UNITED STATES OF AMERICA

FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

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VACCINES AND RELATED BIOLOGICAL

PRODUCTS ADVISORY COMMITTEE

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MEETING

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WEDNESDAY,

MAY 16, 2001

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The Advisory Committee met in the Grand Ballroom, Holiday Inn Gaithersburg, 2 Montgomery Village Avenue, Gaithersburg, Maryland, at 9:00 a.m., Dr. Robert S. Daum, Acting Chair, presiding.

PRESENT:

ROBERT S. DAUM, M.D., Acting Chair

C. ESTUARDO AGUILAR-CORDOVA, M.D., Ph.D.

DONALD BLAIR, Ph.D.

JOHN COFFIN, Ph.D.

JAMES COOK, M.D.

MICHAEL DECKER, M.D.

PAMELA S. DIAZ, M.D., Member

PRESENT (Continued):

ALEX J. VAN DER EB, Ph.D.

WALTER L. FAGGETT, M.D., Member

BARbARA LOE FISHER, Member

JUDITH D. GOLDBERG, Sc.D., member

DIANE E. GRIFFIN, M.D., Ph.D., Member

STEPHEN HUGHES, Ph.D.

SAMUEL L. KATZ, M.D., Member

KWANG SIK KIM, M.D., Member

STEVE KOHL, M.MD., Member

PAMELA McINNES, D.D.S., Msc.(Dent.)

PHILIP MINOR, Ph.D.

LAWRENCE MOULTON, Ph.D.

MARTIN MYERS, M.D.

SUZETTE PRIOLA, Ph.D.

DAVID S. STEPHENS, M.D., Member

SIDNEY WOLFE, M.D.

NANCY CHERRY, Executive Secretary

C-O-N-T-E-N-T-S

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

(9:01 a.m.)

ACTING CHAIRMAN DAUM: Good morning. We will begin our session with turning the floor over to Nancy Cherry, who will read the conflict of interest statement.

MS. CHERRY: First of all, I'd like to welcome you all to this meeting, and then I will read the statement.

The following announcement addresses conflict of interest issues associated with this Session 2 of the Vaccines and Related Biological Products Advisory Committee meeting on May 16th, 2001.

This open session is focused on discussion on adventitious agent testing, tumorigenicity testing, and issues related to residual cell substrate DNA of novel and neoplastic cell substrates used to manufacture viral vaccines.

No temporary voting members have been appointed for this session.

To determine if any conflicts of interest existed, the agency reviewed the submitted agenda and all financial interests reported by the meeting participants. As a result of this review, the following disclosures are being made regarding the

discussion May 16th.

Drs. Griffin, Aguilar-Cordova, and Ketner have each been granted a waiver in accordance with 18 USC 208(b)(3), which permits them to participate fully in the discussions.

Also, in accordance with Section 2635.502 of the Standards of Conduct, Drs. Coffin and Moulton have been granted appearance determinations which permit them to participate fully in the discussions.

Drs. Daum, Goldberg, Griffin, Kim, Stephens, Blair, Priola, Hughes, Cook, McInnes, and Minor have associations with firms that could be or appear to be affected by the Committee discussions. However, in accordance with 18 USC 208 and with the section I referenced above of the Standards of Conduct, it has been determined that none of these associations is sufficient to warrant the need for a waiver, for a written appearance determination or for exclusion.

The agency has determined that the services of Dr. van der Eb as a non-voting guest are essential. Dr. van der Eb has reported that he received a consulting fee for scientific advice on Crucell's human cell line.

In addition, the agency has determined

that the services of Dr. Michael Decker as a non-voting guest from industry are also essential. Dr. Decker is employed by Aventis Pasteur as the Vice President of Medical and Scientific Affairs. He reported a financial interest in a firm that could be affected by the committee discussion.

In addition, Dr. Decker's employer has associations with university researchers and with major vaccine manufacturers.

In the event that the discussions involve specific products or firms not on the agenda and for which FDA's participants have a financial interest, the participants are reminded of the need to exclude themselves from the discussions. Their recusals will be noted for the public record.

With respect to all other meeting participants, we ask in the interest of fairness that you state your name and affiliation and any current or previous financial involvement with any firm whose products you wish to comment on.

Copies of all waivers and appearance determinations addressed in this announcement are available by written request under the Freedom of Information Act.

And I do have one other announcement. The

Committee management specialists that did so much work to put this meeting together are, I guess, both sitting out at the front desk now. Denise Royster is being assisted today by Rosanna Harvey, and if you have any problems, please see them.

ACTING CHAIRMAN DAUM: Thank you very much, Nancy.

There's a peculiar microphone feedback in the room that seems to be resonating around when anyone is speaking. It sounds like someone whispering, and I realize after a while that it's me and it's my echo going around. We had it when Dr. Patriarca was speaking last time also.

Can you give it a thought? Maybe I'm just sitting at the funnel here.

PARTICIPANT: Are you hearing it now?

ACTING CHAIRMAN DAUM: When I speak I am.

Also, cell phones, beepers, all the things you can't use on airplanes, please don't use them here either. Different reason. They really distract the tone of the discussion and the Committee deliberations, and I'd very much be grateful if everybody now thought about whether they have a beeper or cell phone that could ring and disrupt the Committee.

I would like to take a few minutes to go around the table and have people introduce themselves this morning, and I would like to ask that there be a slight discrimination in the process, unless the way we usually do it, and that is we'll start with Dr. Griffin and come down as far as Ms. Fisher, which are the standard Committee members, and then I'm going to ask everybody else, starting with Dr. Myers and working our way around, to not only say who they are and what their affiliation is, but sort of explain how that affiliation gets them here in one sentence or two. Why are they consulting to our Committee for in general or for this particular issue.

I think that would be helpful in terms of orienting everyone toward the discussion. So, Dr. Griffin, would you start us off, please?

DR. GRIFFIN: So I am Diane Griffin from Johns Hopkins. I'm the chair of the Molecular Microbiology and Immunology Department in the School of Public Health, and I'm going to explain a little bit about myself.

I'm interested in the pathogenesis of viral infections.

ACTING CHAIRMAN DAUM: Perfect.

DR. STEPHENS: I'm David Stephens from

Emory University, Director of the Division of Infectious Diseases. I'm a bacteriologist, not a virologist. So I'll pass to the next person.

ACTING CHAIRMAN DAUM: Committee members need to be less explicit in this regard.

(Laughter.)

ACTING CHAIRMAN DAUM: This is not a total expose, but rather an opportunity for the Committee to understand why the consultants that are here today, in fact, are.

Dr. Goldberg.

DR. GOLDBERG: Hi. Judy Goldberg. I'm the Director of Biostatistics at New York University, School of Medicine.

DR. KATZ: I'm Sam Katz, a pediatric infectious disease person from Duke who's spent most of his career studying vaccines.

DR. DIAZ: I'm Pamela Diaz, pediatric infectious disease person and the Director of Infectious Diseases for the Chicago Department of Health.

DR. KOHL: I'm Steve Kohl, pediatric infectious diseases and at the Argonne Health Science University, with an expertise in viral immunology.

DR. KIM: I'm Kwang Sik Kim. I'm head of

pediatric infectious diseases at Johns Hopkins School. My work has been primarily on the pathogenesis of infectious diseases, primarily on bacterial infections in pediatrics.

MS. FISHER: Barbara Loe Fisher, President of the National Vaccine Information Center, a nonprofit organization that's concerned about vaccine safety.

DR. MYERS: I'm Martin Myers. I'm the Director of the National Vaccine Program Office. Background: pediatrician in infectious diseases interested in pathophysiology, particularly animal models of Herpes viral infections; former Chairman of Pediatrics.

MS. McINNES: I'm Pamela McInnes, Deputy Director, Division of Microbiology and Infectious Diseases, National Institute of Allergy and Infectious Diseases. NIAID is, of course, an important funder through public money, expenditure on basic, applied, and clinical research in infectious diseases.

DR. VAN DER EB: I am Alex van der Eb, emeritus professor at the University of Leiden, with expertise in viral transformation and cancer in general. I'm still active in the lab and scientific advisor to Crucell, a member of the Scientific

Advisory Committee.

DR. DECKER: I'm Dr. Michael Decker. I'm a member of the Departments of Preventive Medicine and Infectious Diseases at Vanderbilt University, where for, oh, ten or 15 years I've been actually involved in clinical research and vaccines. Recently I've joined Aventis Pasteur as Vice President for Scientific and Medical Affairs, and I'm here because through a typical federal process, I am the vaccine industry representative to VerPAC.

DR. AGUILAR-CORDOVA: I'm Estuardo Aguilar. I'm with the Harvard Gene Therapy Initiative, and I've been asked to come here primarily because of my work in antiviral vectors and their use in gene therapy applications.

DR. COFFIN: John Coffin. I'm a professor in the Department of Molecular Biology and Microbiology at Tufts University and also part-time Director of the NCI's HIV Drug Resistance Program and also part-time cranberry grower. And I'm here, I guess, because my research over quite a number of years has been engaged in understanding how retroviruses work and how they transform cells and issues related to that.

DR. COOK: I'm Jim Cook. I'm Chief of

Infectious Disease at the University of Illinois, and my research interest is adenoviral early gene expression, especially E1A and how it affects the cell's response to the inflammatory response in host..

DR. BLAIR: I'm Don Blair. I'm Chief of the Oncogene Mechanism Section of the Center for Cancer Research at the NCI and have a long history of interest in DNA biological activity and tumorigenesis.

DR. MOULTON: Larry Moulton. I'm a biostatistician at Johns Hopkins University, and I spend the majority of my time working on vaccine safety and vaccine efficacy studies.

DR. KETNER: I'm Gary Ketner from the Department of Molecular Microbiology at the Johns Hopkins University now Bloomberg School of Public Health, and I'm an adenovirus geneticist.

DR. MINOR: I'm Philip Minor. I'm from the National Institute of Biological Standards and Control in the United Kingdom. We're concerned with quality control and quality issues and regulation of viral vaccines, and we also get involved in viral contamination, issues of biological products.

DR. WOLFE: I'm Sid Wolfe. I'm a general internist by clinical training, and since leaving NIH 30 years ago, I've spent most of my time at the Public

Citizens Health Research Group in activities that relate to the FDA, drugs, biologics, and I think I'm here because we've worked closely, sometimes in an antagonist way, but closely with the FDA for 30 years to try and sort through problems.

This is certainly one of the most interesting and important issues that's come at least to my attention, and I'm glad to be asked to participate.

DR. PRIOLA: I'm Sue Priola from the Rocky Mountain Laboratories, which is an off, off, off campus branch of National Institutes of Health, and I'm here to provide information about infectivity TSE infection, and tissue culture cells and the risks involved.

DR. HUGHES: I'm Steve Hughes. I'm from the HIV Drug Resistance Program of the NCI, and I have a longstanding interest in retroviruses and retroviral vectors.

ACTING CHAIRMAN DAUM: And I'm Robert Daum. I'm from -- I'm with parainfluenza virus infection.

(Laughter.)

