

FOOD AND DRUG ADMINISTRATION
Center for Biologics Evaluation and Research

Meeting of

The Vaccines and Related Biological Products Advisory Committee (VRBPAC)

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P R O C E E D I N G S (8:00 a.m.)

Agenda Item: Call to Order and Opening Remarks, Introduction of Committee

DR. DAUM: Good morning. I would like to welcome everybody to our VRBPAC meeting. We would like to begin by turning the floor over to Don Jehn, who is the Designated Federal Officer for this committee, who will make some remarks and read the Conflict of Interest Statement.

Agenda Item: Conflict of Interest Statement

MR. JENH: Good morning. Thank you, Dr. Daum. Yes, as Dr. Daum said I am the Designated Federal Officer for today's 130th meeting of the Vaccines and Related Biologics Products Advisory Committee. **Today's session is entirely open to the public.** This session is described in the Federal Register notice of July 18, 2012. I would like to request that everybody keep their phones on mute or in the silent mode. Now I am going to read the conflict of interest statement.

The Food and Drug Administration, FDA, is convening the September 19th, 2012 meeting of the Vaccines and Related Biologics Products Advisory Committee under the authority of the Federal Advisory Committee Act, FACA, of 1972. With the exception of the industry rep, all participants of the committee are Special Government Employees, SGEs, or regular federal employees from other agencies, and are subject to federal conflict of interest laws and regulations. The following information on the status of the Advisory Committee's compliance with federal ethics and conflict of interest laws including but not limited to 18 US Code 208 and 712 of the Federal Food, Drug and Cosmetic Act, are being provided to participants at this meeting and to the public.

FDA has determined that all members of this advisory committee are in compliance with the federal ethics and conflict of interest laws. Under 18 US Code 208, Congress has authorized FDA to grant waivers to Special Government Employees and regular government employees who have financial conflicts, when it is determined that the agency's need for a particular individual service outweighs his or her potential financial conflict of interest.

Under 712 of the Food, Drug and Cosmetic Act, Congress has authorized FDA to grant waivers to special government employees and regular government employees with potential financial conflicts when necessary to afford the committee their essential expertise.

Related to the discussion of this meeting, members and consultants of this committee have been screened for potential financial conflict of interest of their own, as well as those imputed to them, including those of their spouse or minor children, and for the purposes of 18 US Code 208, their employers. These interests may include investments, consulting, expert witness testimony, contracts and grants, credos, teaching, speaking, writing patents and royalties, and also primary employment.

At today's meeting the committee will discuss the consideration of the appropriateness of cell lines derived from human tumors for vaccine manufacture. This is a particular matter involving specific parties based on the agenda, and all financial interests reported by members and consultants, no waivers were issued under 18 US Code 208(b)(3) and 712 of the Food, Drug and Cosmetic Act.

Dr. Theodore Tsai is serving as the industry representative, acting on behalf of all related industry. He is employed by Novartis Vaccines. Industry representatives are not special government employees and do not vote. Dr. Carol Tacket has recused herself from today's meeting.

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

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

FDA encourages all of their participants to advise the committee of any financial relationships that you may have with any affected firms, their products, and if known, their direct competitors. Thank you. I'll pass it back to you, Dr. Daum.

DR. DAUM: Thank you Mr. Jehn. Some of you may know that this is the second time I have had the privilege of chairing this committee, and the first time, the meeting took place in the hotel where we stayed. We of course complained bitterly that we didn't like the hotel and didn't like the conference room. But it was sure nice to get up in the morning and throw on your clothes and trudge downstairs to the meeting.

This morning's experience is a little bit different than that, as everybody here knows. And the bus ride from the hotel to here was as pleasant as it could be. But nevertheless, a bus ride. So I would like to thank the committee members for their endurance and willingness to serve. And I much appreciate it, as I am sure the agency.

I would like to begin in our usual style by asking each member to identify themselves, and maybe in one or two sentences at the most state their expertise and the reason that they might be called upon special to serve today, if that is the case. Dr. Tsai, we will start with you if you don't mind.

DR. TSAI: I am Ted Tsai, and I am the industry representative. I work for Novartis Vaccines. I have no special expertise in this area.

DR. LOWY: My name is Doug Lowy. I am Deputy Director of the National Cancer Institute and I have a background in vaccine development as well as study of growth regulatory genes involved in cancer.

DR. COFFIN: I am John Coffin, and I am on the faculty at Tufts Medical School, and I have a part-time job with the National Cancer Institute. And I am basically a fundamental retrovirologist. I work on HIV, I work on evolution of viruses. And I have had a longstanding interest in retroviruses and pathogenesis of disease.

DR. COOK: I am Jim Cook. I am Director of Infectious Disease at Loyola Medical Center in Chicago, and co-director of the Infectious Disease and Immunology Institute there. My expertise as related to this committee has to do with interest in viruses, how they modify mammalian cells, how DNA virus transforms cells-induced tumors in experimental animal models.

DR. SCHOOLNIK: I am Gary Schoolnik from Stanford University. I work in microbial pathogenesis with bacteria, not viruses. I am specialist in internal medicine and infectious disease. I don't have any particular expertise in this topic.

DR. MARCUSE: Ed Marcuse. I am a Pediatric Clinician and Epidemiologist who has had a career-long interest in vaccines, but have no expertise in this topic.

DR. WHARTON: I am Melinda Wharton from the Centers for Disease Control and Prevention. I have been in our US immunization program for many years, involved in vaccine preventable disease prevention and the vaccines that we use to prevent them. But I don't have specific expertise in this topic.

DR. MCINNES: Good morning. I am Pamela McInnes from the National Institutes of Health. My expertise is in vaccine development.

DR. BRADY: Good morning. I am Nathanael Brady. I am in private practice in allergy and immunology in Colorado. I have no particular expertise in this area.

DR. DAUM: Excuse me, before the string of apologists continue, I would like to point out that you guys I think will be surprised at the expertise that you have. And so that you are committee members and chosen for this committee very carefully. And I think that you will find that you have more expertise than you think, and it remains to be determined.

DR. CHEUNG: My name is Ambrose Cheung. I am in the Department of Microbiology at the Geisel School of Medicine at Dartmouth. My background is in infectious diseases. My interest is in Staph aureus pathogenesis, and I am waiting to be convinced about the expertise I am going to have on this topic.

DR. GELLIN: I am Bruce Gellin, the director of the National Vaccine Program Office at HHS. I trained in internal medicine and infectious diseases and spent a lot of time in CDC doing epidemiology.

DR. HUDGENS: I am Michael Hudgens from the University of North Carolina. I am an associate Professor of Biostatistics and Director of the Biostatistics Score for the UNC Center for AIDS Research.

DR. AIR: I am Gillian Air, of the University of Oklahoma Health Sciences Center. I work on influenza virus antibodies and receptor recognition.

DR. GRAY: My name is Greg Gray. I am Professor and Chair of Environmental Global Health at the University of Florida School of Public Health, and I am an infectious disease epidemiologist with a number of studies in influenza and other respiratory viruses.

DR. PIEDRA: I am Pedro Piedra at Baylor College of Medicine. I am in Pediatric Infectious Disease. My area of research is in respiratory viral vaccine.

DR. DAUM: FDA folks?

DR. KRAUSE: I am Phil Krause. I am the Acting Deputy Director of the Office of Vaccines. I have been at the FDA for a little over 20 years, and have over that time worked on viral vaccines, including issues associated with virus detection and qualification of cell substrates, and have been to a lot of meetings, including all of the VRBPAC meetings that I will summarize shortly.

DR. GRUBER: My name is Marion Gruber. I am the Director of the Office of Vaccines.

DR. PEDEN: I am Keith Peden, Chief of the Laboratory of DNA Viruses. My expertise is in virology, molecular genetics and oncogenicity, and as you will hear from me later on, on cell substrates.

DR. DAUM: Thank you. And I am Robert Daum. I am a pediatric infectious disease guy at the University of Chicago now for 24 years, and I work on Staphylococcal pathogenesis and mechanisms of antimicrobial resistance. So with that done, I would like to turn to our agenda this morning.

Agenda Item: Topic: Consideration of the Appropriateness of Cell Lines Derived from Human Tumors for Vaccine Manufacture

DR. DAUM: We have a very complex issue to wrestle with today. And we are first going to hear from Dr. Krause of the FDA who is going to give us an introduction and overview of cell substrates. Dr. Krause is the Acting Deputy Director of OVR and CBER. Good morning, Dr. Krause, and thank you.

Agenda Item: Introduction and Overview of Cell Substrates

DR. KRAUSE: Good morning. In this presentation I am going to go through some introductory material as well as give you an overview of vaccine cell substrates, including the history of how it is that we at CBER, and in fact the VRBPAC itself has considered these issues over the years.

And of course this presentation will be focused on the information that you will need to help support your subsequent discussion on the use of human tumor-derived cells as substrates for vaccines. So first I would just like to go over the agenda, to give you a preview of what you are going to see today.

After I give my presentation you are going to hear from three manufacturers, who are going to discuss three human tumor cell derived cell lines that are proposed for use in different kinds of vaccines. And those manufacturers are PaxVax, Sumagen, and NIAID, which has taken over a vaccine that was previously manufactured and supported by Targeted Genetics.

After the break then you are going to hear from Dr. Keith Peden at FDA, who is going to talk about tumorigenicity as well as DNA as a potential risk factor associated with these kinds of cells.

And then you are going to hear from Dr. Patrick Moore from the University of Pittsburgh, who will talk about use of molecular methods to discover previously unknown or undiscovered human cancer viruses. Dr. Moore, many of you may know, is the co-discoverer of two human tumor viruses using modern methods. And so he has a very unique perspective on that issue.

And then Dr. Arifa Khan will give us a presentation on the evaluation of vaccine cell substrates for adventitious agents that may be present in cells. And of course focus that discussion also on tumor derived cell substrates. Then there will be lunch and then we will have a robust discussion, at which we will discuss the questions which I will present at the end of my presentation.

Just so that we are all on the same page, I would like to go over a few definitions. An adventitious agent is defined as a microorganism that is inadvertently introduced into the

production of a biological product. A cell substrate simply defines the cells that are used to produce a biological product.

And in this case, the cell substrates that we are talking about are generally metazoan cell substrates, cells from multicellular organisms that up until now have generally been used to produce viral vaccines. So there are potential applications for other kinds of vaccines as well.

And the other point that I wanted to make about adventitious agents is that because of the nature of the cells, and also because of the nature of the kinds of assays that are available, the main adventitious agents that we are concerned about today are going to be viral adventitious agents.

A diploid cell strain, which many people also call a diploid cell line, are normal cells with the expected number of chromosomes that senesce after prolonged passage in cell culture. And so the term cell strain used to refer to the fact that the cells would senesce, although that language is a little bit outmoded. And now people call it a cell line because they don't perhaps understand that distinction. But we can call it either today.

A continuous cell line, then, are cells that have been propagated in culture since establishment of a primary culture, and then survival through crisis and senescence. So they have some extra ability over a completely normal cell that allows them to be immortal.

A tumorigenic cell line, then, is defined as cells that form tumors when inoculated into immunocompromised animals. And those immunocompromised animals could be animals that have been deliberately immunosuppressed, or it could also be animals that are neonatal and thus have incompletely developed immune systems. And then a tumor derived cell line, which are really the cells we are talking about today, then, are cells that are linearly descended from tumor cells.

