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VACCINES AND RELATED BIOLOGICAL

PRODUCTS ADVISORY COMMITTEE

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MEETING

+ + + + +

WEDNESDAY,

MAY 16, 2001

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OPEN

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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

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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.D., 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

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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

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1 discussion May 16th.

2 Drs. Griffin, Aguilar-Cordova, and Ketner have
3 each been granted a waiver in accordance with 18 USC
4 208(b)(3), which permits them to participate fully in
5 the discussions.

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

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

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

25 In addition, the agency has determined

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

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

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

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

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

25 And I do have one other announcement. The

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

6 ACTING CHAIRMAN DAUM: Thank you very
7 much, Nancy.

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

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

16 PARTICIPANT: Are you hearing it now?

17 ACTING CHAIRMAN DAUM: When I speak I am.

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

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

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

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

22 I'm interested in the pathogenesis of
23 viral infections.

24 ACTING CHAIRMAN DAUM: Perfect.

25 DR. STEPHENS: I'm David Stephens from

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

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

6 (Laughter.)

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

11 Dr. Goldberg.

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

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

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

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

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

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

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

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

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

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

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1 Advisory Committee.

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

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

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

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

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

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

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

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

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

23 DR. WOLFE: I'm Sid Wolfe. I'm a general
24 internist by clinical training, and since leaving NIH
25 30 years ago, I've spent most of my time at the Public

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

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

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

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

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

23 (Laughter.)

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

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

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

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

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

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

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

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

7 ACTING CHAIRMAN DAUM: Thank you very
8 kindly.

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

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

13 DR. GOLDBERG: Judy Goldberg from New York
14 University.

15 DR. KATZ: Sam Katz from Duke University.

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

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

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

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

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

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

3 DR. COOK: Jim Cook, University of
4 Illinois.

5 DR. BLAIR: Don Blair, NCI.

6 DR. MOULTON: Larry Moulton, Johns Hopkins
7 University.

8 DR. KETNER: Gary Ketner, Johns Hopkins.

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

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

13 DR. HUGHES: Steve Hughes, NCI.

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

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

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

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

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

10 Okay. Thank you.

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

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

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

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

5 Tumorogenicity is the ability of
6 neoplastic cells growing in tissue culture to multiply
7 and develop into tumors when injected into animals,
8 and oncogenicity is the ability of a virus or viral or
9 cellular genes to convert the cells of an injected
10 animal into tumor cells.

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

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

20 Second is the development of virtual
21 vectored HIV vaccines.

22 Finally, progress in understanding
23 carcinogenesis and detecting adventitious agents, and
24 the successful experience with highly purified
25 biologicals that are actually derived from tumor

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1 cells.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

14 Examples of these types of cells in
15 Categories 3 through 5 include HeLa cells and the HUT-
16 78 cells, which is used to propagate HIV virus.

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

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

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1 2000 meeting, which are present on the CBER Web site.

2 Of these five categories, only Categories
3 1 and 2 as examples of designer cell substrates are
4 going to be discussed today.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

4 However, when you compare these to A-549
5 cells, which is the cells derived from human tumors,
6 it only takes about 1,000 cells to produce tumors for
7 50 percent of the mice.

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

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

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

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

25 Due to these observations, the possibility

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

19 (Laughter.)

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

25 Is there Committee input? Dr. Goldberg,

1 and then Dr. Griffin.

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

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

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

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

17 DR. LEWIS: I guess I'm having a little
18 bit of a hard time hearing what you were saying.
19 You're trying to understand how we calculate the TPD-
20 50?

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

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1 a distinction between --

2 DR. LEWIS: Okay.

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

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

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

17 The data on the 549 cells and many of the
18 293 cells were repeated at least twice, and the
19 numbers are basically the same.

20 ACTING CHAIRMAN DAUM: Dr. Griffin.

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

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

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1 other is Ad. 12 transformed?

2 DR. LEWIS: No.

3 DR. GRIFFIN: And they have different --

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

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

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

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

22 DR. GRIFFIN: Thank you.

23 ACTING CHAIRMAN DAUM: Okay. Thank you.

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

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

3 Dr. Hughes.

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

6 Thank you.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

1 consider.

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

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

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

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

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

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

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

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

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

25 Cell lines do change upon prolonged

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

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

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

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1 its properties.

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

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

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

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

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

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

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

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

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

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

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

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

17 Dr. Kim.

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

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

24 ACTING CHAIRMAN DAUM: Would you like to
25 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

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1 high rate because one layer of safety has been
2 removed.

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

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

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

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

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

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

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

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

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

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

20 ACTING CHAIRMAN DAUM: Thank you.

21 Dr. Kohl and then Dr. Myers.

22 DR. KOHL: That was my question.

23 ACTING CHAIRMAN DAUM: Dr. Myers, please.

24 DR. MYERS: I guess I have two questions.

25 On the confidence of the stability of the genome,

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

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

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

11 DR. MYERS: Knock it out?

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

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

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

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

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

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

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

25 Dr. Lewis, then Dr. Aguilar-Cordova.

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

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

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

15 ACTING CHAIRMAN DAUM: Thank you, Dr.
16 Lewis.

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

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

25 And I guess that begs the question to

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

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

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

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

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

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

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

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

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

20 DR. AGUILAR-CORDOVA: Can you hear me?

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

25 So adenoviruses were identified in the

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

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

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

14 Hopefully nobody else has slides.

15 (Laughter.)

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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1 replication of the virus.

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

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

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

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

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

25 And there are two key factors of

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

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

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

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

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

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1 genes in the most common of these vectors.