ACTING CHAIRMAN DAUM: I'm from the University of Chicago. I'm head of the Section of

Pediatric Infectious Diseases there. My interests include antimicrobially induced stress in Gram positive bacteria, and that's my day job, and my closet research concerns clinical evaluation of vaccines and strategies for improving immunization rates in inner city children.

And so with that, I welcome everybody, members and guests, to our meeting. We have obviously a very distinguished panel of consultants today to help us with these important issues.

And at this point I'd like to move on with the body of the meeting and call on Dr. Andrew Lewis from the FDA, who will introduce us to this session on so-called designer cell substrates.

While Dr. Lewis is walking up to the podium, could the FDA folks tell us who they are also and just in the same kind of brief, USA Today format?

DR. PEDEN: Yes, my name is Keith Peden. I'm in the Division of Viral Products in the Office of Vaccines at CBER. We're involved in the regulation of vaccines, and as a nighttime job we do some research on HIV.

DR. KRAUSE: Phil Krause in the Laboratory of DNA Viruses. I'm interested in viral latency and in viral detection.

DR. GOLDING: I'm Hana Golding. I'm the Chief of the Laboratory of Retrovirus Research in Division of Viral Product. I'm very much involved in regulation of HIV vaccine, and my scientific world has been focused on HIV cell entry and HIV vaccine development.

ACTING CHAIRMAN DAUM: Thank you very kindly.

DR. GRIFFIN: I am Diane Griffin from Johns Hopkins.

DR. STEPHENS: I'm David Stephens from Emory University.

DR. GOLDBERG: Judy Goldberg from New York University.

DR. KATZ: Sam Katz from Duke University.

DR. DIAZ: Pamela Diaz, Chicago Department of Health.

DR. KOHL: Steve Kohl, Argonne Health Science University.

DR. KIM: Kwang Sik Kim, Johns Hopkins School of Medicine.

MS. FISHER: Barbara Loe Fisher, National Vaccine Information Center.

DR. MYERS: Martin Myers, National Vaccine Program Office.

DR. COFFIN: John Coffin, Tufts University and sometimes NCI.

DR. COOK: Jim Cook, University of Illinois.

DR. BLAIR: Don Blair, NCI.

DR. MOULTON: Larry Moulton, Johns Hopkins University.

DR. KETNER: Gary Ketner, Johns Hopkins.

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

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

DR. HUGHES: Steve Hughes, NCI.

ACTING CHAIRMAN DAUM: And I'm Robert Daum from the University of Chicago.

DR. LEWIS: And by way of introduction, I'm Andrew Lewis, as it says on this slide. Maybe we need to cut the lights down a bit. Can people see this better now?

I'm the Chief of the Laboratory of DNA Viruses, Division of Viral Products. I came to the FDA about a little over five years ago, having spent basically a 30-year career at the National Institutes of Health studying adenoviruses and adenovirus transformed cells.

My role in introducing today's session is twofold. The first is to review the status of the Office of Vaccines' approach to the use of neoplastic cell substrates for viral vaccine development and, second, to introduce the topic of designer cell substrates and the issues associated with their use for vaccine manufacture.

Is this better? Keith, could you see about focusing this slide? Is that better?

Okay. Thank you.

Several of the topics for discussion today have evolved from studies of viral oncology, using in vitro tissue culture systems in studies of neoplastic development in vivo using animal models.

To understand the terminology that's evolved from these fields that will be used by some of the speakers today, I've defined in this slide what we mean when we say we need neoplastic cells, cell transformation, cell line tumorigenicity and viral oncogenicity.

Neoplastic cells is, for our discussion today, used in its broadest sense to include spontaneously transformed cells, virus transformed cells or other types of immortalized cell lines that may be either tumorigenic or non-tumorigenic.

Transformation is a process by which normal cells are changed by viral or cellular oncogenes or spontaneous events to become immortal neoplastic cells.

Tumorigenicity if the ability of neoplastic cells growing in tissue culture to multiply and develop into tumors when injected into animals, and oncogenicity is the ability of a virus or viral or cellular genes to convert the cells of an injected animal into tumor cells.

Now, the use of neoplastic cells for vaccine manufacture has been discouraged since 1954. A number of factors are contributing to the need to reconsider neoplastic cell substrates for vaccine development, and those factors that are related to the discussion today are presented in this slide.

First, cell lines capable of complimenting the growth of defective viral vectors used as antigen delivery systems and hence of vaccines.

Second is the development of virtual vectored HIV vaccines.

Finally, progress in understanding carcinogenesis and detecting adventitious agents, and the successful experience with highly purified biologicals that are actually derives from tumor

cells.

Discussions regarding issues associated with the use of neoplastic cell substrates were begun in the Office of Vaccines in 1996. The outcome of these initial discussions was the development of a systematic approach to consider and evaluate these issues.

This approach consisted of the five steps, which include identifying the issues, developing appropriate models to evaluate each issue, developing the necessary data to establish the validity of the models used to issue your evaluation, developing criteria to consider levels of risk, and discussing the approaches or this approach in public forums and meetings.

In the initial stages of implementing this approach, six issues were identified. These issues and the concerns they generated are presented in this slide. The issues were discussed in detail before the committee in 1998 and again in May of 2000.

Of the six issues that we identified originally, only Issues 2, 3, and 5 will be the focal points for today's discussion.

Issue 2 includes adventitious agent contamination with the possible transfer of known or

unknown viruses. For purposes of today's discussion, we will include agents of transmissible spongiform encephalopathy under the category of adventitious agents.

Issue 3 includes residual cell substrate DNA contamination with the possible transfer of activated oncogenic and/or infectious genetic information.

And Issue 5 includes viral-viral and viral-cellular interactions with the possibility of transfer of novel or recombinant viruses, and for the issues that we will be dealing with today, this includes replication competent adenoviruses.

Now, to manage the model and risk assessment aspects of the Office of Vaccines' approach, what we're calling a defined risk evaluation was developed. The basic aspect of this evaluation includes assessing quantitative where possible the risk posed by the issues, establishing the probability of a worst case scenario for plausible issues, using available data to evaluate plausible risk individually and cumulatively, and using cumulative data to assess the relative risk of the product.

The concept and implementation of the defined risk evaluation will be presented in more

detail by Drs. Peden and Krause when they discuss residual substrate DNA and with adventitious agent issues later this morning and this afternoon.

To implement the public discussion stage of the CBER approach, our plan was presented to the Advisory Committee in November of 1998. During this meeting, the Committee recommended that we develop the plan into a draft document and present the plan for discussion at an international workshop on cell substrates.

This recommendation was implemented over the next nine months and culminated in a workshop on neoplastic cell substrates that was held in Rockville, Maryland, in September of 1999.

Additional discussions at the Office of Vaccine followed this meeting and the public discussion of neoplastic cell substrates was continued at the May Advisory Committee last year.

Now, to briefly summarize the substance of the Office of Vaccine's presentations at the May 2000 Advisory Committee meeting, neoplastic cell substrates were divided into five categories. Category 1 included human cells used for vaccine manufacture that are transformed by known mechanisms. Since there are no cell lines like this, hypothetical examples include

the diploid WI-38 and MRC-5 cell strains that are immortalized by human telomerase gene.

Category 2 includes early passage human diploid cells transformed by known mechanisms. Examples include the 293 cells and PER.C6 cells that are going to be the focal point of our discussion today.

Category 3 through 5 represent non-human primate cells transformed spontaneously. These include VERO cells, CV-1 cells and BSC-1 cells. All cell lines that are derived from tumors of any species, and those cells lines that are not covered by Categories 1 through 4.

Examples of these types of cells in Categories 3 through 5 include HeLa cells and the HUT-78 cells, which is used to propagate HIV virus.

Now, these categories were developed based on estimations of the difficulties in managing the regulatory issues associated with different types of cells. Possible management approaches were presented for each category.

However, today time doesn't permit me to review the variety of issues and approaches that were raised by cells in each of these categories. This information is available in the transcripts of the May

2000 meeting, which are present on the CBER Web site.

Of these five categories, only Categories 1 and 2 as examples of designer cell substrates are going to be discussed today.

And as I mentioned, the subject of today's meeting is to consider issues associated with designer cell substrates which fall into Categories 1 and 2, as you just saw. For today's discussions, we're defining designer cell substrates as normal human cells. They're neoplastically transformed by a known viral or cellular oncogenes or by immortalizing cellular genes.

Because it's now possible to engineer or design all types of mammalian cells to express desired traits, this definition may need to be altered in the future. In the next talk, Dr. Steve Hughes will present in more detail the development of designer cell substrates and the issues associated with their use.

Like the factors that are stimulating the need to use all types of neoplastic cells and substrates for vaccine development, there are a number of factors behind the need to develop and use designer cell substrates for vaccine development. These factors include the development of cells to complement the replication of bioengineered viral vectors,

increasing experience with viral vectors in gene therapy and the production of biologically active proteins, and hence the development of vaccines and the development of HIV vaccines.

I should like to point out the development and use of bioengineered defective viral vectors to serve as vaccines by delivering immunizing antigens requires the use of cells containing the missing copies of the defective viral genes to assist the growth of the defective vector.

In the third talk this morning, Dr. Aguilar will have much more to say about viral vectors and especially adenovirus vectors as vaccine delivery systems.

The designer cell substrates we'll be considering today include 293 cells, which are human embryonic kidney cells transformed by restriction enzyme flea fragment of the Adenovirus 5 genome. Frank Graham described this cell line in 1977.

PER.C6 cells, which are human embryonic retinal cells that are transformed by a clone fragment of the Adenovirus 5 genome, these cells were described by Frits Fallaux in 1998.

Because there's been very little published on PER.C6 cells and a considerable amount of

information has accumulated on 293 cells since they became available in 1977, much of our discussion today will focus on 293 cells.

The talk by Dr. Alex van der Eb later this morning will discuss the origins and the characteristics of these cell lines.

The regulatory issues associated with the use of designer cell substrates are similar to the issues associated with the use of other types of neoplastic cell substrates. These issues include tumorigenicity and the ability of cells on tumors in animals, residual cell substrate DNA contamination, and the possible contamination with adventitious agents.

And in contrast, the cells are transformed spontaneously, are derived from mammalian tumors that arise in animals or humans. Designer cells have the perceived advantage of starting with cells that are known to be normal and are neoplastically transformed by a known mechanism.

From a regulatory perspective, this type of information provides an additional level of assurance that unknown factors which might be present in the cell substrate of less certain origin are not available to enhance any risk to vaccine recipients.

The issue that tops the list of concerns with the use of designer cell substrates and neoplastic cell substrates, in particular, is their tumorigenicity, which is their potential to grow into tumors when injected into rodents.

For many years assays of tumorigenicity have been used to discriminate between cells that are suitable for vaccine development and those that are not.

The risk believed to be associated with the capacity to produce tumors in animals are noted in this slide. Tumorigenicity has been perceived to be a trait associated with high risk, and due to the possibility of transferring cell components, either DNA or proteins or possibly viruses, with oncogenic activity to vaccine recipients.