A few general comments about vaccines, which of course all of you know. Vaccines are among the very most effective ways to control infectious diseases. And the effectiveness of vaccines is often enhanced by herd immunity.

The safety record of vaccines is excellent, but nonetheless the maintenance of public confidence in vaccines is critical to public health. And an important part of that maintenance of public confidence is open public discussions like the one we are having today, and like the ones we have had over the years in talking about cell substrates. And of course it is not just the discussions, but it is also the underlying science, then, to evaluate these cell substrates, that do play a very important roll, then, in consideration of vaccine safety.

A few more things to say about vaccine cell substrates. We don't approve cell substrates, although we talk about cell substrates and we talk about the risk and benefits cell substrates may bring to a product. But cell substrates are considered in the context of the entire manufacturing process as well as the benefit and risk of the product.

Cell substrates can be difficult to characterize, thus, cell substrates have historically given rise to important regulatory considerations. And I will go through some of that history shortly. Our goal at CBER is to address the issues in a scientifically rational manner, quantitatively when possible. And we will over the morning describe to you how we have done that as well.

This slide has a list of metazoan cell substrates that are currently used for US licensed vaccines. And your handout includes smallpox vaccine, for which the license has been withdrawn so it is not actually a currently licensed vaccine, so this slide does not have it. But this includes animal tissue including embryonated eggs, which are used for live attenuated vaccines, influenza and yellow fever, as well as inactivated influenza vaccines, primary cell cultures, which includes chick embryo fibroblasts at this point, used for live attenuated measles and mumps vaccines as well as for inactivated rabies vaccine.

Diploid cells, both the MRC-5 strain which is used for varicella-zoster virus vaccine, which is a live attenuated vaccine. And the inactivated Hepatitis A and rabies virus vaccines, as well as WI-38, which is used for rubella and adenovirus vaccines. The rubella vaccine is live attenuated, and the adenovirus is a live vaccine.

And then continuous cell lines have been used as well, including vero cells, which have been used now for licensed live attenuated rotavirus, smallpox vaccines, as well as for inactivated vaccines including poliovirus and Japanese encephalitis virus. And there is also an insect cell line, which is used to make one of the Human Papillomavirus vaccines, which is really a virus-like particle. And that is called Hi-5.

So why would we consider introducing even more cell substrates to this mix?

Well, there are number of advantages to contemplating the use of additional cell substrates. One of them is that there can be a virus growth advantage. The yield of virus may be greater. There may in fact be such a difference that a vaccine could be made in a new cell substrate but not in the previously used ones. With new cell substrates there can be more rapid scale-up. There is the ability to bank and thoroughly characterize cells, which provides some significant advantages as well. And there is the potential for adaptation to serum-free growth as well as growth in suspension.

And these kinds of cells, or new cells, can then be considered the enabling technology for a number of different kinds of products, including many genetically-engineered virus vectored vaccines, which require components of other cells that are not present in the virus vector in order to allow the production of the vector. And those components then may change the phenotype of those cells.

Some HIV vaccines, as you will hear a little bit later, as well as for pandemic influenza vaccines, for which the ability to use new kinds of cells to produce vaccines can be an advantage. And at one of our previous meetings the consideration of avian influenza as a pandemic virus was a very significant one, because if you worry about avian influenza it could also influence the egg supply. And so having alternatives to eggs for producing pandemic flu vaccines is potentially important.

So on this slide we have a list of three examples of human tumor derived cells that are proposed for use as vaccine cell substrates. And these are the three that we are going to hear presentations on as soon as I finish: The A549 lung adenocarcinoma cell line, which is proposed for use for adenovirus-vectored vaccines for antigens like influenza, HIV or anthrax.

CEM derived cells, which come from a lymphoblastic T cell leukemia, proposed for use in an inactivated HIV vaccine, and HeLa cells, which comes from cervical carcinoma, proposed for use for an AAV-vectored HIV vaccine. Now for the purpose of the discussion today, we are going to hear about these cell lines but we don't want to focus a discussion on these products in particular.

We really want to use these as examples of the kinds of cells that we are talking about and the kinds of characterization that can be done to try to think about this topic more broadly, to come to some conclusions about what really is needed in order to contemplate the use of these types of cells as vaccine cell substrates.

So the big question that we really have to consider, then, is: Is there a potential risk for making vaccines in tumor derived cells? The theoretical risk comes from the fact that, in contrast to many other products, vaccines are often difficult to purify to high levels. And that then leads to the question of whether residual cellular components from tumor-derived cells could pose a safety concern.

So how have we thought about this in the past? We have really identified three major factors that could potentially convey risk from tumor derived cells. And these include the cells themselves. And of course, if cells were present in vaccine, they could retain their original phenotype.

And if they were tumor-derived cells then maybe they themselves could form tumors in a vaccine recipient. Although they would still be susceptible to rejection by the host immune system, and so it is unlikely that that would be a problem. But nonetheless, that is a theoretical concern.

Cell DNA also is a theoretical concern, both because cell DNA could contain infectious genomes -- and we know that DNA can be picked up by cells and that then could lead to initiation of an infection -- as well as a theoretical oncogenic risk. And then there is the question of adventitious agents.

There are several ways in which tumor derived cells might lead to an increased adventitious agent risk, potentially an increased risk due to more passages in history of the cell, as the cell line was developed. Potential ability of a cell substrate to support the growth of additional viruses, but probably most relevant to the discussion in the potential for a virus to have been involved in tumor development in the first place, because that is one of the differentiating factors of these cells with other cells.

Although in many cases, for instance, papillomaviruses, the virus is incorporated in defective form. There are examples of tumor derived cell lines which still are able to produce infectious virus. And of course, we have to consider the possibility that there is some other factor that we haven't considered that may be associated with these cells as well.

So over the years as we have engaged in this dialog with the VRBPAC over the introduction of new cell lines into vaccine manufacture and vaccine investigations, what approaches have we used? For cells, we decided fairly early on that it was very easy to simply incorporate a manufacturing step that was certain to guarantee that cells were completely removed during manufacture.

And so the cells themselves aren't a real concern, because we are sure that they are all removed. So the major discussions have really focused on DNA and on adventitious agents. For DNA there have really been three kinds of things that have been introduced over the last decade to try to address risks or theoretical risks associated with DNA. One of them has been the introduction of some extended tumorigenicity testing, which Dr. Peden will describe.

There also has been the introduction of animal oncogenicity testing, specifically of DNA that has been taken from the cells, to see whether the DNA can be shown to be oncogenic in various animal models, as well as fragmentation and removal of DNA during manufacture of the product.

For adventitious agents there have been a couple of additional approaches that have been added over the last decade to think about, to develop an approach to see whether there might be additional adventitious agents in these cells.

And this has included oncogenicity testing, then, of lysates of the cells, in similar animal models to those used for the DNA, to see whether there is some component likely in an adventitious agent which could cause a tumor in those animal models, at least, as well as in vitro virus induction studies. And Dr. Khan will describe both of these assays in more detail. In addition, of course, for some vaccines, although the extent to which this can be done varies from vaccine to vaccine, there can be inactivation and purification of vaccines during manufacture. And of course the risk then also for adventitious agents is mitigated by cell banking and use of prequalified reagents.

And then just a point to make here is that the risk may also be influenced by the route of vaccine administration, for instance, oral versus parenteral. But for the discussion that we are going to have today, we will think about these vaccines mostly as parenteral vaccines, because that is a worst case. And so if we can come to some conclusion about what to do with parenteral vaccines, it will I think be obvious what to do with orally administered vaccines.

I am now going to give you a little bit more of a history of how cell substrates have been thought about over the last 50 or so years. But to do that I am first going to give you a

little bit more of an expanded view of the cell substrates which have been used, not just for licensed vaccines but also for investigational vaccines.

We already talked about animal tissue, eggs, the original smallpox vaccine also was made in animal tissue, as well as primary cells, human diploid cells, and what continuous cells are. Over the last decade, with the advisory committee we have talked about manufacture of investigational vaccines in cells that have the capability of developing tumorigenic phenotypes. And that would include vero cells, cells that are transformed by a known mechanism, and that includes cells which have been transformed, for instance, with adenovirus genes to allow the production of defective adenovirus vectors.

We have talked about the use of other tumorigenic cells, for instance, the use of the MDCK cells, which have been proposed for use in manufacturing influenza virus vaccines. And today of course we are talking about tumor-derived cells.

In the meantime, not really a topic of advisory committee discussions but of interest just because this also represents an expansion of the kinds of cell substrates that have been considered, CBER has also allowed investigational products to go forward in avian stem cells, plant-derived cells, as well as additional insect-derived cells.

So let's go back now 50 years, to the mid-1950s, and think about how cell substrates were viewed at that time. At that time, of course, people were very worried about polio and there was an important effort to develop poliovirus vaccines. And one of the major considerations had to be what kind of cells these vaccines would be made in.

In 1954 the Armed Forces Epidemiology Board had a very important meeting at which they recommended the use of normal cells for vaccine production. And back then, the notion of using human cells wasn't even on the table because there was a concern that human cells might contain human adventitious agents, and the notion of normal cells was literally due to a fear that there might be some oncogenic risk associated with cells which were not completely normal in some respect.

And so the main concerns back then, as they are today, were human adventitious agents and potential oncogenicity. And even now some currently used vaccines are made in primary cells. This includes influenza virus vaccines, measles, mumps and rabies vaccines.

In 1960, as primary cells were being used, the virus SV40 was discovered as an adventitious agent in poliovirus vaccines. And the vaccine was manufactured in primary rhesus monkey kidney cells, and it turned out that SV40 could grow in those cells without causing any cytopathic effect, and so it was not recognized, using the testing that was being used at the time.

And so millions received SV40 contaminated vaccines in the late 1950s and early 1960s. The cell supernatants of these cells actually did cause tumors in laboratory animals and cytopathic effect in primary African green monkey kidney cells. And that was observed also around that time. And so the solution was to develop a new cell substrate for these vaccines, which meant that these vaccines then were manufactured in African green monkey kidney cells in which, if there was cell contamination, cytopathic effect would be evident and it would be possible to identify that. And testing regimens were also expanded.

The other thing that happened, of course, was the vaccine seeds which were already proving to be very successful at preventing polio, were treated with anti-SV40 neutralizing antibodies. And as quickly as possible, then, SV40 free vaccines were developed.

Of interest, in the 1990s a number of investigators reported the presence of SV40 DNA in some human malignancies using PCR assays. And the conclusion of that story is really that over the years it became clear that these studies didn't rule out PCR contamination, and there has been no confirmed association of SV40 virus with malignancies. And so it doesn't seem as though that was a real finding.

Moreover, epidemiological studies suggest not adverse sequelae to vaccinated children. But it does show how an advantageous agent event can pop up and cause additional concern and the need for a lot of additional studies, even many, many years later. So the next landmark in my story is actually 19 years later, when the US licensed a rubella vaccine that included the use of human diploid cells.

This is an interesting story, so I will spend a little bit of extra time on this. Congenital rubella syndrome was first recognized in 1941. And rubella was first cultured in 1962. And this was incredibly fortuitous, because there was a worldwide epidemic from 1963 to 1965, and the ability to culture this virus at that time allowed people to define rubella as the responsible pathogen, as well as to really understand the nature of the disease.