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

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

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

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

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

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

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

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

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

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1 and then it also uses some integrants.

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

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

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

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

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

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

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

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

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

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

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

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1 generation than the second generation.

2 And here is again using liver enzymes,
3 which is another known toxicity of this adenoviral
4 vectors, and you can see that one times ten to the
5 11th, one times ten to the 12th, and then only a three
6 times ten to the 12th is there a difference, and
7 again, at one times ten to the 13th there's no
8 difference.

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

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

18 This is the level of expression through
19 time. These are weeks, and you can see with the first
20 generation it's a peak of expression within the first
21 few days, leading to baseline, no expression later.
22 These are just different doses of the vector ranging
23 from 3.2 times ten to the 11th to 1.2 times ten to the
24 10th.

25 And you can see that with the gutless

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

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

11 But it is not only the adenoviral genes
12 that can induce the immune response. In fact, it is
13 also the trans-gene that can induce the response.
14 This is some data from Morall, et al., in PNAS in
15 1999, and what we see is in the little light blue
16 color is a gutless vector, and it's human alpha-1
17 anti-trypsin again, and in the multi-color lines is a
18 first generation vector. This is in baboons.

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

25 But there was one animal with a gutless

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

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

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

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

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

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

15 So it turns out in that particular case it
16 was the CO₂ that was seeping in, dropping the pH, and
17 there was a seven log differential that could be lost
18 in a very short period of time.

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

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

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

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

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

13 That's all I have.

14 ACTING CHAIRMAN DAUM: Thank you very
15 much.

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

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

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

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

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

18 Can you comment on that?

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

25 The one difference would be that they do

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

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

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

12 ACTING CHAIRMAN DAUM: Dr. Kohl, please.

13 DR. KOHL: Two questions.

14 Thanks for your talk. It was enjoyable
15 and elucidating.

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

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

25 However, there is the potential for

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

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

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

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

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

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1 DR. KOHL: Who does the mechanism?

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

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

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

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

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

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

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

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

11 So it could go either way.

12 ACTING CHAIRMAN DAUM: Thank you.

13 Dr. Katz, please.

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

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

22 ACTING CHAIRMAN DAUM: Dr. van der Eb.

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

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

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

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

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

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

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

24 DR. AGUILAR-CORDOVA: Right.

25 ACTING CHAIRMAN DAUM: Two last questions,

1 please.

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

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

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

24 ACTING CHAIRMAN DAUM: Thank you, Dr.
25 Cook.

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1 Dr. Blair.

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

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

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

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

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

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

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

25 ACTING CHAIRMAN DAUM: We're ready to

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1 continue with the open session.

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

6 Welcome, Dr. van der Eb.

7 DR. VAN DER EB: Thank you.

8 So what I would like to do is to describe
9 to you how and why we have made two different cell
10 lines, adenovirus transformed human embryo cell lines
11 which are called 293 and PER.C6. Both cell lines were
12 made in my lab, and also the cells, the starting
13 material, was prepared by myself at the University of
14 Leiden. The 293 cell was made by Frank Graham in 1973
15 from human embryonic kidney cells that were made from
16 fetal tissue one year ago by myself one year before
17 that, so that was in 19 -- probably in 1972, whereas
18 the PER.C6 cell was made by Ron Bout and Frits Fallaux
19 in 1995 from an embryonic retina cultures that were
20 made from fetal tissue by me ten years before that, in
21 1985.

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

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

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

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

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

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

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1 but human cells could not be transformed.

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

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

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

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

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

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

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

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

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

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

9 One year later we had resection enzyme
10 available to make pure DNA, but the first and also the
11 293 cell was made with sheared Adenovirus 5 DNA, then
12 simply scored for transformed colonies as we did with
13 the rodent cultures.

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

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

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

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

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

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

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

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

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1 virus was not yet used at that time.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

25 And then the culture started to recover

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

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

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

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

25 And adenoviral vectors, in contrast to

1 retroviral vectors, in fact, have as have been already
2 said today a deletion in the E1 gene and in the place
3 of the E1 gene, you can clone the gene of interest,
4 and the reason that E1 deleted adenovirus was chosen
5 was, in fact, the present or the availability of the
6 293 cells, which turned out to be very suitable
7 packaging cell line for these first generation viral
8 vectors because they expressed the E1 genes.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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1 could be transformed by adenoviruses, Adenovirus 5 and
2 12, and that was, again, based on some other studies
3 in animals, and therefore, we decided to take human
4 embryonic retina cells.

5 So they can be transformed by Adenovirus
6 5, and also at least in some of the cases that we have
7 studied, there is no real crisis. So the cells become
8 transformed and then go on to become immortal without
9 a real crisis in which the whole culture stops the
10 fighting.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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1 of Hepatitis B virus, I believe.

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

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

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

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

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

25 The serum sources were of noncommercial

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

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

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

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

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

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

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

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

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

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

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

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

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

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

25 ACTING CHAIRMAN DAUM: Thank you.

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

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

4 DR. VAN DER EB: No.

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

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

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

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

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

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

23 DR. VAN DER EB: Yeah.

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

1 DR. VAN DER EB: Yeah.

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

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

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

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

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

25 ACTING CHAIRMAN DAUM: Thank you.

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1 Dr. Minor, please.

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

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

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

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

16 ACTING CHAIRMAN DAUM: Dr. Minor.

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

22 DR. VAN DER EB: Yeah.

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

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

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

9 ACTING CHAIRMAN DAUM: Thank you.

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

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

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