However, proteins from tumor cells are unable to sustain neoplastic development, and they're unable to transform cells. This leaves cell DNA and oncogenic viruses as the risk factors associated with cell substrates that are tumorigenic.

In order for the Committee to appreciate what we mean when we talk about tumorigenicity of adenovirus transformed human cells, studies on the tumorigenicity of 293 cells are presented in the next

slide, and they're compared with A-549 cells, a cell line that was established from a human lung tumor.

I have to apologize for the transfer of our information by computer to the people making the slides because I became Lew is rather than Lewis, and the mouse obviously suffered a discrepancy as well.

But in this slide, what we're looking at are a series of tumorigenicity assays, one done by Frank Graham and two done by myself. In the original description of the 293 cell line, Graham reported that the cells weakly tumorigenic, and they produced tumors in only three of 20 animals inoculated with -- and I think this may be hard to see -- but that's ten million cells per mouse.

We repeated this experiment ten years later, did a little more detailed inoculations and the animals inoculated with 100 million cells per mouse, ten million cells per mouse, and a million cells per mouse, and we basically discovered or found, got the same results that Frank Graham got in that of the number of cells required to produce tumors in mice was somewhere in the range of ten million cells.

The way that these data are reported is in terms of the TPD-50 value, which is tumor producing dose at a 50 percent endpoint. That's the number of

cells that's required to produce tumors in 50 percent of the mice, and these numbers are basically comparable.

However, when you compare these to A-549 cells, which is the cells derived from human tumors, it only takes about 1,000 cells to produce tumors for 50 percent of the mice.

Therefore, the A-549 cells are about 1,000 to 10,000-fold more efficient in inducing tumors in animals than are the 293 cells.

Dr. Jim Cook is going to have a lot more to say about tumorigenicity of adenovirus transformed cells later this morning.

The potential risk associated with residual cell substrate DNA in vaccines prepared in designer cells represents another concern. DNA from neoplastic cells can contain activated oncogenes, viral oncogenes, the genomes of oncogenic viruses, latent viruses, as well as retrovirus proviruses.

Clone cellular oncogenes can induce tumors in rodents, and DNA from oncogenic viruses and cloned viral oncogenes can also induce tumors in rodents. Latent viral genomes in retrovirus proviruses sequestered in cell DNA can be infectious.

Due to these observations, the possibility

must be considered that residual DNA from designer cell substrates could transfer either neoplastic activity or infectious virus genomes to vaccine recipients.

The talk by Dr. Peden later this morning is going to cover in detail the issues associated with the use of residual DNA.

The third concern associated with the use of designer cell substrates is the possibility of adventitious agent contamination. All cell substrates are subjected to possible contamination with adventitious agents. Due to their laboratory origins, the designer cell substrates might represent a risk of adventitious agent contamination because they're neoplastically transformed and may be tumorigenic. Designer cell substrates might represent a risk of contamination with unknown, possibly latent oncogenic agents.

Dr. Krause in his talk this afternoon will address the issues specifically associated with evaluating designer cell substrates for adventitious agents.

I'd like to conclude my talk by saying that today we are facing a transition. By considering the issues associated with the use of Adenovirus 5

transformed cells, such as 293 cells, we're confronted with the first of the truly novel neoplastic cell substrates that we've discussed with the Committee over the past three years.

As these cells fall into the category of being tumorigenic, they represent a transition from the previous way of thinking about cell substrates that goes back over four decades to future ways of thinking about cell substrates.

As with most of these types of situations, this transition presents risks that must be confronted. However, this transition also presents the possibility of future rewards. Those rewards will come from the ability to maximize the benefits that can be obtained by the application of molecular technology to the development of safe and effective vaccines.

The challenge facing us today is to objectively review the data that's available on these types of cells, determine what these data tell us about their potential to produce safe and effective vaccines.

I think that's the end of the slides. To assist CBER and the Committee in this review, we've invited those individuals who have introduced

themselves to you whose work qualifies them as experts with sufficient experience with defective viral complementing cell systems and the issues they raise to review the relevant data before the Committee, to answer Committee questions, and to offer their opinions regarding the issues that need to be addressed.

Before they begin to speak, I'd like to just take this opportunity to thank them for the time that they have used to assist the Office of Vaccines, the Committee, and the public in these discussions.

This concludes my talk. I'd be happy to try to answer any questions.

ACTING CHAIRMAN DAUM: Thank you very much, Dr. Lewis. That provides a useful setting for us to continue hearing about this issue.

It also reminds some of us that it's time for our annual visual screening test.

(Laughter.)

ACTING CHAIRMAN DAUM: We do have the opportunity run behind a little bit here in terms of scheduling if there are Committee questions. Alternatively, we can get some more information on the table and then initiate discussion.

Is there Committee input? Dr. Goldberg,

and then Dr. Griffin.

DR. GOLDBERG: Yeah, just on your table of tumorigenicity where you show the rates of tumorigenicity in the 293 cells in the nude mice, can you give me some feel for how you feel that you can distinguish these levels?

For example, you have -- I can't see. I'm sorry -- you don't observe any tumors in four nude mice at ten to the sixth in one experiment and in another experiment you observe four of four.

And you know, any calculations I do would suggest that you really with four animals can't distinguish.

So can you give me some feel for what other information you're bringing to bear on this to make the distinctions about what the TPD-50 is?

DR. LEWIS: I guess I'm having a little bit of a hard time hearing what you were saying. You're trying to understand how we calculate the TPD-50?

DR. GOLDBERG: No, I think I do know how you do that, but my concern or my question really is: how do you feel that based on these experiments with four mice at each of these dose levels that you can really estimate the TPD-50 with any certainty to make

a distinction between --

DR. LEWIS: Okay.

DR. GOLDBERG: -- ten to the three and ten to the sixth, for example?

DR. LEWIS: Basically, the data that was used to do this came from a series of titrations that we did on Adenovirus 12 transformed mouse cells. These assays were repeated ten times, and each time they were done in four mice, but nude mice are expensive, and each time they were done the standard deviation of those assays was about plus or minus .6 of a log. Okay?

So based on the information that we obtained with that, we are reasonably confident that this represents an accurate way of reflecting this type of information.

The data on the 549 cells and many of the 293 cells were repeated at least twice, and the numbers are basically the same.

ACTING CHAIRMAN DAUM: Dr. Griffin.

DR. GRIFFIN: Well, I guess I was being puzzled by the same table. And maybe I just missed this, and I got a little clued in what you just said.

The difference between the 293 cells and the A-549 cells is one is Ad. 5 transformed and the

other is Ad. 12 transformed?

DR. LEWIS: No.

DR. GRIFFIN: And they have different --

DR. LEWIS: No. A-549 cells are a cell line was established from a human. I believe it's an oat cell (phonetic) carcinoma. Okay? And they were established directly from the human tumor in the tissue culture. They are not virus transformed. They are a cell line that developed from a human tumor that developed in nature, a spontaneous tumor in the human.

DR. GRIFFIN: So the point to be made from this is that cells differ in how likely they are --

DR. LEWIS: Well, yes, that's one point. The second point is that it takes a large number of Adenovirus 5 transformed cells to produce tumors. This is true both in adenovirus transformed mouse cells, Adenovirus 5 transformed hamster cells, as well as adenovirus transformed human cells.

They fall into a category that most people would define as weakly tumorigenic, and this is a characteristic of Adenovirus 5 transformed cells.

DR. GRIFFIN: Thank you.

ACTING CHAIRMAN DAUM: Okay. Thank you.

I'd like to move on then at this point -- thank you very much, Dr. Lewis -- to Dr. Steve Hughes'

presentation, entitled "Designer Cell Substrates for Vaccine Development: Concepts and Issues."

Dr. Hughes.

DR. HUGHES: This is somewhat smudged. I may challenge people's optical state once again.

Thank you.

Since this subject has been so ably introduced by Andy, I'll try and go through this quickly.

Basically the question to consider, of course, is how designer cell substrates, in fact, differ from other permanent cell lines with transformed cells, and basically in the past, spontaneous transformation has been used to establish cell lines, and that simply means you take cells from an animal usually or an embryo and passage them in culture, and it's a particular characteristic of rodent cells that after some period of passage the cells undergo some sort of change, which we still don't understand clearly, that alters both their ability to grow permanently in culture and alters some of their physical and biological properties.

The other way that cells have been immortalized or immortal cells have been derived is as was just mentioned, from tumors taken from either

humans or animals, and in some cases these can be established directly in culture, and in some cases these tumor cells are then serially passaged in animals, and both of these methods have been used to establish a number of types of cells lines that basic researchers, such as myself, use routinely in the laboratory.

And it's very convenient, but it has a particular disadvantage, and that is that in neither case do we have any clear notion of what changes have taken place in these cells, what it is about these cells that differentiates them from the normal cells that don't have the properties of either being transformed or growing forever in culture.

And so one of the things that I think makes everyone a little nervous about these types of cells is not necessarily that they have something specific wrong with them, but, in fact, the very fact that we don't know what it is that has changed them. We don't know how they differ from the normal cells that everyone feels reasonably comfortable with.

And as an alternative to that kind of idea, what's meant, as Andy has just told you, by a designer cell substrate that differentiates it from these two types of cells is that one now can take

specific -- which I have not spelled correctly -- specific DNA segments either derived from virus, derived from cells, that can change the growth properties of normal cells, and in so doing, we now have something in which we understand what agent it is that is causing the cells to behave differently.

And that gives us some particular handle and some particular feeling that we have at least some idea of what's going on.

This does not, of course, eliminate all the worries that one might have. There are issues. One of the issues is that there is the question of whether this specific DNA that when it's added actually has some sort of risk associated with it.

Of course if this DNA segment is capable of causing the cells to grow forever in culture, it may have oncogenic potential, and in fact, as you've just heard discussed by Andy, there is some reason to think that in the case of the adeno early region that there is some oncogenic potential of that.

So you really would worry about carrying the DNA if you're using a vaccine preparation with the vaccine material that you're going to use.

So there is actually still a question of the degree to which this is a serious concern, and

you'll hear more about that later today. I would also be pleased to tell you one of the things that was discussed the last time this group met was that there should be in a sense a collaboration between the NCI and the FDA to try and get a more quantitative assessment of what the risk is in terms of using defined amounts of defined oncogenic DNA segments.

I'm pleased to say that that interaction has reached the point where it's funded and that there will be some quantitative studies to try and establish exactly what the risk is at least from some defined DNA segments.

As Andy has also mentioned, there is the issue of adventitious agents, that is to say, that any cell, whether it's a cell that is permanent in culture, whether it's a normal diploid fibroblast can be infected with virus, can have other agents associated with it.

And in both of these cases, as I just tried to allude in the DNA, in part the question here is both understanding what sorts of things pose risks and, secondly, trying to understand how it is we can determine what agents, in particular for adventitious agents, what adventitious agents might be present.

And so what I've tried to say is that the

issue, I think, from the DNA is at least in part one of risk assessment, and I think that there actually are reasonable ways of defining what the risks are.