Also, of course, the ability to culture this virus led to the ability to very quickly develop some vaccine candidates. And by 1969 there were four vaccine candidates. One of these candidates was the strain RA 27/3, developed by Stan Plotkin. And that was produced in WI-38 human diploid cells.

You will remember that the concern from the fifties had been that vaccines shouldn't be made in human cells because of a theoretical concern that there might be human adventitious agents, as well as the concern that perhaps human cells might be more likely to have some oncogenic risk.

And so this vaccine was the one of these four vaccines that was not licensed in the US in 1969. The other three were licensed. However, over the ensuing decade it became clear that the three vaccines that were used in the US were not nearly as effective as the RA 27/3 vaccine, and so finally then 10 years later the strain was approved in the US, in 1979.

But there again, we have these same issues, the question of adventitious agents and oncogenicity that were at least considered and in this case were considered a major reason why the current rubella vaccine had its relatively delayed entry into the US market. And of course in the meantime now, human diploid cells are considered one of the preferred vaccine cell substrates. And varicella-zoster virus, rabies and adenovirus vaccines are made in these cells.

In the mid-1980s as the biotechnology revolution got started it became necessary, or people started contemplating making vaccines in continuous cells. These are cells, then, that have survived, passed senescence, and are immortal. And so a couple of vaccine cell substrates were introduced in the 1980s. Vero cells were used for inactivated poliovirus vaccine. However, with very strict limits on the amount of DNA per vaccine dose.

This was based on calculations assuming single hit models of carcinogenesis. I actually think Dr. Lowy was at the meetings where some of these limits were established. And at that time, also in the mid to late eighties, Chinese hamster ovary cells, which are also immortal cells, were used for highly purified investigational subunit vaccines, none of which ultimately got licensed because they didn't work well in their efficacy studies.

In the mid 1990s, an endogenous avian retrovirus was identified in avian cells. And what happened there was a more sensitive PCR test was developed for the enzyme reverse transcriptase. And every retrovirus contains reverse transcriptase, and in fact at that time reverse transcriptase testing was done to evaluate vaccines. But not with an assay that was nearly as sensitive as this PCR based assay.

Because previously the reverse transcriptase was detected by an incorporation in radioactive nucleotides, which was a relatively insensitive method. But with the advent of PCR, the ability of reverse transcriptase to create DNA then could be detected using PCR. So the new assay was considerably more sensitive than the old. And using this new assay, very small amounts of reverse transcriptase could be detected in avian cells.

And so this test in 1996 showed that previously undetectable quantities of RT were present in some avian cell produced vaccines. However, additional studies showed that

EAV is a defective particle and does not induce productive infections in culture. The long safety record of hens' egg as well as chick embryo fibroblast produced vaccines, together with the absence of evidence of potential harm to humans were also important considerations. And both the World Health Organization as well as the VRBPAC determined the benefits of these vaccines outweighed any theoretical risk. But an epilogue to this story is that this new test, then, became incorporated as part of routine vaccine testing. And so we now use this as a more sensitive way to make sure that retroviruses are not present in vaccines.

In 1997 there was an important meeting that was sponsored by the International Association of Biological Standardization and the World Health Organization in which the DNA limit for biological for parenteral use -- and here not only vaccines were considered but also biological therapeutic products -- produced in continuous cells was increased to 10 nanograms per dose.

This was due to changing understanding of what the oncogenicity, what it took in order to cause tumors, the two-step model. But at the same time there was a caveat raised during that meeting, actually during the FDA participants, that the DNA shouldn't contain infectious virus genomes because there was concern that if there were entire virus genomes in the DNA, that amount of DNA might not completely rule out the potential for infectivity.

In 1998, OVRB began a series of consultations with the VRBPAC. This was based on the need to further consider expansion of cell substrates, the same kinds of considerations that bring us here today. And of interest, one of the cell lines that was brought up in 1998 was the '78 cell line. It was a human T cell lymphoma for an inactivated therapeutic HIV vaccine. And you will hear more about an analogous vaccine shortly.

CBER proposed to the advisory committee a Defined Risks Approach, which meant that where possible we would use quantitative approaches to consider the impact of potential risk factors associated with new cell substrates. And so based on that approach, then, we came back to the VRBPAC to talk about a series of additional cell substrates which were being proposed for various kinds of investigational vaccines.

The first of those subsequent meetings was in 2000, where we talked with you about vero cells for live attenuated vaccines. Vero cells, as you have already heard, are continuous African green monkey kidney derived cell line. They have the capacity to become tumorigenic, with additional passage in cell culture, although they do not cause tumors when they are inoculated into immunocompromised rodents at the passages where they had been and have been used for vaccine manufacture.

Use of vero cells for a variety of vaccines, including live attenuated rotavirus vaccines, was considered. And VRBPAC at that time strongly recommended making sure that there were no whole cells in the vaccines, as we have already discussed, and generally agreed with the use of well characterized vero cells, however, at non-tumorigenic passage levels with control on residual DNA, both its amount and size, as well as with thorough testing for adventitious agents.

In 2001 we had another discussion with the VRBPAC in which in vitro transformed human cell lines were discussed. And this included the PER.C6 cell line, which was proposed for use to produce adenovirus vectored HIV vaccines. And VRBPAC at that time generally supported the use of these cells for such products, with controls, again, on residual cell DNA and thorough adventitious agent testing.

At that time, though, the VRBPAC recommended the oncogenicity testing in animals for both cell DNA and cell lysates, which I described briefly a little bit earlier and which we will hear more about from Doctors Peden and Khan. In 2005 we came to the VRBPAC to discuss the use of tumorigenic MDCK cells for inactivated vaccines. So Madin Darby Canine Kidney cells were proposed for use in production of inactivated influenza vaccines.

MDCK cells have the potential to become tumorigenic, and many tumorigenic variants of MDCK cells have been reported. And in this case the cells that were proposed for use for these vaccines

were tumorigenic. Which meant that if you put the cells into immunocompromised animals they would cause tumors. However, these cells have substantial advantages for influenza virus growth.

The characterization that was presented by the manufacturers at that time included comprehensive adventitious agent evaluation, as well as residual DNA quantity and size reduction. And the VRBPAC generally supported the use of these cells for inactivated influenza vaccines. Concerns expressed by the VRBPAC, though, did include the difficulty of assessing the oncogenic activity of cell substrate components.

In 2008 we came back to the VRBPAC to discuss the use of non-tumorigenic MDCK cells for live influenza vaccines. So these were non-tumorigenic cells, although MDCK cells clearly had the capacity to become tumorigenic. But these were for live vaccines, which could not be purified to the same degree as an inactivated vaccine could.

The committee voted, however, that this vaccine could enter Phase I studies, although concerns were raised regarding the size of residual cellular DNA in the product. It was more a question about the assays used than the actual size, as well as the adequacy of the oncogenicity studies used to characterize the cells. And VRBPAC members at that point, and I think it was Dr. Coffin at the time, pointed out that newer technologies could, over time, begin to further improve cell substrate characterization.

In 2010 we had a discussion with the VRBPAC, and I think that includes many of the members who are here today, on porcine circovirus, which was found in rotavirus vaccines. And porcine circovirus DNA was identified in a rotavirus vaccine by the Delwart lab in California using massively parallel sequencing techniques and other molecular studies that were consistent with the presence of PCV, infectious PCV in one rotavirus vaccine, and the presence of infectious PCV was indeed confirmed. And porcine trypsin was considered the likely source.

The VRBPAC discussion included the fact that PCV-1 was present throughout development in this rotavirus vaccine, as well as in the cells used to produce the vaccine. There was also a component of vaccines tested and shown to be safe in the original clinical trials, and all subsequent post-marketing follow up.

The fact that PCV-1 is a ubiquitous virus to which humans are frequently exposed without known adverse consequences, the fact that some therapeutic products are chronically administered and contain high levels of PCV-1 without known adverse sequelae, as well as in the context of the known benefit of rotavirus vaccines.

The VRBPAC recommended the development of PCV free rotavirus vaccines and also at that time recommended further consideration of how new assays could be used to reduce the likelihood of adventitious agents in vaccines.

In 2010 the FDA published a final updated guidance document. A draft guidance document had been published in 2006, on characterization and qualification of cell substrates and other biological starting materials used in the production of viral vaccines for the prevention and treatment of infectious diseases.

And so that summarized our thinking, not only of what tests should be used but also of an approach to thinking about how cell substrates should be characterized. We wanted that approach to be used, knowing that we wouldn't be able to predict what would happen in the future. And that guidance document, of course, was a part of your briefing package.

Another point to be made here, and that is that this advisory committee deals with the vaccines that are regulated by the Office of Vaccines Research and Review. And so those are specifically the viral vaccines for the prevention and treatment of infectious diseases. And so we don't deal with vaccines that are used, for instance, for cancer or for other kinds of non-infectious disease therapeutic indications.

Just to show you that in addition to the VRBPAC meetings that I have described, members of the Office of Vaccines have been intimately involved in the organization as well as presentation at a

large number of workshops that have introduced the introduction of novel cell substrates for vaccines.

So there has been a very robust international scientific dialog on this topic as well. This included a CBER-sponsored meeting in 1999 called Evolving Scientific and Regulatory Perspectives on Cell Substrates for Vaccine Development, and various other meetings that have been sponsored variously by IABS, NIAID, PDA and FDA, including three meetings just in 2011.

Just to give you a perspective, these kinds of cells, tumor-derived cells, are not contemplated for use only in vaccines, of course. There are other vaccines, other products that are produced themselves as well. And this includes therapeutic biological. Human Burkitts lymphoma cells, for instance, were initially used to produce interferon. And these cells were not only tumor cells but they also contained the Epstein-Barr virus genome.

Baby hamster kidney cells have been used for recombinant Factor VIIa. These cells are tumorigenic. Chinese hamster ovary cells, who are tumorigenic and also contain endogenous retroviruses, which over time have been proven to be non-infectious, have been used for many products including recombinant Factor VIII as well as many other therapeutic biological.

And a number of Murine Hybridoma cells, which are tumor-related cells, obviously, have been used to produce monoclonal antibodies. And these cells contain both endogenous retroviruses as well as have, over the years, had various other potential murine components that have needed to be dealt with. And then 293 Human Embryonic Kidney cells have been used for other therapeutic products. And these cells include Adenovirus DNA sequences as well.

These kinds of cells also have been used for investigational gene therapy vectors, which have gone into people, including rodent cells, BHK21, which are tumorigenic, as well as 293 and PER.C6, both of which have adenovirus sequences in them. As well as A549 and HeLa cells, which we are going to be talking about today.

So to summarize the overview which I have given you here, then, the number of cell substrates considered acceptable for vaccine production has been gradually increasing. This increases the potential for development of novel vaccines that address important public health needs. And in the US this process has included frequent VRBPAC consultation, of which this meeting is an additional step.

Concerns about potential oncogenicity associated with not just tumorigenic cells, but as you can see, go back to the 1950s, really have been thought about in the context of oncogenicity and adventitious agents. And the oncogenicity has generally boiled down to thinking, especially with tumorigenic cells, also about DNA and adventitious agents, as Dr. Peden will describe.

And I think it is important to think about where historically adventitious agent issues have arisen. Some of these episodes have come about and were related to the use of primary cells, and in fact many people view primary cells as those which have the greatest risk for adventitious agent, because they come directly from a living organism. And they can't really be banked. They have to be used immediately. And so you are using fresh cells every time. And so you can't do the kind of thorough characterization and testing that you can do on cells that can be banked.

