be determined by the epidemiology of worldwide flu and what happens in six months or what happens in the next three or four years. That was actually my point earlier on, so I'm not sure we really know what it is.

And I also would like to commend again the OVRR's approach to this. I think it has been very good. And to me, actually, I was very convinced. I'm not -- I may be more naive, but fairly convinced by the safety of the processes that we are now using. And I think it is fairly convincing. But I think everybody is going to be -- the more data you can get prior to the time and to use the available database that we already have seems reasonable also, which is some of the human population has already been immunized. Dr. Minor?

DR. MINOR: This is just one quick sentence about long-term follow-up of this particular product. I think you have to bear in mind that it is used in the elderly, a group which I'm rapidly

approaching myself, and therefore the opportunity for long-term follow-up may be quite limited. That's all.

CHAIR OVERTURF: Are there any other questions, comments? Any comments from the FDA or any issues that they want us to specifically address? I'm ready to go ahead and adjourn the meeting. I will tell the Committee Members need to remove everything that they don't wish to have removed otherwise from the room. We don't want to leave anything in the room overnight.

Okay. The meeting is adjourned. Thank you.

(Whereupon, the meeting was concluded at 4:48 p.m.)