One can take, particularly if one knows what DNA segments one is dealing with; one can take those DNA segments; one can take defined amounts of those DNA segments; one can inject them into animal models, and one can define the oncogenicity, and based on that, one can get some reasonable measure, some idea of what it is that we're facing in terms of the risk.

And in the case of adventitious agents, one of the nice things about modern molecular biology and biotechnology is that we now have much better ways of looking for at least nucleic acid bearing agents, and you'll hear, I think, a little later today in considerably more detail than I intend to discuss the sorts of things that are under consideration as ways of doing this.

So the question then becomes given that we have these tools and given that we have these problems, what sorts of things should we do. How should we go about trying to be as safe as possible?

And I think one of the things, and I think it's going to come up in considerably more detail, is

culture history. You would like to know where the cells have come from. You would like to know where they have spent time. It's sort of like worrying about where your teenage children go at night.

And there are a couple of things that I think are worth discussing, although probably only briefly, that might not be sort of immediately obvious if one just thinks about passage or culture history, and it's been alluded to, I believe, earlier that, in fact, the source of the serum and what might be in the serum turns out to be a substantial consideration.

And this is true for both agents like BSE and, of course, for viruses as well. And I think there's another issue that I don't believe has been discussed in any particular detail, but actually I think does matter not even so much for designer cell substrates, but for substrates that are derived in a sense directly from tumor material, and that's the idea that it's one of the traditional methods for deriving particularly cell lines from human tumors, is to passage the cells in mice.

And there's a particular consideration which is one which makes for one of John Coffin's favorite stories, that suggests that there is an element of risk here that people don't always

consider.

Mice, of course, contain several families of endogenous retroviruses, and some of these endogenous retroviruses preferentially replicate in cells derived from mice, and some actually replicate preferentially in non-rodent cells.

And one of the things that happens when, for example, human cells are passaged through nude mice is that that provides a wonderful opportunity actually for xenotropic viruses -- these are the viruses that like to replicate in non-rodent cells -- to actually infect the human cells.

And it's quite possible actually in this kind of culture history to add an adventitious agent that one would really not normally think would be one you'd have to look for in a cell derived from a human or a primate.

So these sorts of considerations, I think, are very important and certainly I think we have to give substantial consideration to having a defined culture history not so much because it will necessarily rule out all possibility of adventitious agents, but we can understand if we know the culture history what sorts of adventitious agents we should look for.

And one of the problems in searching for adventitious agents with, for example, the nucleic acid technology that will be used for many viruses is you only find the things you look for, and if you know what to look for, it makes your job much easier.

And the final thing that I think is perhaps the most challenging part of the problem, the part of the problem that I'm not sure I have a particularly good idea yet how to resolve, is the issue of the stability of the genotype or the phenotype of the cells.

And the reason this is a consideration actually goes back to the idea that I introduced the talk with that, in fact, you can derive cells, cell lines by simply passage in culture; that, in fact, there is such a thing as spontaneous transformation.

And of course, not only is there spontaneous transformation, but upon passage the properties of the cells in culture upon prolonged passage can change. They don't have to change, but change can occur.

Now, that means that, in fact, the phenotype, for sure, and probably the underlying genotype has altered during the passage of the cells.

Cell lines do change upon prolonged

passage in cell culture, and so the question then arises if that is true, how do we gain confidence that the properties of the cells after some period in culture, in fact, match the properties of the cells with which we began. That is to say if we're quite confident that we've made a designer cell line that has the desirable properties and has only the changes we put in and then we passage it for a long time, given that the cell lines can change, how do we know that the cell line hasn't changed?

And that seems to me to be one of the substantial requests that we need to consider, and of course, one of the old standards of tissue culture people is simply to use cells that have been passaged a relatively small number of times, and that, of course, because these changes appear to be spontaneous, some sort of genetic accident, by using low passage cells, the chances that some change has taken place seems to be better. The possibility that there's a change seems to be less.

But the final thing that I think we ought to at least begin to think about is the idea that we might at least in some cases give consideration to using some sort of regulatable system to drive the expression of the gene that causes the cell to change

its properties.

And if we imagine, for example, that we have some sort of promoter that has a switch on it that we can turn on and off so that we can turn on and off the gene that we're interested in that is causing the cells to be transformed, then if that is the cell -- I'm sorry -- that is, if the gene we've added is, in fact, the agent that changes the properties of the cell, if we switch that gene off, then the cells' properties ought to fall back to that of the starting cell, which was not permanent or transformed.

And I mean, it may be that I'm throwing this out as an idea, not as a solution. You may not want to use necessarily an inducible promoter, but the idea that I think is central here is somehow to find a way to regulate the expression of the gene you're interested in, whether it's some sort of dominant negative effect either at the protein level, at the nucleic acid level or the inducible promoter.

The idea that we want to, I think, think about is can we validate, can we determine after some passage that the agent that we think is changing the properties of the cell is, in fact, the responsible agent -- I'm sorry -- the gene, the designer gene that we've added, or have there been some additional

changes in the genotype and phenotype that are influencing the behavior of the cell.

And I think giving some consideration to that idea will be important, and I think at that point I'll stop, and if there are questions I'd be happy to answer them.

ACTING CHAIRMAN DAUM: Okay. I'd like the questions at this time to be focused mainly on Dr. Hughes' presentation. There will be plenty of time for more general discussion later.

DR. COFFIN: Steve, there's an issue you didn't raise actually that comes up particularly when one is considering these cells for growth of viral vaccines, and that is the potential of the cells to actually contribute genes to the vaccine virus itself by some sort of recombination and the consequences of that, and I think that's an issue that could arise, particularly if the retroviruses of a cell line has picked up an endogenous xenotropic virus or with the early genes of adenovirus.

DR. HUGHES: I deliberately, as i'm sure you're aware, avoided that issue both because I think there will be consideration of the recombination issue by others later and because I believe that the issues are somewhat different for adenovirus, which I think

we're supposed to be focused on today, and retroviruses.

And I think the issue of recombination and the mechanics of recombination particularly as they pertain to retroviruses are a bit beyond the scope of the discussion we have here. So that was a deliberate omission.

But I certainly think that as Dr. Coffin points out that the issue that he raises is a real one, and that we should give very careful consideration to issues of not only what the viruses can do to cells but, in fact, in some more complicated sense what the cells or things in the cells can do to the viruses.

ACTING CHAIRMAN DAUM: Thank you, Dr. Coffin and Dr. Hughes.

Dr. Kim.

DR. KIM: Are there any designer cell substrates on the horizon or on the radar screen that are shown not to be oncogenic or less likely to be oncogenic?

DR. HUGHES: I'm not qualified to answer that.

ACTING CHAIRMAN DAUM: Would you like to try, in looking for answers to this question?

(Laughter.)

ACTING CHAIRMAN DAUM: Sorry, but I do recognize you are number two in line.

Dr. Aguilar-Cordova.

DR. AGUILAR-CORDOVA: Yes. You talk about the transformation of cells and for a cell to become tumorigenic there's some old data showing a series of events. So if one has only one agent, wouldn't that just make ten minus one and the oncogenic event may still be there on that genotype?

DR. HUGHES: I'm not quite sure I precisely understand your question, but it certainly is the case that we now believe that for most tumors multiple genetic changes are needed, but many of the things that we regard as tumorigenic, whether they're chemical agents or viral agents, and these studies have been confirmed by genetic manipulation of mice, that anything we do that moves us one step closer to the required number, be it two, three, five, whatever it is, if we add any one thing to the list of changes, if we make any of the changes, that by doing that, by making the change, you do bring the cell closer to a transformed phenotype, and that you can show, for example, in mice by the p53 knockout mice, which have only a single change, get spontaneous tumors at a very

high rate because one layer of safety has been removed.

And I think that's the sort of thing that we would be nervous about, and of course, some of the cells may have more than one change so that you could actually from the DNA, depending on what the cell substrate was, actually deliver more than one of the things necessary to drive a cell in an animal or a human towards the transformed phenotype.

So the single one is not good, and some of the cells may have had more than one. So I think it makes us feel better that there are more than one, but I don't think it means that things were perfectly safe.

ACTING CHAIRMAN DAUM: Dr. Minor, please. Then Dr. Kohl.

DR. MINOR: The tumorigenicity assays done in rodents for very, very good technical reasons clearly, but is it possible that there are actually species effects; that if you took the immune response out of things, that you would find a different tumorigenicity ranking in a different species?

I mean, how relevant are the rodents do you think to a human situation?

DR. HUGHES: I think the answer is -- and

this is by definition a speculation because the experiments, in general, can only be done in rodents. So you have to sort of extrapolate.

But there is enough good data, I think, from chemical carcinogenesis to make one believe that, in fact, there are very strong species effects in some cases.

And I think that's a concern, but I think the choice in some sense experimentally is between doing the experiments in rodents, in which you have the worry that it may not perfectly reflect what happens in humans, and not doing the experiment at all.

And while I have some reservations of the exact sort you mention, in terms of worrying about doing the experiments in rodents and applying it to humans, I certainly would rather have rodent data and try and worry about the extrapolation than have no data at all.

ACTING CHAIRMAN DAUM: Thank you.

Dr. Kohl and then Dr. Myers.

DR. KOHL: That was my question.

ACTING CHAIRMAN DAUM: Dr. Myers, please.

DR. MYERS: I guess I have two questions. On the confidence of the stability of the genome,

would you be more confident if the designer segment were excised and the tumorigenicity was lost?

And the second question is related to that, and that is could you tell us about the relevance of the tumorigenicity limited to nude-nude mice?

DR. HUGHES: I don't think it's probably technically feasible to excise the segment, but I think there are ways of setting up the experiment so that you can interfere with the expression.

DR. MYERS: Knock it out?

DR. HUGHES: Knockout technology is probably not the easiest, but the point I'm really trying to get at is what I think you want to look at is not necessarily the precise technology or necessarily even to limit yourself to a precise technology, but to be able to somehow develop either a technology or technologies that will allow you to ask the question that, in a sense, you're posing.

If you then interfere with or obliterate the expression of the thing you think is driving the cells towards this permanent tumorigenic phenotype, does that, in fact, change the behavior of the cells as you would expect?

And if you can do that, I think you're

quite confident that there's nothing else going on or nothing substantial going on. I don't know that I -- I think actually in terms of responding to the nude mouse question I'd feel more comfortable probably deferring to my colleague Don Blair, who I think is much more of an expert on tumorigenicity in nude mice than I am.

DR. BLAIR: Well, I guess the question is, if I understand it, is in an immunocompromised animal does the fact that the cell is tumorigenic have any relevance to normal situations, and I guess, you know, the argument would be that at least by demonstrating the tumorigenicity in the nude, you've shown the potential, and the failure to be tumorigenic in an immunocompetent system presumably arises from the immune response which could at some stage, at some mechanism be lost or be modified.

So I think, you know, the demonstration of the tumorigenicity in a immunocompromised system is important because it does demonstrate that there is that potential, as opposed to no potential or what presumably is no potential at all.

ACTING CHAIRMAN DAUM: Thank you for clarifying, Dr. Blair.