On the other hand, identification of porcine circovirus and vero cells showed the potential for adventitious agents to be present in continuous cells as well. And at present we are considering issues associated with the use of vaccines that are produced in cells derived from human tumors. So one important thing to consider as you go through your discussion is not just what could be in cells, because that is an issue that we face every time we need to think about a cell substrate in a vaccine. But what is it that makes these human tumor cells different from other cells? And specifically, are there more risks, and if there are more risks, or if there are different types of risks, how one might mitigate those risks.

So in that light, then, I just have this slide which I have already presented that I think summarizes a lot of what you are going to hear through the morning, that really this issue

has boiled down to thinking about cells which we remove, DNA which Dr. Peden will describe, and adventitious agents, which Dr. Khan will describe.

The final slide then here has the questions for the committee, which I will come back and present this afternoon. These will include a discussion of safety concerns associated with the use of human tumor-derived cell lines for production of preventive and therapeutic vaccines for infectious diseases. And then to please discuss approaches that could be used to address any concerns.

And in that context, to consider the role of the manufacturing process, whether a vaccine is live versus inactivated, the adequacy of current approaches as they have been described to mitigate potential risk, as well as then the potential use of new technologies to mitigate potential risk. So thank you very much, and I even have a few minutes left for questions.

DR. DAUM: Thank you very much, Dr. Krause, for so elegantly posing this nice, simple question for the committee to consider. And we have about eight or nine minutes left for clarifying questions regarding your presentation.

DR. LOWY: I was curious whether something has happened because you have looked at this question a number of times. Has something happened that precipitated the necessity or the desirability of having this advisory meeting?

DR. KRAUSE: No not really, although we have come back with this question with different types of cells. But we have never explicitly considered the use of cells that are derived directly from human tumors. So the previous discussions have generally been of cells which have been considered normal in some way, but then, for instance, for the PER.C6, were made to be tumorigenic by manipulating the cells, or cells, for instance, the vero cells, which were a continuous cell but could become tumorigenic.

But I think there is a distinction that most of these previous discussions centered around tumorigenicity as an endpoint, which might or might not be as relevant to risk in humans as the fact that the cell is directly derived from a human tumor.

DR. DAUM: Maybe Chairman's prerogative. I have a question. You mentioned that your office doesn't regulate vaccines intended to alter the course of a cancer event in a patient, and that there is, I presume there is another agency of FDA that does that.

DR. KRAUSE: That is also done in the Center for Biologics, but those vaccines are considered by our Office of Cell Tissues and Gene Therapy, if I have their name correct. OCTGT.

DR. DAUM: So now for my question. What regulatory process are they going through, and are they going through similar considerations to what you presented here for us to consider today?

DR. KRAUSE: I think they are taking the same considerations into account, but of course they have a different use for the vaccines. And so if they are contemplating using a vaccine for somebody who already has a tumor, then they may be dealing with a very different risk/benefit calculation than we are when we are thinking about vaccines that could be given to otherwise healthy individuals, including children.

DR. DAUM: Fair enough, but you actually didn't mention risk/benefit calculations in your talk. And so I think that is a valid consideration and might be good sometime to have a joint session with them or to hear that they are on the same page with us in terms of these considerations. I think actually risk/benefit considerations are very important. Other committee comments or questions? Okay, I guess you were real clear, and thank you very much.

We are going to move to hear from three manufacturers now in sequence, that are proposing to manufacture vaccines that use these tumorigenic cell lines. The committee should be reminded of something that Dr. Krause said, and that is that we are not really here today to consider these products per se, but we are here to consider the issues that we would like to advise the agency to consider in helping the company continue the manufacturing process, what should they be concerned about, what should they be watching for.

So with that, I will call on the first speaker, who is Dr. Tim Mayall, who is the Senior Director of R&D at a company called PaxVax. Good morning. And his topic will be A549, which I suspect many at the table have worked with or know about, a novel cell substrate enabling production of new vaccine candidates. Welcome, Dr. Mayall.

Agenda Item: A549: A Novel Cell Substrate Enabling Production of New Vaccine Candidates

DR. MAYALL: Thank you. Good morning ladies and gentlemen, members of the committee. My name is Tim Mayall and I am the Senior Director of Research and Development at PaxVax, a vaccine company in San Diego, California. It is my honor to represent PaxVax today. And we thank the committee for the invitation to present our recommendation for A549 as a vaccine cell substrate.

I will summarize today the characterization of the A549 cell line. That is, the culmination of five years of work that has been at the core of PaxVax's technology from the company's inception. It has resulted in a master file submission in 2011 to support a planned IND filing next year.

In the time I have available this morning I will lay out a path for how we chose A549 as a candidate cell substrate to the extensive characterization that we have completed. As you will see, a vision or purpose of PaxVax is the development of novel vaccines with a potential global unmet medical need. A robust high yield cell line is an integral part of this, and I will discuss both the rationale for the selection of A549 cells and the implications of our most advanced programs.

Next, for those of you not familiar with the A549 cell line, I will provide an overview on the origin of the cell line and current regulatory status of our massive cell bank. Importantly, I will then review the information contained in the master file that pertains to several aspects of A549 evaluation, for vaccine production. Finally, in summarizing the characterization, I hope to have addressed any of the questions that may have arisen around the use of A549, and conclude with our recommendation.

PaxVax is a vaccine company that was founded on the premise of creating novel vaccines that strive to meet large scale global needs while aligning with WHO recommendations for an ideal vaccine. That is, a safe, effective vaccine that is both affordable and can be delivered simply.

Our vaccine production platform is based on the previously developed US military live Adenovirus 4 and Adenovirus 7 vaccine, and like the military vaccine has a desirable attribute of oral delivery. The platform offers flexibility. Using reliable and fast cloning methods we can insert antigens relevant to the targeted disease, such as those indicated on the right of this slide. These vaccine candidates are required to be produced in large quantities, and rapidly in some cases. In the beginning it was clear that we would have to identify a new cell substrate that would offer greater production capacity for our lead vaccine candidates. To achieve this, we went through a selection process, which identified A549 as the ideal cell substrate. We began by looking at the currently accepted vaccine cell substrates.

The known species specificity of Adenovirus quickly ruled out non-human primary cells and non-human cell lines such as vero or MDCK. We evaluated human diploid cells MRC-5 and WI-38, as they are used for the licensed vaccines. In the case of WI-38, they are used for the production of the US military Adenovirus 4 and Adenovirus 7 vaccine.

With our recombinant Adenovirus vaccines we experience typically lower virus yield compared to wild type Ad4. This rules out the human diploid cells for use due to low productivity, and I will show examples in a moment. Finally, we considered continuous human cell lines. We screened some of the most common ones by determination of virus yield. Representative production data from the different cell lines is shown to the right for both Ad4 wild type and our pandemic flu candidate.

A549 was identified as the highest producer, and in combination with our favorable cell culture

conditions, the optimal candidate. Let me now discuss the benefits of A549 in relation to our lead vaccine programs. In brief, we find that vaccine development in critical areas of PaxVax will be potentially impeded without the ability to utilize A549.

While we were developing A549, we relied on MRC-5 cells to begin our proof of concept work, with the H5 pandemic influenza vaccine, and to enter a Phase 1 clinical trial. However, we are aware that the low yield in MRC-5 may well limit large scale clinical trials and will not support potential pandemic needs.

In a second program sponsored by NIH/DMID, we are developing two potential anthrax vaccine candidates. The yields in MRC-5 were so low that we would be unable to generate enough vaccine for a Phase 1 study for our planned IND. We therefore have an immediate need to be able to use A549 to produce clinical material, so as not to impede advancement to an anthrax clinical study.

A third lead program in development is focused on vaccines for HIV. The immunogenicity data from our pandemic influenza Phase 1 study showed good cellular responses with oral priming and a very good antibody response following a heterologous protein boost, which is thought to be the ideal profile for an effective HIV vaccine. We are actively pursuing three lead HIV vaccine candidates. Firstly, HIV gag. Phase 1 material has been produced in an MRC-5 for the Ad4 gag vaccine candidate. But the limitations on large scale production likely hinder further development.

The other HIV vaccine candidates are based on the HIV envelope protein. Our second vaccine candidate is a truncated envelope gp145, development of which is supported by NIH/DAIDS. This Ad4 M vaccine has a low production yield in MRC-5 and the gag factor I just mentioned.

The third candidate is a full length envelope vaccine candidate, gp160, which is of great interest to the HIV vaccine community. But literature shows gp160 to be difficult to express from other vector effect systems. Like the anthrax vaccines, the production of Ad4 gp160 has only been possible in A549 cells. At this time, further development of this program is in jeopardy without the use of the suitable cell substrate, such as A549 cells.

To give you a visual perspective on some of these programs, the next slide looks at four of PaxVax's key programs and the relative yields of virus that we have observed. You can clearly see the magnitude of difference in yield between MRC-5 and A549 for our key disease targets. A549 is better than MRC-5 by 10 to 40 fold. For our anthrax and HIV gp160 vaccines, this demonstrates the immediate need for A549 cells for further development. In the case of pandemic influenza and HIV gp145 vaccines, although production has been initiated in MRC-5 cells, there is a critical need to use A549 cells to enable later, larger clinical trials, and for further development.

I have given you a justification for our selection of A549 cells, for the development of our vaccine platform. Now let me focus on A549 in a lot more detail. A549 is a well-known cell line being used in many applications in research. It was one of a number of cell lines generated in the early seventies from different neoplastic tissues.

A549 was derived from the solid lung tumor of a 58 year old Caucasian male. As an intensely studied cell line, the basic molecular mechanism of transformation has been elucidated. The genetic signature is associated with smoking and one dominant onc gene has been identified, mutated K-RAS. We received a vial from ATCC, from which our massive cell bank was generated.

With the support of the Wellcome Trust and NIH and the guidance of CBER, we have performed a comprehensive characterization of the cells. This, as I mentioned earlier, is detailed in a biologics master file submitted to CBER in 2011. Although I am focusing on PaxVax vaccines, we stand behind a provision in the Wellcome Trust award that will provide access to the PaxVax A549 cells to other parties in order to provide low cost vaccines to developing countries. For the remainder of my talk, I will focus on the content of the master file, i.e., the characterization of A549 PaxVax cell substrate. I will break this down into three topics

for investigation. Adventitious agent testing for both known and unknown agents -- this includes chemical induction studies for investigation of potential viral contaminants that can reside in a latent state, and a new method, massively parallel sequencing, that offers a detailed analysis of the genetic environment, looking for trace sequences from viral contaminations.

Oncogenicity, as defined as the ability of DNA or acellular materials to induce tumor formation in animals. And then tumorigenicity, defined as the ability of intact viable cells to establish a tumor in an animal. Finally, I will review the PaxVax manufacturing process for its ability to clear intact viable cells, cellular DNA, and protein from the final drug product.

Let's start with what we will call standard adventitious agent testing. This is the first of three slides I will not go into in detail due to the shortness of this presentation. They capture the different agents analyzed. This slide shows the general screens we performed for adventitious agents, including microbial and viable agents on the master cell bank, or MCB, or on the end of production cells, EOPC. All results were negative.

This next slide summarizes the specific non-human viral agents, both bovine, porcine, and rodent derived. All results were negative. And finally this next slide, the specific and human viral agents. Again, all results were negative. In summary, after comprehensive testing, no adventitious agents were identified or detected.

Another major safety concern in the use of normal cell substrates is the potential presence of latent and occult viruses, which may not be detected by currently used conventional assays. One way to detect such viruses is to induce them to proliferate. Chemicals offer one of the best ways of doing this.

The induction conditions were optimized with guidance from Dr. Arifa Khan of CBER, according to the published algorithm. Different inducers were used for either DNA viruses, phorbol acetate, TPA, and sodium butyrate, or RNA viruses, nucleotide analogs IdU or AzaC.

No viruses were detected using broad approach methods such as TEM or PERT for retroviruses, or following co-cultivation with indicator cell lines. Degenerative PCR was used to look for related viruses from the Herpes virus, Polyomavirus, Papillomavirus, and Adenovirus families. All results were negative for latent or occult viruses.

I will summarize now our final investigation for adventitious agents, which was our work with massively parallel sequencing, or MPS. MPS became available in 2000, as the next advance in technologies to sequence or read the genetic code of DNA. The big step forward in this technology was new instrumentation, and chemistries that allow massive quantities of sequenced data to be generated in parallel.

This information is then mined, using bioinformatics to look for sequences that are similar to those found in a curated reference virus database. We worked with our colleagues at BioReliance, Drs. David Onions and John Kolman, who are here today, to look at two compartments. Within the cell we used cellular transcriptome analysis to interrogate the actively transcribed RNA species for foreign sequences.

We also studied any protective nucleic acids that were present in the extracellular environment, which may come from a productive virus. This was expanded to include investigation of the culture media components as well, as I will discuss next. We performed MPS on the A549 cells in 2009. One benefit of this technology is the ability to revisit the data at later times.

We have the analysis repeat again this year, with a database of viruses that had expanded from 300,000 in 2009 to more than 1 million sequences today. We received the same result when looking for homology to the virus database. No viral sequence was detected in a cellular transcriptome.

Both times that we analyzed the extracellular media from outside of the cells we detected sequence fragments from three bovine parvoviruses. This contamination was traced back to the lot of serum used to prepare the A549 cells for MPS. One of these viruses represents a

virus nearly identified in cattle, which had not been described in serum before, demonstrating the power of this method.

Specific high sensitivity QPCR assays were developed, which confirmed the presence of these sequences was isolated to this serum lot, and that the A549 massive cell bank itself was negative for these bovine viral sequences.

To conclude MPS did not detect transcripts from replicating latent or transforming viruses in the A549 cells. The value of MPS was confirmed by its ability to detect traces of viral sequences from the serum used to culture the cells. Consequently, this has led to changes in our screening of future production serum loss.

We conclude from the culmination of this data from our adventitious agent studies, from both broad detection at general screens, extensive type-specific viral assays, chemical inductions for latent occult viruses and from MPS that no adventitious agents are detected in A549 cells. A related observation that came from the MPS data was that there are no predisposition mutations in the PRNP gene, which is associated with transmissible spongiform encephalopathies.

Following adventitious agents, the potential for oncogenicity was a second topic for analysis. As a reminder, oncogenicity is defined as the ability of DNA or acellular materials to induce tumor formation in animals. Using standard methods we looked at both A549 cell lysate and isolated A549 genomic DNA, neither of which induced tumors in nude mice, rats or hamsters. This data is consistent with other public reports, including data in a more susceptible mouse model, the CD3 Epsilon mice, which Dr. Keith Peden will be presenting later today.

A third topic for analysis was tumorigenicity. Tumorigenicity is defined as the ability of the intact cells to establish a tumor in an animal. A549 are known to be tumorigenic. And as you can see, we confirm this in athymic nude mice. Tumorigenicity would only be an issue if intact viable A549 cells remain in our final drug product. We believe this is not the case for our purified, orally delivered Adenovirus vaccines.

Therefore, to conclude, on both oncogenicity and tumorigenicity I will draw them together and discuss the manufacturing process and how it relates to clearance of cellular material and cells. Although A549 cell substrate has no detectable adventitious agents or oncogenic characteristics, processed clearance addresses any concerns about the potential presence of any transforming genes in the residual DNA and the presence of intact viable cells in the final drug product.

As I am about to discuss, our process involves multiple orthogonal or independent purification steps that will clear intact cells, cellular DNA and proteins. Firstly, cell destruction is achieved early on with detergent lysis and freeze-thaw steps, which destroy the cells in order to release the Adenoviral vaccine.

Several filtration steps in the process include filtration with 0.2 micron filters which remove intact A549 cells. We will naturally be validating the clearance of intact cells by the process prior to our planned IND. DNA clearance begins with benzonase treatment, which is a nucleic acid digester. And subsequent steps of anion exchange chromatography and high molecular weight diafiltration are also DNA clearance steps. All these process steps result in the purified bulk drug substance, which is lyophilized and filled into enteric coated capsules for oral administration.

The question of residual DNA is an important one, so let me discuss the study of that in more detail. Cancer is a multi-step process with oncogenicity most likely requiring more than one change to occur. Residual DNA would therefore need to be present at a high enough amount and in a large enough fragment size to accommodate an oncogene.

Having already discussed the several steps above to degrade and remove residual DNA, we looked at five small-scale bulk drug substance lots of wild type Ad4, with a high sensitivity qPCR assay. All five samples were below the level of detection of the assay, which when we back calculate to a clinical dose of 10 to the power of 10 viral particles, is less than 0.013 nanograms per dose.

As we have not been able to detect residual DNA, it has made determination of fragment size impractical. I will stress, however, that we use Benzonase in an exhaustive

digestion step which has the capacity to reduce DNA to only a few nucleotides. In the remaining moments, let me conclude my presentation.

To review, I presented a cogent rationale for our selection of A549 as a production cell substrate for PaxVax adenovirus based vaccines. Principally with yields 10 to 40 fold higher than the MRC-5 human diploid cells, A549 are acquired for large scale recombinant Ad4 based vaccine production at a scale necessary to meet global unmet medical needs.

I believe we have made a compelling argument for the absence of additional risks on the human continuous A549 cell line, ruling out adventitious agents through extensive characterization from general screens, specific viral PCR assays, chemical induction and MPS. We demonstrated a lack of oncogenicity of A549 DNA or cell lysates instead of animal models.

Finally, I detailed a multi-step orthogonal manufacturing process that provides effective clearance of residual cells or DNA and ensures confidence in the final drug product, a purified, live, oral replication competent adenoviral vaccine. We would therefore ask the committee to respectfully consider that the A549 cell substrate be accepted for use for the production of human vaccines. Thank you for your consideration. I will hand it back to the Chairman. Thank you.

DR. DAUM: Thank you, Dr. Mayall, for your presentation. We have a few minutes generated by your being succinct and also by being a little bit ahead of schedule. So I would like to call on committee members to ask questions of Dr. Mayall and his presentation. Yes, could you state your name for the recording?

DR. COFFIN: John Coffin. A couple of points of clarification. You mentioned a bovine virus in some lots. I didn't catch the name of the virus. Could you tell us what it was?

DR. MAYALL: In the database it is known as AAV-Go.1, but we believe it probably is a bovine virus.

DR. COFFIN: And you mentioned PCR tests for residual DNA. What was the target of those? What sequence is being amplified? Or sequences? I think it was more than one.

DR. MAYALL: John, do you remember what the sequence is?

DR. COFFIN: I was just curious whether you are looking at a single copy gene or a multi copy line element or something like that.

DR. MAYALL: I think we actually used a line element. It was one of the ones we looked at.

MR. KOLMAN: I am John Kolman from BioReliance. Our residual DNA tests do target multicopy loci. I am not 100 percent sure which one was used in this case. I am actually trying to find that out now.

DR. DAUM: Other committee questions? Ambrose? Dr. Cheung?

DR. CHEUNG: Yes, you mentioned something about follow up on the bovine virus sequence. You relayed the fact that it related to a serum source. Have you found a source of serum that is free of these virus sequences?

DR. MAYALL: Yes. We can screen several cells.

DR. CHEUNG: And with your benzonase treatment and then you do the residual PCR afterwards, are you 100 percent confident those were removed? So when you have your sample, you are true to the baseline to digestive DNA, and then you do PCR. Have you done PCR on that specific bovine virus sequence to see it has actually gone?

DR. MAYALL: Yes, we use the screen assay. I believe it is a release assay on our materials. Yes.

DR. CHEUNG: Yes they are gone?

DR. MAYALL: Yes, they are gone. Sorry, they are not there to start with, because we pre-screen the serum.

DR. MCINNES: If you could put slide 4 on, which is headed A549 is an ideal substrate for Ad4 based vaccine production, you looked at the different cell lines. It is a graph, a histogram. So I am just interested in this other promising looking candidate, HuTu80, and what do you know about that? You didn't talk about it at all. You headed straight to A549. I am just

interested in whether you worked up HuTu80? No?

DR. MAYALL: We looked at several other cell lines actually on this graph. HuTu80 was one that we had in the lab at the time. We focused mainly on A549 because there are other characteristics of A549 that make it a favorable cell line. It typically can be grown as suspension, serum free, which I believe we didn't know the status of HuTu80 at the time, so there were other characteristics that we applied to this selection.

DR. DAUM: I have one question for you. You presented a great deal of, I think, fairly compelling biological data that the -- you have done an extensive amount of testing with the cell line to rule out DNA and adventitious agents, and the things we are gathered to be concerned about today. And you concluded that the substrate be accepted for the use of production of human vaccines.

But you didn't say anything about the issues that we are here together about. And that is, one, the biologic testing of course, which I think you have been very thorough about. **But two, there is also a public perception issue and there is a risk-benefit issue,** which Dr. Krause raised. And I wonder if you would comment on the issues that we are gathered here to discuss today. What would you ask FDA to do in terms of regulating the biology, which you have done extensive amounts of? **And what do you think of the risk-benefit and public perception issues?** That is a nice simple question to end on.

DR. MAYALL: Sure, a simple question.

DR. DAUM: Well they are relevant. It is what we are here to discuss.

DR. MAYALL: Exactly. We believe that the modern methods, obviously we had a good background here on cell substrates. Dr. Peden, Dr. Krause, which I think details a process that the CBER has gone through in terms of selecting cell lines. And I think we have got to a point now with the technologies that a human cell line can be an acceptable cell substrate, that there is extensive ability to test and prove it is as safe as any of the other cell lines out there.

And so I really think that we have got to that point of making a cell line such as A549 a viable candidate as a cell substrate. And I think the risk-benefits now are certainly in our favor.

DR. DAUM: I think we should probably stop in terms of time. I am going to thank you very much, Dr. Mayall, for your fine presentation. We may return to some of the issues that you have raised this afternoon in our discussion. We hope you will be here to clarify if we need you to. Our next speaker is Dr. Seung Ho Choo. He is from a company called GMP Manufacturing Sumagen Company, Limited. His topic is history and characterization of the A3.01 cell line and its tumorigenic evaluation.

Welcome, Dr. Choo, but before you start I would like to ask Dr. Kester to introduce himself, because he came in late, so that we know who he is.

COL. KESTER: Good morning. I am Colonel Kent Kester, Uniformed Services University of Health Science, Bethesda, Maryland.

DR. DAUM: Thank you, and welcome, Dr. Choo.