Dr. Lewis, then Dr. Aguilar-Cordova.

DR. LEWIS: Just in response to Dr. Kim's question about immortalized cells that are not tumorigenic, the only system that we're aware of in which that occurs by immortalizing cells with the human telomerase gene data suggests that it takes three different genes, telomerase, ras, and SV40 to transform a normal cell to a cell that is, in fact, tumorigenic.

You can immortalize cells with hTERT with a telomerase gene, and those cells, as far as I understand right now from what we're aware of in the literature, are not tumorigenic.

But so far nobody has proposed one of those as a designer cell substrate for our attention.

ACTING CHAIRMAN DAUM: Thank you, Dr. Lewis.

DR. AGUILAR-CORDOVA: My question actually follows very well on that, and it's a follow-up on what I started to say.

So if it's a series of events, would a particular event, whether it be the telomerase, the SV40 T antigen or myc, be oncogenic depending on the background of the cell that it hits so that they're complementing oncogenes?

And I guess that begs the question to

whether any cell, tumorigenic or normal, is any safer depending on what the target cell is. It would appear normal if it only has an activated end myc, for example. It wouldn't appear tumorigenic, nor immortalized perhaps.

DR. HUGHES: Those of us who have spent many, many hours, days peering through microscopes at cells that had single oncogenes delivered by, for example, retroviruses that give very high levels of expression could in many cases see the effects either by change in morphology, change in growth pattern, change in behavior of adding a single gene.

So I think there are certainly reasons to believe that even if it takes multiple changes to produce the Frank phenotypic tumorigenic phenotype in vivo, that the adding of individual oncogenes one at a time or in some cases removing or ablating tumor suppressor genes actually does substantially change the properties of the cells, even if it's not sufficient necessarily to drive the cell to its full Frank transformed phenotype.

So I actually believe it is important to in some sense keep track, and I also think, as I tried to say earlier, that because these things are, in fact, additive as far as we know in humans, that

providing one or two steps in the direction of the oncogenicity is something we'd like to avoid, I think.

One could argue in many cases that chemical carcinogenesis provides exactly one or two of the changes and the long duration that you see to the development of the tumor after the initial exposure to the chemical insult actually represents the fact that the chemical may have changed only one or two things, and the rest must occur spontaneously later.

But that still enhances the risk substantially, and I think those are things that we need to worry about.

ACTING CHAIRMAN DAUM: Thank you very much, Dr. Hughes.

I think we'll move on, if you would. That was a very helpful presentation, to Dr. Aguilar-Cordova -- I hope I'm not butchering your name -- who will tell us about adenovirus biology as related to development and use of adenovirus vectors.

DR. AGUILAR-CORDOVA: Can you hear me?

Yeah. So I'm just going to give a general, generic background on the adenoviruses so that we can use this for further discussion and their use as vectors.

So adenoviruses were identified in the

early '50s from an adenoid tumor, from an adenoid tissue, thus the name adenoviruses, and it's associated with some fairly common illnesses in the lay group referred as the "common cold," some eye inflammations, et cetera.

It's composed of a linear, double stranded DNA encapsidated in a protein shell; has no envelope, and there are many different types of adenoviruses in nature.

They are primarily classified based on the organism of origin, and so there are two major groups: Mastadenoviruses and the Aviadenoviruses, those that come from mammals and those that come from birds.

Hopefully nobody else has slides.

(Laughter.)

DR. AGUILAR-CORDOVA: The further characterization is in the antigenicity of the terminal knob in the fiber protein and hexon epitopes and, thus, the serotype, and you'll hear about Serotype 2, Serotype 5, the most commonly used, and there are many other different serotypes, and also by hemagglutination, binding of the fiber protein to red blood cells.

And it turns out that some groups of adenoviruses have more tumorigenic ability in rodent

cells. None have been shown, as you will hear probably later on, to be tumorigenic in humans.

The virus is icosahedral. It has 240 hexons, 12 for each of 20 triangular phases and 12 pentons, and as you can see, it has these little fibers. The fibers and the pentons and the hexons are what constitute the serotype of the virus.

Inside that icosahedral protein base there is a double stranded genome flanked by two terminal repeats and some proteins that go with that genome, and here's the list of them.

Primarily the terminal protein is important to keep the stability of the genome and to condense it.

The gene structure is linear as well, but it does express from both strands. What you've heard here is E1A and E1B. The E1 region, these are critical for expression of other genes of the virus, and thus the majority of the vectors that are used in adenoviruses are vectors in which these two genes have been deleted and replaced by the gene of interest.

There are two origins of replication. In the ITRs, inverted terminal repeats, transcription units include five early genes, the E1A and E1B that I just mentioned, the E2 region, the E4 region, and

the E3 region. I'll talk a little bit more about these. There are two delayed early and one major late transcript that includes late one through five.

The E1A, there are two proteins, and this is host activation, and what this really does is activate the transcription and induce hosts to enter S phase, and that activates a transcription of most of the other adenoviral genes.

E1B is also two proteins, and it induces cell growth, and we know no what E1A and E1B do this in part by binding to some of the cellular genes that regulate cell cycle and expansion, like p53 and retinal blastomagy (phonetic).

E2 has three proteins. It's involved in virus reproduction, DNA replication, in particular. E3, there are four proteins. It's believed that this is protection from viral infection, and thus it down regulates the ability of the cell that has the virus to be immunogenic.

One of the down functions is that it down regulates the expression of MHC Class 1, and thus the host can't recognize the other proteins going on.

And the E4, there are at least four proteins, and it has miscellaneous activities, such as regulation of transcription, MRNA transport, and DNA

replication of the virus.

The late genes, there are five of them. They're mostly involved in the structure of the virus itself and the stabilization of that core.

The viral life cycle and replication is really two faces. There are early events, which occur in the first six hours after infection, and that includes absorption, penetration of the virus into the cell, disassembly of the virion core, and transcription and translation of the early genes.

One that begins, the late events by definition start, and that's in the next 18 hours or so, and that is when there is the construction of new virions.

And we have approximately ten to 50,000. Traditionally it was said there's about 10,000 virions per cell. We know now that there are -- when we produce them in the laboratory, we can produce a lot more than that. So wild type probably also does more than that.

Now, the next step, of course, is that's the virus. How does one use the virus to optimize gene transmission and, therefore, its potential use in gene therapy?

And there are two key factors of

adenoviruses that actually have allowed us to use this variance as good, effective vectors. One is that we can package up to 105 percent of its capacity. So when we take out the E1A or the E1B genes, that gives a little bit of space, and then you have actually a five percent wiggle room there.

And the other very important issue is that one can manipulate the virus in a circular form. That is, the ITRs can be circularized in a plasmid-like structure, and one can clone and change contents that way.

So given those two factors, we can then manipulate the virus easily and make it an efficient vector.

This obviously you will not be able to read, but it is to show that there are many different viral vector vehicles that one can use. The most common ones are retroviruses, adenoviruses, and associated viruses and Herpes viruses, and they all have pluses and minuses depending on the use that one will have for them.

Retroviruses are often used as an advantage, which is that it will enter the cell efficient, integrate so there will be stable expression for long terms, and that one has no viral

genes in the most common of these vectors.

And the disadvantages is that they're hard to produce. They have a limited insert size, and that they may integrate randomly and thus cause mutation.

On the flip side for adenoviruses, the advantages often listed are that it enters cells sufficiently, produces high expression of therapeutic gene, the transgene of interest; does not integrate into the host chromosome, and thus the disadvantages that are often related that it's not a long-term expresser; that the viral genes are often in the vector so that it's immunogenic, and it gets disposed of by the host fairly quickly.

In the case of vaccines, this may be a very useful disadvantage. So it may not be a disadvantage.

The same as for the other vector types, there are advantages and disadvantages depending on what one needs to use them for.

So typically on first generation vectors what one does is put the gene of interest instead of the E1A/E1B region. The E3 region is often deleted to create a little bit more space. The E3 region is totally for all apparent reasons irrelevant for in vitro expansion.

But as you can see, there would be a lot more of the viral genes that are still within the vector, and even though there is no E1A, there is some leakage and some expression of those viral genes after transduction.

So typically then one would create this in the laboratory, clone whatever gene of interest one wants, use that plasma to transfect a packaging cell. In this case we're talking about 293 cells or PER.C6 cells. These are cells that express the E1A/E1B constitutively. So they can entrance, complement the deficiency of this vector, and then one can produce a lot of those virions in the laboratory.

And theoretically once that virion then gets used to infect the target cell, it will not make anymore virions because it will not have this E1A/E1B region.

In that type of vector, the virus has approximately 8 kb of space for foreign DNA, as I mentioned, its replication deficiency. One can product them in very high titers in the laboratory, often close to ten to the 13th viral particles per milliliter. It affects a variety of tissues. It goes through a receptacle CAR for coxsackievirua adenovirus receptor, and it's fairly prevalent throughout nature,

and then it also uses some integrants.

And it can get into nonreplicating tissues, such as antigen presenting cells, and it can have high levels of transgene expression.

Now, there have been many different evolutions of the types of adenoviral vectors that are currently in use. What I've described to you so far is what is referred to as the first generation. That's E1 minus, and it can be E3 positive or minus.

The second generation vectors or so-called second generation is E1 minus as well, and then they had an additional mutation either in the E2 gene or E4 gene, and again, E3 positive or negative.

They haven't been quite referred to as any generation. I just called it generation X here. They are the ones that are E1A positive, E1B minus, and then the E3 region that's again positive or minus, and various farther generations like X.1 here is E1A and E1B with a conditional promoter so that they replicate only specific tissue types in which that promoter is active.

And the final generation at least so far is X.2, which are helper dependent, and these are closer to what a retroviral vector would be like in as far as viral gene content. Everything has been

deleted from the vector backbone, except the ITRs in the packaging sequence, and it has been replaced by some DNA content.

It does require at least 28 kb of DNA. So often these vectors come with stuffer DNA of some origin.

I want to give you a couple of examples though of how this generation of changes may not be that critical in the development of vectors for their use. For example, this is data from O'Neill, et al., in which they were using a second generation and comparing it to a first generation vector.

Here the dark bars are a vector that has the E1 and the E2 region deleted, deletions in the E1 and the E2 region, and the light bars are just an E1 vector. At one time it was ten to the -- these are dose per kilogram -- one times ten to the 12th, three times ten to the 12th, and one times ten to the 13th, and this is the platelet count of the animals or mice.

As one can see, the toxicity was perhaps slightly different. One times ten to the 12th dose, but it equilibrates very quickly at three times ten to the 12th and one times ten to the 13th.

So the thrombocytopenia that is often caused by this virus was no different in a first

generation than the second generation.

And here is again using liver enzymes, which is another known toxicity of this adenoviral vectors, and you can see that one times ten to the 11th, one times ten to the 12th, and then only a three times ten to the 12th is there a difference, and again, at one times ten to the 13th there's no difference.

So maybe the changes from first to second generation might give a slight window of difference in as far as the toxicity, but the profile seems to be the same.