Agenda Item: History and Characterization of the A3.01 Cell Line and its Tumorigenic Evaluation

DR. CHOO: Hello everyone. Thank you for giving me the opportunity to present Sumagen's research to this committee. Today I am going to present the history and characterization of A3.01 cell line derived from CM and its tumorigenic and oncogenic evaluation. In this presentation I will introduce our A3.01 cell line and Sumagen's killed whole HIV/AIDS vaccine briefly. And I will show you the data for selection of the cell substrate.

Also, I will present the history and the risk assessment and general and target specific adventitious agent tests. And finally I will present tumorigenic and oncogenic evaluations. Let me introduce the A3.01 cell and Sumagen's killed whole HIV/AIDS vaccine. The A3.01 cell is the cell substrate for Sumagen's HIV vaccine. It is a human T-lymphocyte. This is the first time it has been introduced for vaccine manufacture.

One of our greatest challenges in development of an HIV vaccine is the selection of the

appropriate antigen. No one knows what is the best immunogen to protect the human body against HIV infection. Sumagen has focused on the killed whole vaccine rather than the subunit vaccine used by the other companies. Sumagen HIV/AIDS vaccine has SAV001-H. It is the first genetically modified killed whole HIV/AIDS vaccine which has been double inactivated using both chemical and physical methods.

It has been confirmed it could induce a strong humoral and cellular immune response in non-human primate studies. This vaccine was originally invented at the University of Western Ontario in Canada, and developed by Sumagen Company. And it was approved for phase 1 clinical trial by US FDA. The study is presently ongoing in the USA.

There are three challenges to develop an inactivated HIV vaccine: safety, production and maintaining the immunogenicity intact during the inactivation process. Because there is the killed whole HIV vaccine, the safety issue is very important. To ensure it is safe, Sumagen made genetically modified virulent HIV. And the vaccine was double inactivated, both chemical and physical methods to make non-infectious, safe vaccine.

The other huge challenge is the production of HIV vaccine in large scale. In order to accomplish this, Sumagen generates an excellent strain showing high production yields through the genetic modification, and we selected the proper cell strain for this strain. I will discuss this more later.

Sumagen also successfully developed the process to produce the HIV vaccine in large quantity. Now we have the capacity for vaccine production. Generally during the inactivation process, the protein structure, which is an important immunogen, can be changed by chemical reaction. But Sumagen's HIV vaccine was inactivated by aldrithiol-2, which we called AT-2. It is a strong oxidizing agent. It can induce part of the bond between free SH group and the proteins.

It has been confirmed that it can only modify the nuclear capsid of HIV proteins. Therefore, major immunogens like gp120 and PTNE4 are not affected by this chemical reaction. As well, the vaccine was double inactivated with gamma irradiation, which mainly affected the nucleic acids. So our vaccine, SAV001-H, is a genetically modified and double inactivated, safe and effective HIV vaccine.

One of the key genetic modifications of Sumagen HIV vaccine is the replacement of env signal sequence with melittin signal sequence. This picture is the result of pulse-chase analysis for gp120 expression and secretion. This is the gp120 of the natural signal sequence, and this is the gp120 with the melittin signal sequence. Gp120 expression was checked in the cell, and secretion was checked in the medium during the eight-hour time period.

As you can see here, the gp120 was expressed well in both cases. But it was more efficiently secreted in the case of gp120 with the melittin signal sequence than natural signal sequence. The replacement of the env signal sequence with the melittin signal sequence caused the secretion of gp120 to increase dramatically. Obviously, it is helpful to make HIV production increase.

After the completion of the genetic modification, we tested HIV expression in several different cell lines. Wild type and genetically modified recombinant HIV were injected into our A3.01 cell, H9, PM1 and PBMC. The level of HIV expression was analyzed by P24 analyzer during the 16 days after infection. In this picture the filled box is wild type and open triangle is genetically modified Sumagen-HIV. The others is partially modified HIV. As you can see here, the recombinant Sumagen HIV expressed higher than wild type HIV in most cell lines. And the level of the expression of both recombinant and wild type HIV expressed higher in A3.01 cell line. Sumagen thinks it is critical to use the T cell line to produce HIV vaccine. Consequently, Sumagen selected A3.01 cell substrate for HIV/AIDS vaccine manufacturing.

I will show you the A3.01 cell line history. A3.01 cell line is the human T cell line derived from CEM cells, which was isolated from four years old female with the acute lymphoblastic leukemia by Dr. Foley in 1965. The CEM cell was cultured in the presence of 8-Azoguanine to develop the HAT sensitive A3.01 cell line by Dr. Folks in 1985. A3.01 cell line is CD4 receptor positive, and

sensitive for HIV infection. The master cell bank was produced under the cGMP compliance at CMO in the USA in 2007.

To use the A3.01 cell line as a new substrate for vaccine manufacturing there are some potential risks to evaluate. There is no information about the cell characteristics and no information about adventitious agents and tumorigenic and oncogenic properties. Therefore, we assessed all of this with some characterization studies, general and other target-specific adventitious agent tests following USA USFDA guidance, and we also did in vivo tumorigenicity tests with live cells and oncogenicity tests with cell lysate and DNA. For the cell characteristics we did isoenzyme analysis, karyotyping, PrP genomic sequencing and the cellular morphology and cell growth characteristics. As you can see from the results here, it is a compound A3.01 cell is human origin and karyotypically abnormal as expected.

It has normal PrP gene sequence. It means there is no concern about prion proteins. The morphology of A3.01 is the lymphoblast-like cell as expected, and the cell growth, grows as single cell in suspension. And the doubling time is approximately 28 hours in RPMI 1640 with 10 percent FBS.

To detect the adventitious agent we do several general adventitious agent tests. We perform the sterility and mycoplasma tests, and we perform the in vivo adventitious virus detection with cell and supernatant in newborn and adult mice, and embryonated chicken eggs.

Also we did in vitro adventitious virus detection with the cell and supernatant in MRC-5, HeLa, Vero and CEM-A cell lines. Also, we did in vitro bovine adventitious virus detection. So through all these tests we confirmed that no adventitious virus agent was detected in Sumagen A3.01 MCB.

We have also done the target specific adventitious agent tests. We performed that it was RT assay to detect retrovirus and the TEM assay to detect virus including retrovirus. And we also have done the PCR assay to detect certain different viruses, and also we did PCR assay, essentially to discover the human polyoma virus, which is BK/JC and WU/Ki virus. Through all these tests it was confirmed, no adventitious agents were detected in Sumagen A3.01 MCB. Essentially we are performing whole transcriptome analyses to detect unknown adventitious agents, as the FDA recommended.

Sumagen evaluated the A3.01 cell tumorigenicity in other assigning nude mice. 10 to the three, and 10 to the five, and 10 to the seventh cell was prepared from over 25 Sumagen MCB, and injected subcutaneously in 10 nude mice for each group, and observed for four months to evaluate the tumor generation.

As you can see from the data, 10 percent of 10 to the five cell-injected mice, and 90 percent of 10 to the seven cell-injected mice has tumor. Therefore, Sumagen considered the A3.01 cell showed tumorigenic phenotype in high concentrated cell suspension.

To evaluate the oncogenicity, the cell lysates and DNA were isolated from 25 passaged A3.01 cells and injected subcutaneously in newborn nude mice, newborn rats, and newborn hamsters. 10 to the seven equivalent to cell lysate, and 100 micron of cellular DNA was injected into 15 to 20 of each animal for each group. During the four months observation period, no tumor was found in any of the animals. So we can conclude that the Sumagen A3.01 cell has no oncogenic phenotype.

In conclusion, I will summarize this presentation. The A3.01 cell line is a human T cell line, and the best cell line for manufacturing of Sumagen HIV/AIDS vaccine. No adventitious agent was detected in Sumagen A3.01 MCB. Sumagen A3.01 MCB has tumorigenic phenotype at high cell concentration. However, there was no oncogenic phenotype in various animal species. Thank you so much.

DR. DAUM: Thank you very much. I would like to take advantage of the fact that we have three minutes or so for committee questions.

DR. LOWY: I have two different kinds of questions. The first deals with the cell line. Since it is derived from a child with acute lymphoblastic lymphoma, have you looked for whether there might be gene rearrangements, as occur frequently in such leukemias?

DR. CHOO: That is a good question. We actually did a karyotyping study and we found there is no second sex chromosome. And we found some deletion and replacement in several chromosomes.

DR. LOWY: I was also curious about the inactivation and the attenuation of the virus. You said that it was avirulent, and I was wondering what the basis for that was. And a slide also said that it was effective, that the vaccine was effective. And I was wondering what the basis of that was.

DR. CHOO: We just focused here on the A3.01 cell line, but during the genetic modification we deleted from HIV genome, which is not expressed in the HIV virus. So it was a compound. Therefore we deleted that gene. It is non-virulent, avirulent.

DR. DAUM: I am going to try to squeeze in Dr. Cheung and Dr. Coffin before we go onto the next speaker.

DR. CHEUNG: So in your presentation about tumorigenic potential you mentioned that if you inject 10 to the fifth and 10 to the seventh cells in the mice, they develop tumor. But when you do it for 10 to the three they don't. Is it possible you didn't observe the mice long enough at 10 to the three?

Because it seems like you have a tumor at 10 to the fifth and you do it too long, if you don't have it maybe it is the duration of observation that makes a difference. I think the same thing applies for the A549, too. That is a similar finding.

DR. CHOO: At the time we just followed our FDA guidance to test this tumorigenicity test. So we think we didn't look at long periods or so. This is our data.

DR. COFFIN: To address Dr. Lowy's questions, it happens I was a summer technician working in Dr. Foley's group on this very cell line when it was first isolating, doing karyotyping. And as I recollect, it has a micro chromosome, like a Philadelphia chromosome, suggesting an oncogeny arrangement that I think we observed at the time.

DR. CHOO: Sorry, please repeat and make sure, I don't understand.

DR. DAUM: Dr. Coffin, he would like you to repeat your comments.

DR. COFFIN: Yes, the karyotyping that was done way back in the sixties, I think, suggests that the presence of something like a Philadelphia chromosome, which is a chromosome derived from a rearrangement of BCR-ABL, and that gives rise to, basically activates the c-abl oncogene.

DR. DAUM: Dr. Choo I would like to thank you for your presentation. I hope you will be around this afternoon, because we may need to question you some more about your presentation. I appreciate it. Our third speaker of this morning's trilogy is Dr. Rebecca Sheets, captain of USPHS, now at the NIH NIAID. And her topic is are HeLa cells an acceptable vaccine substrate. Welcome, Dr. Sheets.

Agenda Item: Are HeLa Cells an Acceptable Vaccine Substrate?

DR. SHEETS: Thank you. Good morning, and I would like to thank my former colleagues from CBER for inviting me to come present this morning regarding this important issue. I will correct slightly a comment Dr. Daum said earlier. I am actually not a manufacturer. But I represent a division of the Institute which is a funding organization and we support the research community as well as manufacturers who are developing HIV vaccines in order to achieve our mission.

Our mission is to end the AIDS epidemic. So while this is a pressing, urgent and global public health need that is currently unmet by the available prevention methods that are currently in use, it is certainly a goal that we have to consider novel cell substrates, novel vectors and a variety of novel approaches,

Because there have been historically safety concerns as well as evidence of lack of efficacy on the part of one or the other on the traditional kinds of vaccines, which is the live attenuated and inactivated vaccines.

DR. DAUM: Correction accepted. But those of us with an NIAID grant had to report a conflict for this presentation. So please continue.

DR. SHEETS: Okay, thank you. As far as I am aware, I have no conflicts of interest. I am going to talk to you today why we need cell substrates. I have already given you a flavor of that, that there are certain kinds of vaccines that might require cell substrates that are novel from those that Dr. Krause presented earlier. I am also going to give you my take on the evolving cell substrate policy and then move into why we should consider HeLa cells.

I will talk to you about the HeLa cell bank characterization that was done to support one of our projects, and then sort of outline what differences there might be between HeLa cells and other cell substrates that have historically been considered more acceptable, and what do those differences actually mean in terms of product safety. And then I will draw to a conclusion.

So over the past 60 plus year, cells that have been propagated in culture have been safely and successfully used to produce viral vaccines. And without cell culture we would not have a number of important public health tools that we currently have, and that Dr. Krause has also introduced. As Dr. Krause said, in the early days only, quote, normal cells were considered appropriate or permissible. However, as time passed there was a recognition that human diploid cells and other cell lines that remained diploid in culture -- for example, fetal rhesus lung cells -- could be appropriate cell substrates for the production of vaccines. In the nineties we started to look at immortalized cell lines. And these cell lines could be aneuploid as opposed to diploid, but still non-tumorigenic. And so the vero cell became considered a more acceptable cell substrate. In the 2000s we began to look at engineered or non-engineered cell lines that actually were tumorigenic in animals. And this committee has considered those cell lines as well in the past.

And now we are poised to consider tumor-derived cell lines such as the A549 cell line that you heard from PaxVax earlier, and HeLa cells, which I am going to tell you about now. So over time, primary cells, while still in use, have really fallen out of favor because they cannot be banked and well-characterized prior to use in production, and they must be sourced each time. So there is always that risk of introducing an adventitious agent from the primary source. Whereas, cell lines can be banked. They can be thoroughly and exhaustively tested prior to their use in production. And therefore, just the use of a cell bank can lower your risk of adventitious agent contamination.

So what has really driven this evolution? I think in large part we have a much better understanding of cell biology and the molecular basis of cancer than we did 60 years ago. We certainly have better tools to characterize cells and to characterize products. We have a greater experience with the various different cell lines, including a lot of experience from the veterinary field, as well as the biotech therapeutics field that the vaccines, human vaccines field, can trade on.

And finally we really do need to support the development of HIV/AIDS vaccines as well as improved influenza virus vaccines, particularly those that would be necessary to prevent a pandemic. And so it is because of these things that it has really driven us to move forward and reconsider what has historically been a reticence against using certain types of cells.

We know now that tumors arise from genetic changes that result in inappropriate expression or knock out of what started as a proto-oncogene and might get turned into an oncogene. In other words, cancer is in the DNA. Viruses that cause cancer often express proteins that interact with cellular oncoproteins or which are themselves oncoproteins. And so again, it is in the nucleic acid of the virus as well that encodes these oncoproteins. We also know now how to more carefully measure cell residuals in products. For example, how to measure host cell nucleic acids and host cell proteins, which in traditional vaccines were not measured. We have improved methods for detection of viruses and viral nucleic acids, and we also have improved purification processes to remove or destroy cell residuals in viruses that might have been present. And I should add that we now operate more in an era of current good manufacturing practices, which was not in place in the 1950s and 1960s.

So what about HeLa cells? HeLa cells derived from a woman with cervical cancer and were established as a cell line in the 1950s. And we know that the cause of that cancer was Human Papillomavirus type 18. The nucleic acid remains of this virus in HeLa cells are the cause for the cellular transformation in culture, in other words, the immortalized growth. And this is known because you can use RNA to silence the expression of these genes, and you will revert the phenotype. And I have a reference at the end for that paper.

The cell transformation can be reversed, and so we really do have a handle on why these cells formed a tumor in the first place. And in addition, in terms of product manufacture, they grow readily in culture and they are easily engineered. So they actually are easy to transfect with nucleic acids that you want to uptake, and express a foreign gene. There are many cell lines that are very refractory to that kind of cell transfection.

So of course, as Dr. Krause mentioned earlier, we have to consider not just the cell line but its use, and what kind of product we are trying to develop. AAV vectors, or adeno-associated viruses, can be engineered by entirely removing the viral protein genes and simply using the ends of the virus, of the nucleic acid, to insert your foreign gene. So essentially you are getting rid of all of the viral parts and just keeping the framework and inserting your gene.

But when you do that, you can't make a virus any longer. And so the only way to encapsulate that nucleic acid to use it as a viral vector is if you provide the viral capsid gene in trans. And what that means is that you have to put it into the cell line, so that because it is no longer being expressed from the virus it now has to come from the cell line. So you need a packaging cell line.

Also, you can only replicate that viral vector in the context of the viral replicase gene and other needed genes that are provided from the cell line. So when we have to consider alternatives, what are the alternatives to using HeLa cells. So efforts were undertaken by the vaccine champion, and funded actually by our agency to establish a packaging cell line based on vero cells instead of HeLa cells.

And while that was successful, there are several issues that arose that made it apparent that this was really not the optimal way to move forward. And that is because in part it requires a change from the human helper virus, which we have quite a bit of experience with, to a simian helper virus. And use of that virus for a helper may introduce unique safety concerns that really we have much less handle on than the human virus we have more experience with.

In addition, the yield in manufacturability of AAV on vero cells has not been established. And the robustness of that packaging cell line for commercialization, in other words, scale up to a commercial scale or even for clinical trial manufacture, is undetermined.

Another alternative that might be considered is 293 cells, which you have heard a little bit about today. And this won't work because the E1 gene that is already present from the adeno type 5 virus actually will interact with the AAV rep gene and interfere with production. So HeLa cells were chosen, and like all cell substrates they had to be characterized. So they were engineered to have the necessary equipment to be a packaging cell line, and they were amplified to establish a master cell bank. Actually, several cell lines were developed by the manufacturer I am speaking regarding, which is targeted genetics, which is unfortunately no longer in business.

But these banks, as well as their end of production passage level cells, were thoroughly characterized prior to use in production. They were tested for identity, first to establish that they were actually human cells, then to demonstrate that they were actually HeLa cells, and finally to distinguish between the other HeLa cell lines that were used to package other vaccines that the manufacturer was also making.

And then they were also sequenced to show that the engineer inserted gene was the correct gene. They were also tested thoroughly for adventitious agents. And so essentially this is the same thing that would be done for all cell substrates. All the usual tests were done and they were all negative. I am not going to read the list to you, but you have it in your slides.

In addition to these usual tests that would have been done, the manufacturer did do additional tests, including doing a retrovirus test under induction conditions, looking for bovine

and porcine circoviruses which at the time the banks were originally established was not routinely done, but after the contamination of the vero cells that was added later and was performed. And then they used a test that is normally done on rodent cell lines, even though this is a human cell line, and so they looked for potential murine contaminants that may have come in from raw materials or something of that nature. All of these were also negative.

They performed tests for tumorigenicity on two master cell banks and a bank that was established that represented the end of production passage level. And this is reported in a manuscript that I will also cite at the end of this presentation. And they did this not in sort of the usual way that would be done for diploid cells, but rather in a manner to actually establish a tumor producing dose because they knew that the cells were tumorigenic, being that they were from a tumor.

They use culture media as a negative control, and they used another human tumor cell line as a positive control, and they did this dose ranging from 10 cells to 10 to the seventh cells per mouse, using 10 mice per group. And the TPD values were thus established, and what they showed was that their HeLa cell banks had TPD values that were right in line with what FDA had previously reported from their own experience with tumorigenicity testing of HeLa cell lines, which is on the order of a little less than 10 to the fifth.

They also did tests for oncogenicity including lysates, and in a moment I will tell you about the DNA. The purpose of testing cell lysates is, when a cell is lysed it has a potential to release theoretical oncogenic or latent agents that -- and I say theoretical because they are theoretically present in the cell and the test is there to find them.

They tested more than 10 to the seventh cells in newborn rats and hamsters. And these were followed for five months. They used a large sample size and no tumors were observed in any group. They also tested the cellular DNA more than 200 micrograms, I think as much as 400 micrograms per animal was injected into 30 animals per group and followed again for five months.

The four groups received either a negative control, which was buffer, a diploid cell DNA, which was used as a cellular negative control, the master cell bank, or an end of production passage level cell bank DNA. Again, no tumors were observed in any group.

Further, they did a risk assessment for TSE agents. And the purpose of that was that because HeLa cells were established quite a long time ago and there was this legacy where the bovine serum use was undocumented prior to the manufacturer actually establishing the cell banks.

And documenting serum use in accordance with CGMP, they made a theoretical calculation assuming that the cell line was exposed. So this is really a theoretical risk assessment approach. And then they calculated the dilution factor that would occur had the cell line actually been exposed. And I will tell you in a moment the results of that analysis or calculation.

In addition, because it is theorized that the cells which express a normal human gene that is the precursor to prions, whether or not that might convert. And there are certain mutations that are associated with familial disease of that nature. In addition, they did a Western block to look for that protease resistant form, which is considered to be the harbinger of the disease form.

So the results of this risk analysis is that they calculated the risk to be less than one infectious dose of a BSE agent per 10 to the 19th doses of vaccine, had there actually been a BSE exposure and legacy. In addition they found no protease resistant PRP protein, using an antibody that would have detected by human TSE and BSE. And I have listed here the limits of detection of that method. It is not a particularly sensitive method. Unfortunately, it is really the only tool we have available, because there is not a validated and sensitive method to test for this. In addition, they showed that the sequence was normal at the known mutation points and that the cell line was heterozygous in that it had both a normal full length gene as well as a gene that was slightly truncated at a known, octopeptide repeat region which is variable. So that region is known to vary. So this is essentially normal.

Again, we have to take the cell line in the context of the product that is being manufactured, and so the product is also characterized for residual cells and other cellular residuals, as well as the viral residuals that are present in the cell. Because the production process uses a helper virus, they also test for residual helper virus, and then the normal sort of bio-safety parameters that are performed for products.

The residual host cell DNA from a preparation of vaccine was assessed by PCR for the E6, in other words, the Human Papillomavirus that was the cause of the transformation. And the lower limit of detection of that assay is on the order of 25 copies per mill. So the total cellular DNA that was measured was essentially measured as 60 picograms per human dose of vaccine. And this is below the sort of limit that was originally discussed by WHO and which Dr. Krause presented earlier.

*In addition, because they use benzonase, which is an enzyme that digests DNA in the manufacturing process, the E6 gene fragment residuals that might have been present were essentially unmeasurable. They were below the level of detection of the assay. So essentially, whatever was there from the cell substrate was essentially digested to unmeasurable levels.

In addition, they looked at cell clearance and the manufacturing process included six different filtration steps. So the manufacturer calculated the removal clearance to be on the order of 10 to the 22. And since a single dose of vaccine is derived from 10 to the eighth cells, this gives what they calculated to be a 10 to the 14th fold margin of safety per dose.

Likewise, they assessed viral clearance. So even though they are purifying a virus, they do have the capacity to clear other viruses from the product. And they tested particularly for the helper virus, which is the Adenovirus Type 5. But they also looked at model viruses, which include bovine viral diarrhoeal virus and Simian Virus 40. So the Ad5 is a relevant virus and the other two are considered to be model viruses, both of which are small viruses that would be relatively resistant to clearance. And they looked at one that was both enveloped and non-enveloped.