Now, this was not the case when we got to the generation X.2, the gutless or helper dependent adenoviral vectors, and here what we see is a first generation vector with alpha-1 anti-trypsin, and this is a gutless vector with the same insert.

This is the level of expression through time. These are weeks, and you can see with the first generation it's a peak of expression within the first few days, leading to baseline, no expression later. These are just different doses of the vector ranging from 3.2 times ten to the 11th to 1.2 times ten to the 10th.

And you can see that with the gutless

vector, expression is much prolonged. Putatively, this may be due to the fact that there are genes, viral genes in the first generation that are still being expressed and that are still being immunogenicity against that content of genes.

And here is the toxicity profile. This is the liver enzyme toxicity profile, and you can see a peak. That is very clear in the first generation vectors, and it does not occur at any of the doses tested here with the gutless vector.

But it is not only the adenoviral genes that can induce the immune response. In fact, it is also the trans-gene that can induce the response. This is some data from Morall, et al., in PNAS in 1999, and what we see is in the little light blue color is a gutless vector, and it's human alpha-1 anti-trypsin again, and in the multi-color lines is a first generation vector. This is in baboons.

And what we see is that two of the three animals that received the gutless vector have a very long term expression. This is out 100 weeks here, and all of the first generation vector animals had lost all detectable expression by 20 weeks. Most of it was lost by ten weeks.

But there was one animal with a gutless

that actually lost all expression by ten weeks, and that animal, in fact, had developed an immune response to the alpha-1 anti-trypsin.

So these vectors can be used to generate an immune response to the coded gene. Of course, in addition to the variables that I just spoke about, there's variables for the analyses of the vectors, and I will just go very, very brief couple of slides on this mostly to give you a sense of what is not known rather than what is known. I will not go through all of the safety testing of quality control that's normally done for all viral agents.

And in order to do that, I want to just give you an idea of how these vectors are typically quantified, and so we have the quantity of particles that we can detect easily, but then we also need to know of those particles how many of them are functional and can transduce the gene of interest into the target cells.

Often what we do is we just layer a soup fully of particles on top of some target cells. The problem with this is that these particles, depending on how deep this soup is, how long the assay gets allowed to progress, and many other variables, most of these particles will never reach one of the target

cells. So may not be detected, and whether they get detected one time or another is quite variable.

So much so that we sent out an identical preparation to six laboratories, and we had a two log differential in the determined titer from those laboratories, all experienced with adenoviruses and also how they get handled may be very specific. This is just a bit of data from a paper by Neiber Hoffman, et al., and you see here you can't tell the distance, but there's a seven log differential on the vector that was shipped just across town into a clinical setting as an experiment for shipment only from a vector that was shipped on dry ice and a vector that was not shipped on dry ice.

So it turns out in that particular case it was the CO_2 that was seeping in, dropping the pH, and there was a seven log differential that could be lost in a very short period of time.

So in conclusion, adenoviruses, I think, I've shown that due to their biology they can be converted into efficient gene transfer vehicles. They're not inherently dangerous, even as wild type viruses.

Not all adenoviral vectors have equivalent toxicity profiles. Additional safety of the second

generation vectors may be transient and only in a small, temporal stage.

And this is actually very clear because standardization of the dose specifications is necessary, and I'm told there is a very standard way of determining the potency and the quantity of these entities. It is very difficult to analyze the data as a whole, and thus, assurance of clinical potency.

And just as a last mention, there is currently a working group that is developing a standard of wild type adenoviruses so that all of these things can be compared and quantified.

That's all I have.

ACTING CHAIRMAN DAUM: Thank you very much.

That was extremely helpful. It raises many questions for us to consider.

Dr. Coffin and then Dr. Kohl and then Dr. Stephens, Dr. Katz.

DR. COFFIN: You said something early on regarding the difference between adenoviral vectors and retroviral vectors that I think has something of the status of an urban legend without ever actually having been subject to a real test, and that is the idea that retrovirus vectors might have some greater

danger because of the property of integrating their DNA into a cell, in the cell DNA, whereas adenoviruses don't do that.

That's actually not true, of course. Adenoviruses do, of course, -- adenovirus DNA does get integrated after infection at some low event, and I believe that the -- I don't know exactly what the numbers are and I don't know if anybody knows exactly what the numbers are, but it's quite likely that you make up that entire difference in efficiency in integration by the difference in the doses of adenoviral vectors versus retroviral vectors that you give, and in fact, the probability of the integration of a fragment of adenovirus DNA may be equally high as a probability of retrovirus DNA integration after administration of these as gene therapy or vaccine vehicles.

Can you comment on that?

DR. AGUILAR-CORDOVA: The fact is that they do integrate occasionally. It's not their standard method of operation, but they do integrate occasionally. I didn't mean to imply that they were more dangerous or not. I was just reading off a list of often stated events.

The one difference would be that they do

no carry a promoter enhancer in there, in the LTR. So at least the promotion enhancement effect of the integration would be different.

DR. COFFIN: But there must be one somewhere in the vector or it wouldn't be any good as a vector, and often more than one, of course, if --

DR. AGUILAR-CORDOVA: Often, yes, and so how they compare as far as integration I can't comment. I don't know that there is any data. There is data showing that they do integrate on occasion, yes, and they persist.

ACTING CHAIRMAN DAUM: Dr. Kohl, please.

DR. KOHL: Two questions.

Thanks for your talk. It was enjoyable and elucidating.

You mentioned the concept of leakage. Could you elaborate that a little further, and can you get enough leakage from a gene that supposedly is deleted that you can get a competent virus injected into the host?

DR. AGUILAR-CORDOVA: So the leakage that I was speaking about was from the genes that were not deleted. Obviously the one that's deleted can't leak on there.

However, there is the potential for

recombination with the gene inside the packaging cell and thus leading to a replication competent virus. I believe that that will be spoken to as far as why the PER.C6 cells were developed, and the difference between them and the 293 cells.

DR. KOHL: Can you elaborate a little bit more on the toxicities of the adenoviral vector? What causes that? What's the mechanism of the toxicity? The platelets, the liver function transmission and other toxicities as well?

DR. AGUILAR-CORDOVA: Yes. So what we know are what the toxicities are, and particularly, of course, as you've heard, there was an incident in the University of Pennsylvania where a young man died due to a large dose of an adenoviral vector directly injected into their hepatic artery.

In that case what was seen was a DIC like syndrome with upper respiratory distress, and what we have seen in many animal models is an elevated liver enzyme content, often transient and recoverable, and not just animal models. Many Phase 1 studies have seen the same thing.

Thrombocytopenia, believed but not shown to be caused due to endothelial cell damage and leakage because it is consumptive thrombocytopenia.

DR. KOHL: Who does the mechanism?

DR. AGUILAR-CORDOVA: The mechanism is probably cytopathic effects of the vector and potentially an immunological response to the original infection.

ACTING CHAIRMAN DAUM: I have Dr. Stephens, Dr. Katz, Dr. van der Eb.

DR. STEPHENS: In this discussion, there's some at least in my mind confusion about the issues of gene therapy vectors versus vaccine vectors, and I'd like you to kind of clarify that issue for us, if you would.

More specifically, the question relates to the E3 sequence and whether you think that should be in or out of the vaccine delivery vector.

DR. AGUILAR-CORDOVA: Sure. So not just in this discussion is there a confusion of vaccine or gene therapy. I think as a member of the RAC we've had that discussion a lot, too, what should be accepted or not, and many of the gene therapy applications especially in cancer are, in fact, vaccines. We're trying to vaccinate against cancer.

And Dr. Ginsberg started working with an E3 region in early '80s, I believe, or before, and he's big proponent of leaving the E3 region in when

one wants to create an immune response because in that situation, it is possible that the adenovirus itself is an adjuvant to whatever one wants to create an immune response against.

The flip side of that is that in some studies in my laboratory and others, when transducing a gene that one wants to create a CTL response against, for example, with an adenoviral vector, we tend to get an awful lot of CTL against adenovirus and very little to none against the gene of interest.

So it could go either way.

ACTING CHAIRMAN DAUM: Thank you.

Dr. Katz, please.

DR. KATZ: What is the receptor for adenovirus and what cells express the receptor?

DR. AGUILAR-CORDOVA: The known receptor, and there are probably others, the known receptor is a module called CAR, coxsackie adenovirus receptor, and its distribution is fairly ubiquitous. Epithelial cells are especially high expressers, and it also uses some integrants as co-receptors.

ACTING CHAIRMAN DAUM: Dr. van der Eb.

DR. VAN DER EB: I'd like to come back to the issue of leakage, leakage that you mentioned. Even a deleted or undeleted vectors are supposed not

to express the rest of the viral genome that is still present in the vector. That is because the E1A gene is the master switch that directs the expression of the rest of the viral genome.

Now, it's known already for a rather long time that leakage may occur and expression of the rest of the viral genome occurs when very high multiplicities of infection are used.

For reason then this creates a kind of E1A-like activity in the cell and leads to expression of the rest of the viral genome with consequent reaction of the host cell immunological reaction of the host.

DR. AGUILAR-CORDOVA: Right. So in order for efficient transcription of the other genes, the E1 region is necessary. However, it seems like there is some gene expression, although maybe not at sufficient levels to produce virions and to create the E1.

But given the data, there seems to be some expression of the other viral genes even in its absence.

DR. VAN DER EB: Is the data of Tom Shenk from long ago?

DR. AGUILAR-CORDOVA: Right.

ACTING CHAIRMAN DAUM: Two last questions,

please.

DR. COOK: I'd just like to respond to the question about E3 in or out. E3's job from the virus' point of view is to shut down Class 1 expression or travel to the surface through Golgi mechanisms. So theoretically if that were uniformly true and you were trying to make a vaccine and it required expression of that peptide in the cell in which E3 was co-expressed, it would be a good idea maybe not to have E3 present.

The truth is when you infect with adenovirus in normal human cells or cells that don't express E1A, that phenomenon is very late in infection. It doesn't happen until probably 48 to 72 hours after infection with that virus.

So chances are the peptide expression could occur, depending on what the kinetics are. If the cell co-expresses E1A, the E3 effect is much greater. So it might depend on how you rate the system, but theoretically, at least, one would have to consider whether E3 is downregulating Class 1 expression on the surface and whether that alters antigen presentation or peptide expression on the surface of the gene of interest.

ACTING CHAIRMAN DAUM: Thank you, Dr. Cook.

Dr. Blair.

DR. BLAIR: Yeah. This comes out of my retroviral background, but is there any evidence that there's an encapsidation of cell DNA/RNA protein in the virus as it's assembled?

DR. AGUILAR-CORDOVA: Not that I know of. Not at the level that we've seen in retroviruses certainly with the RNAs, especially of viral-like proteins or viral-like particles and so on.

But certainly there's the possibility of that, and certainly there's the possibility of recombination events, non-specific recombination events that would package random pieces of DNA.

ACTING CHAIRMAN DAUM: Thank you very much, Dr. Aguilar-Cordova.

And we will shorten the 20-minute scheduled break to a 15-minute break. I have 10:40. We'll reassemble at 10:55 and continue with Dr. van der Eb.

(Whereupon, the foregoing matter went off the record at 10:39 a.m. and went back on the record at 10:58 a.m.)

ACTING CHAIRMAN DAUM: Would everybody please settle down as quickly as they can?

ACTING CHAIRMAN DAUM: We're ready to

continue with the open session.

We will now call on Dr. Alex van der Eb to tell us about adenovirus transformation of human cells and the development of 293 and PER.C6 cells for the manufacture of defective adenovirus vaccines.

Welcome, Dr. van der Eb.

DR. VAN DER EB: Thank you.

So what I would like to do is to describe to you how and why we have made two different cell lines, adenovirus transformed human embryo cell lines which are called 293 and PER.C6. Both cell lines were made in my lab, and also the cells, the starting material, was prepared by myself at the University of Leiden. The 293 cell was made by Frank Graham in 1973 from human embryonic kidney cells that were made from fetal tissue one year ago by myself one year before that, so that was in 19 -- probably in 1972, whereas the PER.C6 cell was made by Ron Bout and Frits Fallaux in 1995 from an embryonic retina cultures that were made from fetal tissue by me ten years before that, in 1985.

This just shows you again the adenovirus genome and you have seen it already. The interest in this virus was due to the fact that the viruses can transform cells in tissue culture. In fact, all human

adenoviruses or almost all can transform cells in tissue culture, and also that certain types of adenoviruses can induce tumors in experimental animals.

The transforming region -- oh, I hope it survived -- the transforming region is associated with the left-most about ten percent of the genome that harbors the E1 region.

We became interested in transforming -- in the question whether human cells could be transformed, and therefore, I will tell you how we got the transformed human cells, and it all started actually in 1972 when Frank Graham in my lab developed the calcium phosphate DNA transfection technique, which made it possible in the first place to make infectious virus with intact viral DNA.

If you transfect the intact viral DNA of Adenovirus Type 5 into permissive human cells you get infectious virus, but it also turned out -- do you have a pointer here? There is no pointer? Okay. Thank you.

And it turns out that not only it was possible to get infectious virus by transfecting human cells with the intact viral DNA, but also purified DNA proved capable of transforming cultured rodent cells,

but human cells could not be transformed.

And the reason is that these human cells got destroyed by the viralytic (phonetic) reaction. If the DNA was sheared, however, up to 3 mega delta Daltons, up to three million Daltons, it turned out that the transforming potential of rodent cells still remained intact, indicating that only a portion of the viral genome, a rather small proportion of the viral genome is necessary for transformation.

As I said, purified Adenovirus 5 DNA transfected into permissive human cells yields effective virus, but human cells could not be transformed, everywhere interested in transforming human cells by adenoviruses just in order to find out whether that is possible.

But we found some evidence that permissive human cells could be perhaps transformed from the fact that semi-permissive rodent cell cultures could be transformed if the DNA of adenovirus was sheared into smaller pieces, and these were Syrian hamster kidney cells.

The transforming activity, and this was done with detailed shearing studies at that time; there were no restriction enzymes. There was no DNA cloning at that time; that the transforming activity

associated with the 11 left percent, left-most 11 percent of the adenovirus genome, and this all affect the basis of the transformation by adenovirus of the human cells with fragments of adenoviruses.

So the reason why we wanted to transform human cells is just to answer the question whether human cells can be transformed at all by adenoviruses, human adenovirus, and if so, which part of the adenovirus DNA is required to transform cells? Is that the same area that is also needed for transformation of rodent cell or is it less or is it more?

And then can we simply develop a model to study transformation of human cells? And that was at that time important because although there was no evidence that human adenoviruses have anything to do with cancer in humans, it was still an open issue, an open question, and in fact, it is at this moment still, although clearly there is no evidence that human adenoviruses have anything to do with cancer in men.

So the method that we followed was take human embryonic kidney cultures. Why kidney cultures? And that is mainly because of the fact that the rodent system, the rodent model that we used were always baby

rat kidney or baby mouse kidney or baby hamster kidney. The kidney cells were very suitable for these transformation studies with sheared adenovirus DNA.

When we transfected these human embryonic kidney cultures with sheared purified Adenovirus 5 DNA with the calcium phosphate technique using carrier DNA of salmon sperms. So this was not restriction enzyme fragments. They were just not yet usable.

One year later we had resection enzyme available to make pure DNA, but the first and also the 293 cell was made with sheared Adenovirus 5 DNA, then simply scored for transformed colonies as we did with the rodent cultures.

So the kidney material, the fetal kidney material was as follows. The kidney of the fetus was, with an unknown family history, was obtained in 1972 probably. The precise date is not known anymore.

The fetus, as far as I can remember was completely normal. Nothing was wrong. The reasons for the abortion were unknown to me. I probably knew it at that time, but it got lost, all this information.

The kidneys of the fetus were then isolated and the kidney cells were isolated in the so-called still air cabinet. There were no laminar flow

hoods at that time, and this is simply a still air cabinet that was also used all over for tissue culture and worked quite well. There was UV lights in it just to sterilize it, and that was all.

So as we did also for the rat kidney cells, the surrounding membranes were removed as completely as possible, and the kidneys were then minced with scissors, trypsinized, and the cells that were recovered after removing the trypsin were cultured in medium containing bovine serum, calf serum. That is what we know.

And this calf serum was obtained not from a commercial source. We either got it from somebody else, from another lab, or we made it ourselves from blood, calif blood.

Rodent, monkey, and other human cell cultures took place in the same general area at that time. So there was one cell culture room, and there all of the experiments, all the cell culture work was being done.

There was also experiments with viruses, but that was in a separate virus cultured unit, and we used in addition to Adenovirus 5 whole viruses, also the oncogenic Adenovirus 12, as well as SV40 and possibly also already Herpes virus, but maybe Herpes

virus was not yet used at that time.

So the method was DNA from wild type Adenovirus 5, was isolated from virions. So we had to prepare the DNA by first growing and purifying the virions, and the DNA was then fragmented by shearing in this case through a 22 gauge needle up to about eight million Daltons. There was no cloning strategy at that time, and the DNA fragments were transfected as I already indicated with salmon sperm DNA with the calcium technique.

The results were rather disappointing. In the first experiment of quite a number of dishes there were not a single transformed colony. So we repeated it. Again, no transformed colony.

However, after many other experiments, we found finally one transformed colony which was visible in the cultures, and that colony appeared 33 days after transfection was seen, 33 days after transfection.

This colony, this single colony was picked and established and became the 293 cell.

There were two colonies here mentioned, and that is because one, the second colony, was only seen after the cells at the end of the experiment, the cultures were fixed and stained, and one other colony

was seen at the edge of a dish which we had missed.

So the single transformed colony which would give rise to 293 was very difficult to expand. The cells hardly grew at all, and after five months in culture, it was possible to freeze down the number of ampules. Only three ampules, passage four was that.

And at that time the cells started to grow faster, but still relatively poorly and a doubling time was at least a week or more than a week.

So it appeared from these experiments that human cells are resistant to transformation by Adenovirus 5. Although these cells replicate extremely well in replicating Adenovirus 5, they cannot be transformed with the same DNA that also transforms rodent cells quite efficiently.

So up to this moment, it's still unclear why human cells are resistant to transformation by adenoviruses. One possibility is that the 293 cell came out of a cell line that had some kind of a mutation so that it became permissive to transformation.

Another possibility is that since this is primary human embryonic kidney which consists, of course, of many different cell types, that there is one -- that there are very few cells in the whole

culture which are permissive to transformation, and that this particular cell came from one of those transformation prone cells.

We will never know probably, but one possibility is that the 293 cell is actually a neural cell that was present in that culture. We don't know, and this is something that occurred to me when I was traveling here to Gaithersburg, and so that is a possibility that can probably be tested because neural cells appear to be more prone to transformation by adenovirus.

We also tried human diploid skin fibroblasts for transformation, never any positive result. We also have tried human embryonic lung cells. No positive transformation.

Anyway, around page 13, the cells went into crisis, the same type of crisis that is also seen when SP40 transformation is followed in human diploid fibroblasts, for example. They always go in crisis.

This crisis lasted nearly three months. During that time, the cells remained on the dish or gradually started to die, some of them at least. So you have to defeat the cultures for a long time. Nothing happens. There is no cell division.

And then the culture started to recover

for some reason not in the same way as usually in the case of SV40, but apparently cells all over the plate begin to grow, whatever that means. We don't know what happens in this crisis base.

After crisis, when telomerase is activated, apparently when the cells start recovering, the cells were subcultured and the growth rate increased significantly. Several ambules were shipped then by Frank Graham to McMaster in Canada, where he went to Anestilles (phonetic) in 1974, and the data have been published in several papers.

I would like to show here also the part of the adenovirus genome present in the 293 cells. It is not completely sharp, but it doesn't really matter, and this is the left-most 4,041 nucleotides. There is also some E4 region present in these cells, which is not expressed, however. So this is in the 293 cells.

Now, in addition to basic research, adenovirus DNA also became interesting. Adenoviruses became interesting as factors for gene therapy. So this occurred in the '80s when people started to think about gene therapy, introducing genes into cells, first retroviral vectors, and also later adenoviral vectors.

And adenoviral vectors, in contrast to

retroviral vectors, in fact, have as have been already said today a deletion in the E1 gene and in the place of the E1 gene, you can clone the gene of interest, and the reason that E1 deleted adenovirus was chosen was, in fact, the present or the availability of the 293 cells, which turned out to be very suitable packaging cell line for these first generation viral vectors because they expressed the E1 genes.

So E1 deleted recombinant adenovirus vectors were being used more and more for gene transfer purposes. Adenovirus vectors, you've heard it, are quite suitable for similar reasons. They have certain disadvantages also compared to retroviral vectors, for example, but they certainly have a number of important advantages.

The cells to grow the replication deficient E1 deleted adenovirus vectors were also available, and those were the 293 cells, and in fact, already in 1994 the first clinical study with an E1 deleted adenovirus vector was done, was made by Crystal in '94, which probably was the, if I remember correctly, the CFTR gene. That was the first clinical trial gene in an adenovirus vector.

So there was a new use now for the 293 cells. Two, nine, three for quite some period of time

was the only packaging cell line available for growth of adenovirus vectors.

E1 deleted vectors for gene transfer became common practice. More and more groups started to use adenoviruses as a factor, but there were also limitations to the available packaging cell line, 293. It became apparent at that time, and that is that due to recombination between E1 sequences from the 293 cells into the E1 deleted vector could occur, and this gave rise to formation of replication competent adenovirus, RCA, and it turned out to be very difficult to produce large batches of RCA free vector, and of course, the RCA is almost identical to the vector with the gene of interest, and therefore cannot be physically separated from the vector.

So it was clear that 293 was not really the ideal vector for gene therapy used, and therefore, we decided around 19 -- oh, this first, just to show you here, here is the recombinant vector in which the E1 gene is deleted, and instead of it, the gene of interest can be inserted here.

And here are the 293 cells with a proportion of the adenovirus genome integrated with the E1 gene and P9, protein 9, and there turns out to be quite a considerable overlap at both sides between

the recombinant adenovirus vector, first generation vectors of both sides of the E1 region.

And be recombination you can get back RCA, which is essentially, again, the wild type adenovirus.

So the combination of the E1 gene in the vector yields wild type virus, which is capable of replicating. This could cause toxicity, high concentrations perhaps of virus in places where you don't want it. It could give rise to uncontrolled dissemination not only of the wild type virus, but also of the recombinant factor that replicates together with it in the same cell.

It could theoretically also yield new virus strains in the case of capsid modified vector where the capsid is modified in such a way, for example, that it can attach to other receptors and other cells. So in that case when that becomes replication competent you can say that this is a partially new virus that you have created.

Also, it could yield, give rise to replication deficient E1 containing viruses in the case of multiply deleted vectors, for example, vectors that in addition to deletion of E1 also deleted in E2, E2A, for example, when E1 is reinserted into the vector, it will still be replication deficient because

the E2A gene is absent, but this vector, in theory, is immortalizing or transformation competent virus that you have created.

So in 1995, Brahm Bout (phonetic), Frits Fallaux, Brahm Bout from IntroGene and Frits Fallaux from our university Gene Therapy Group, decided that we should try and make a new helper cell line and matching factor in such a way that there is no sequence overlap between the factor and the advanced sequences in the cell line.

And indeed, in order to make a new system that allowed pharmaceutical production of adenovirus vectors, three of RCA. It should also meet pharmaceutical standards. If you start all over again you can just as well try to do that, and it could be the basis for the manufacture of multiply deleted Adenovirus 5 vectors also.

So we choose the human embryonic retina cells at that time. Why not kidney cells? Simply because these cells were so resistant to adenovirus transformation that we didn't think it would be worthwhile to try it all.

Human embryonic retina was chosen because Gallimore had shown not long before that that human embryonic retina was permissive to transformation,

could be transformed by adenoviruses, Adenovirus 5 and 12, and that was, again, based on some other studies in animals, and therefore, we decided to take human embryonic retina cells.

So they can be transformed by Adenovirus 5, and also at least in some of the cases that we have studied, there is no real crisis. So the cells become transformed and then go on to become immortal without a real crisis in which the whole culture stops the fighting.

Transformation is still a rather low efficiency, but anyway, there is transformation, and it is reproducible.

So I isolated retina from a fetus, from a healthy fetus as far as could be seen, of 18 weeks old. There was nothing special with a family history or the pregnancy was completely normal up to the 18 weeks, and it turned out to be a socially indicated abortus, abortus provocatus, and that was simply because the woman wanted to get rid of the fetus.

We got this. There was permission, et cetera, and that was, however, was in 1985, ten years before this.

This shows that the cells were isolated in October '85, Leiden University in my lab. They were

then isolated in separate cell culture area, which contained a laminar air flow cabinet, and that was we did it in the cell culture area of the three different cell culture rooms that we had available at that time. That was only devoted to diploid cell cultures, human cell cultures.

The cell culture media were, of course, from certified supplies. At that time already '85, I should say, the cells were frozen, stored in liquid nitrogen, and in 1995 one of these files was thawed for the generation of the PER.C6 cells.

We used defined E1, identifiers E1 DNA construct, in order to eliminate sequence homology between the cells and the vectors, and that would allow RCA contamination free E1 deleted vector production.

The E1 genes in the PER.C6 cells were regulated not by the E1A promoter, but by the PGK promoter, and the whole thing was all sequenced and functionally characterized. I can show you if you are interested the data on expression of the viral genes.

Transfection was carried out at Leiden University. There was no carrier DNA used. In '95 was this. The transfection yielded a number of different colonies after about 18 days, and one of

several of those were isolated. One of them, Clone 6, finally was established and gave rise to PER.C6, and Clone 6 was chosen because it gave the highest yield of viruses and also had rather high expression of E1A and E1B gene products.

These cells did not go through crisis, but it is possible that in some case a crisis appears, as I remember from experiments of Phil Gallimore, that sometimes a short crisis may be observed.

So after the transformation event at the university in Leiden and after the colonies were picked, everything was transferred to IntroGene, which was close by also in Leiden, in fact, in the same building, and the whole documentation control was done by them.

In the dedicated cell culture area defined materials were used, of course. Cell banks were laid down at IntroGene, passage 29, 33, and 36.

This shows you the information. The recombinant identifiers itself it shown here. The P9 gene is still present at the right hand and, in fact, of E1, and here is the area where E1 is deleted and the gene of interest is inserted.

Then the PER.C6 cell was transformed by an E1 gene construct with a PGK promoter and a polyacid

of Hepatitis B virus, I believe.

And here is no sequence homology and real RCA has never been observed in many, many different experiments.

And this is the final slide just showing you some comparisons between 293 and PER.C6. Again, I remind you that both cell lines were made in my lab for different reasons.

The objective, as I indicated, is for 293 -- was basic research, and we have done many different transformation studies after that, not transformation studies, but gene expression studies with human embryonic kidney cells in the years following that up to now, I would say.

PER.C6 was made just for pharmaceutical manufacturing of adenovirus vectors. As to RCA free, PER.C6 is RCA free. Two, nine, three is not.

The history documentation of the cell line has been carried out completely for PER.C6 and was not done at that time for 293. We had no donor information on 293 or what was available got lost, and this is available for PER.C6. Containment at that time was a little primitive perhaps and was now done in a laminar flow cabinet.

The serum sources were of noncommercial

use. Probably I have made it myself. Certified samples -- supplies were now used for serum and medium, et cetera.

Crisis free history just means that 293 had a crisis, was not crisis free at the long crisis, and these cells, PER.C6 had no crisis for some reason.

And then pharmaceutical industry standard. I realize that this sounds a bit commercial, but PER.C6 were made for that particular purpose. Also, as far as I know, more than 50 different companies have taken license for PER.C6.

Two, nine, three was not in the same way characterized, is in the public domain, whereas PER.C6 is licensed.

So I think I'm at the end if somebody wants to see the data again of virus production and so on, but I don't think that's very important.

ACTING CHAIRMAN DAUM: Thank you very much, Dr. van der Eb.

We'll take a couple of questions. Dr. Decker, then Ms. Fisher. Dr. Kohl.

DR. DECKER: Did you say that adenovirus is not capable human diploid cell transformer because of cytolysis? The human cell --

DR. VAN DER EB: Yeah, yeah. Well, yes.

What I said is that you -- if you take intact DNA or a virus and you put it on human diploid cells, the types that we have used is the embryonic kidney, the skin, embryonic lung. Then you see lytic reaction, and that will just wipe out a whole culture area.

DR. DECKER: Does that imply that an attenuated and human diploid cell adapted might then adapting it that way so you didn't get the cytolysis might unmask a transformation capability?

DR. VAN DER EB: Theoretically that is a possibility. I don't believe it because the three different types of diploid human cells that we have tested were so resistent to transformation just by DNA, also by fragments of the DNA, resection fragments of the DNA. We did it later also, but I don't believe that that is a big issue.

What I think is that there might be other tissues, cells and tissues in the human body that can be transformed, for example, retina cells. It could be that neural cells are also transformed.

It is known that Adenovirus Type 12 is more clearly -- much more efficient in transforming neural cells of neural origin than Adenovirus 5, but this is five that we are talking about here.

ACTING CHAIRMAN DAUM: Thank you.

Ms. Fisher, then Dr. Kohl and Dr. Minor.

MS. FISHER: From your chart you said that there was no cell crisis with the --

DR. VAN DER EB: No.

MS. FISHER: -- use of PER.C6, but before that you said that there was a short crisis observed.

DR. VAN DER EB: There have been, I believe, a short type of crisis observed by Gallimore in some cases, but he just described that the cells slowed down a little bit, and during one or two weeks did not seem to grow and then took off again.

So you can perhaps not say that that is a crisis. I don't know. That could be a crisis, but the whole culture just took off again and continued.

ACTING CHAIRMAN DAUM: Dr. Kohl, then Dr. Minor, please.

DR. KOHL: Regarding the possibility of prion transmittable diseases, can you tell us more specifically about the fetal calf history of PER.C6, especially back in '85?

You said it was from certified sources, but I'd like --

DR. VAN DER EB: Yeah.

DR. KOHL: -- to know more about that, number one.

DR. VAN DER EB: Yeah.

DR. KOHL: And, number two, can you tell us about the neurological history of the mother and the father of the fetus?

DR. VAN DER EB: I can, yes. As to the source of the serum, we were able to trace back that -- let me see where I have it -- that the serum was obtained in August of 1985 from -- it was Flow, Flow serum, and it was not exactly stated where the serum came from in this particular case, but the Flow serum samples that we got in the years before and afterwards were all from North American sources at that time.

Also we had sometimes GIBCO, also North American sources. It was certainly not European source.

Yes, we got these cells. These serum samples were selected by the University of Rotterdam for growth of diploid cells very carefully, and they usually get something like seven different samples either from Flow or GIBCO or both, and they test which one is the best for cloning of human diploid cells.

And if they select one, the batch is large enough so that they can have enough for half a year, and we had the other half.

ACTING CHAIRMAN DAUM: Thank you.

Dr. Minor, please.

DR. VAN DER EB: Oh, prion. No, you had another question?

DR. KOHL: The neurological histories of the mother and the father.

DR. VAN DER EB: Both the mother and the father. The mother was completely normal. That I know and had -- there was nothing wrong with the mother. She had at least two children afterwards in the same hospital in Leiden, which were completely healthy.

The father was not known, not to the hospital anymore, what was written down, and unknown father, and that was, in fact, the reason why the abortion was requested.

ACTING CHAIRMAN DAUM: Dr. Minor.

DR. MINOR: You may have said this and I missed it. Is there anything know about the copy number of E1 in the PER.C6 and the site of integration of the DNA? In other words, is E1 really all there is to it?

DR. VAN DER EB: Yeah.

DR. MINOR: Or is it where it's actually put in the --

DR. VAN DER EB: No, E1 is the only thing

that's present that are in PER.C6. I can't exactly remember. Maybe somebody in the audience can correct me. I think about six or seven copies in it, which are all located close to each other. So they may be in that kind of tandem repeat that you often see after transfection with calcium phosphate, and it's only -- that is, I think, only one side on one chromosome is integrated, nothing else.

ACTING CHAIRMAN DAUM: Thank you.

I think at this point we're going to thank Dr. van der Eb very much for another informative presentation, and ask Dr. Cook to tell us about adenovirus transformed cell tumorigenicity and transformed cell host interactions that determine their tumor forming capacity.

DR. COOK: So what I'd like to do is focus on tumor development per se to start with in experimental tumorigenicity models and how that might relate to the question at hand and then talk a little bit about the ability of the E1A gene of this E1 region of Adeno. 5 to sensitize cells in which it's expressed to immunological injury. That's been our area of interest and I think it to some extent explains the lack of tumorigenicity of these cells in immunocompetent animals.