So I have told you all about how they have assessed both the cell line and the product to address the risks. So really how does this differ from cell substrates that are already in use? One of the ways that it differs is that the cells do actually cause tumors in animals at doses less than 10 to the seventh cells per animal, which is different from, for example, MRC-5 cells that don't cause tumors, or something like 293 cells which does cause tumors but only really at that high dose.

In addition, the residual cell DNA could contain unmeasurable amounts of oncogene DNA. In other words, the E6 and E7 genes, derived from the Human Papillomavirus. But this DNA would be degraded to sizes less than that of an intact gene. And in that way, it is felt that the risk is mitigated.

So then why should we consider this to be safe or suitable for vaccine production? We should consider it that way because the product purification processes do remove intact cells with a large margin of safety. The processes also degrade residual cellular nucleic acids and purify away other cellular residuals.

The processes are capable of removing model viruses with a significant margin of safety, had there actually been any undetected viruses present. In other words, the processes could have removed viruses had we missed any adventitious agents in the testing. The final product does not contain HeLa cells, and the majority of cellular residuals are processed away very similar to other biological, for example, monoclonal antibodies. And significantly more thoroughly than some of our traditional licensed vaccines that were made in primary cells or diploid cells, and which are essentially minimally purified.

So in conclusion, the AAV vectored vaccines can be successfully made in an engineered packaging cell line that is based on HeLa cells which have been banked and thoroughly characterized as well as the products being thoroughly characterized. Such vaccine candidates may be safely given to humans.

And while the clinical experience with these vaccines is limited, there is much more experience with therapeutics based on this AAV approach. But the clinical experience with the vaccines in healthy adults is limited thus far to about 150.

So in summary, cell lines derived from human tumors can be banked and well-characterized before they are used in product manufacture. Such cell lines propagate well in culture, and often they are more readily genetically engineered than would be some of the diploid cell lines. And they will support the growth of viral vectors that require either genes for complementation or genes for packaging.

So this capability can mean the difference between successful production of a viral vector and the inability to produce it. And these viral vectors and vaccines have the potential to fill unmet public health needs, but they have to be able to be manufactured and tested in the clinic before that potential can be fulfilled.

Such well-characterized cell lines, like other non-tumor derived cell lines, can be safe and suitable cell substrates for the production of preventive vaccines. And in fact, NIAID does support several projects using human tumor cell lines, including the project for biodefense that you have heard about and a potential project for HIV vaccine which you have also heard about earlier this morning using the A549 cells from PaxVax.

In addition, we are supporting and/or collaborating on two other AAV vector projects, the purpose of which is to use those vectors for gene transfer to essentially passively immunize, if you will. In other words, instead of encoding an antigen they are being used to encode an antibody. And those antibodies express broad cross neutralization against HIV. So we need to acknowledge all the people who really have seen this project through over the years, including my team leader who is the program officer for the project, Dr. Michael Pensiero, the vaccine champion Dr. Phil Johnson, and the Regulatory Affairs director who was formerly at Targeted Genetics, is now at Bill and Melinda Gates Foundation, Pervin Anklesaria.

Also, some of the initial funding for the original HeLa cell line which is in the publication was funded by IAVI, and we have had funding with Dr. Johnson in Targeted Genetics through an HIV vaccine design and development team. And here are the references, which I will leave up while I address any questions the committee may have.

DR. DAUM: Thank you very much, Dr. Sheets. We are a few minutes over, as most of you are probably aware. If there are one or two burning, clarifying questions we can take them. If not, we will adjourn into a break.

DR. COFFIN: Two quick ones. Is any transcriptome or massively parallel sequencing analysis being done?

DR. SHEETS: So I have to give a little historical context. At the time this project was initiated, which was more than a decade ago, that was not a widely-available technique. And at the time that the vaccines were manufactured for our program, and at essentially the time that Targeted Genetics went out of business, while that technique was becoming available, essentially we ended up with a product that no longer was moving forward because of the company going out of business. So that technique was not undertaken.

DR. COFFIN: But going forward, do you think it should be done?

DR. SHEETS: I think that is a matter of discussion, whether or not -- certainly there is a lot of information that comes out of that technique. It is definitely useful for characterization. I think the value in terms of quality assurance for products has yet to be established. There is really not a lot of baseline data, although that is certainly changing day by day.

And I think in the near future we will have a better handle on whether or not that technique is really broadly applicable for the characterization of cell lines. So I think we are maybe on the cusp of being there, but we may not quite be there yet. But certainly it is something that one should consider.

DR. DAUM: And as Chairman, I would point out that we would like you to revisit that this afternoon, and offer your opinion, and other members of the committee as well as to

whether that should be part of the evaluation.

DR. COFFIN: It seems like a good time to get her opinion on this topic as well, though.

DR. SHEETS: I think the current status, though, is that that approach, generally when you think about product characterization and cell line characterization, you have to think of it in terms of quality assurance of a product. And so, quality assurance requires a high standard of rigor, including validation of the method. So I think the validation is perhaps not there yet. But it is in progress, I think it is going to be a bit difficult to validate. But certainly I think that is being undertaken.

So I think that is part of the reason it hasn't really fully been firmly established as a method at present, is its validation status.

DR. COFFIN: Quickly, is the status of other potentially cooperating oncogenes such as ras and myc and so on in these cells known?

DR. SHEETS: I know that the E6 and the E7 genes will bind to and interrupt the function of both RB and P53. I am not thoroughly versed with the oncogenes you mention, in this cell line.

DR. DAUM: We are going to have one more question from Dr. Cheung and then we are going to have a break.

DR. CHEUNG: So obviously bovine serum is used for a lot of the tissue culture. And I think you raised a very good point about BSE, which is a disease of latency and potentially took a long time to develop. Have you looked at using serum-free media in culturing the HeLa cells?

DR. SHEETS: That is a good question. And in fact, a lot of the projects that we support are moving towards growth in a serum-free condition, or in an animal protein free condition. Not all cell lines can be grown without serum. So while there are improved, chemically-defined media that are available for culture, not all cell lines do well in the absence of serum. So serum will continue to be used in the production of vaccines for the foreseeable future.

But certainly manufacturers are looking towards whether or not they can establish their cell line for growth in media that do not contain serum. One of the issues with that is that often the growth kinetics and growth characteristics of the cell line, including some of these in vitro and characteristics in animals such as tumorigenicity, may be altered by not having the presence of the growth factors that come from bovine serum.

DR. CHEUNG: Can you comment specifically on serum free growth on the HeLa cells, as opposed to all the other cells?:

DR. SHEETS: I am not in a position where I can answer with regard to this particular company's manufacturing process. I think in general cell lines like HeLa cells can be grown in a serum-free mode, because they do grow more readily than some of the, for example, diploid cells. So MRC-5 cells probably really do need the serum, whereas some of these human tumor cell lines could be adapted for serum-free growth, and thus I think that is another way you are mitigating the risk.

DR. DAUM: Thank you very much, Dr. Cheung. Thank you very much, Dr. Sheets. The committee will now take a break.

(Housekeeping details)

(Brief recess)

DR. DAUM: So there are now three presentations on this subject between us and lunch. The first one is by Dr. Keith Peden, the chief of a long series of acronyms, LDNAV, DVP, OVRP and CBER. His topic is tumorigenic cells and cells derived from human tumors: DNA is a potential risk. Welcome, Dr. Peden.

Agenda Item: Tumorigenic Cells and Cells Derived from Human Tumors: DNA as a Potential Risk Factor.

DR. PEDEN: Thank you. My charge today is to discuss this tumorigenicity of cells and cells derived from human tumors, specifically with emphasis on DNA as a risk factor.

What we really want to discuss today is what factors from human derived cells are a concern. And I think we can help this by discussing the mechanisms by which cells become neoplastically transformed and how that information might inform us as to the potential risks of using such cells. And as you heard earlier on from Dr. Krause, adventitious agents, especially oncogenic viruses and oncogenes, both viral and cellular, could be involved in that. And then the second part of the talk would discuss approaches taken to address residual DNA from the cell substrates. We mentioned a bit about tumorigenicity assays, oncogenicity assays with DNA, and reduction in the amounts and size of DNA.

So the concerns with the use of tumorigenic cells or cells derived from human tumors, really, as Phil Krause said, the possible presence of tumorigenic cells in the vaccine, the presence of adventitious agents, particularly oncogenic viruses, and oncogenic materials derived from the cell themselves.

I want to discuss what potential mechanisms there are for neoplastic transformation. And can understanding the mechanism whereby cells become neoplastically transformed assist us in identifying concerns and also developing risk mitigation strategies. So there are several known mechanisms for neoplastic transformation. There is transformation by oncogenic viruses, there are genetic changes in the cell and the cell genome, and epigenetic processes.

First of all, I would like to discuss the transformation by oncogenic viruses. Viruses were known to cause cancer in animals many years ago. And the Rous sarcoma virus of chickens, Shope Papillomavirus in rabbits and mouse polyoma viruses were among the first oncogenic viruses described.

Once the viruses were known to cause cancer in animals, it only became a matter of time before they were discovered in humans. And now we have several viruses listed down here that are known to be involved in tumor induction in humans -- EBV, HTLV-1, Hepatitis B virus, Hepatitis C virus, Human Papillomavirus, and two viruses that the next speaker, Dr. Moore, was intimately involved in identifying -- HHV-8 and MCV.

So how do oncogenic viruses transform? Well, they can transform by direct conversion through the activity of dominant oncogenes. And in DNA viruses, the Papillomavirus uses the oncogenes E6 and E7 mainly, and adenoviruses E1A and E1B. And in the case of retroviruses, acute transforming retroviruses, they have their own oncogenes. For example, MC29 as v-myc and Rous sarcoma virus as v-src.

There is another way of transformation by oncogenic viruses and that is neoplastic conversion through insertional mutagenesis. And this could be through the activation over dominant proto-oncogene, the cellular oncogene, by replication-competent retrovirus. For example, avian leucosis, sarcoma virus in chickens induce leukemias and sarcomas in chickens. And Murine leukemia viruses obviously induce leukemia in mice.

But they are also an example of replication defective retrovirus inducing tumors and the most visible example of that was using a gamma retrovirus vector to induce leukemias in two gene therapy trials in London and Paris, and for the human SCID-X1 defect. And the oncogenes found to be involved in that were the LMO-2 oncogene and CCND2.

In addition, retroviruses and viruses can disrupt human suppressor genes, and the example in mice is a friend leukemia virus, which inserts into the tumor suppressor gene p53. But in the absence of haplo-insufficiency, inactivation of tumor suppressor genes requires inactivation of the other allele by genetic or epigenetic means, termed loss of heterozygosity. So this mechanism is far less probable.

However, even tumor induction by acute oncogenic viruses requires additional oncogenic events. The best example is the Human Papillomavirus, where even infection with a high risk of Papillomavirus types is not sufficient to induce cervical cancer. Mutation in other genes and perhaps also epigenetic events are required. If a single infection-induced cervical cancer after infection with HPV, say, 16, then teenagers will be getting cervical cancer. And as we know, cervical cancer is a disease of older people.

Also, induction by leukemia over the acute transforming viruses requires additional events. And this is all consistent with the multi-stage nature of carcinogenesis that we have come to understand. So the second neoplastic transformation, genetic changes.

So what are these? Well, they are activation of proto-oncogenes, for example, K-ras and c-myc. They could be activating dominant proto-onc genes by chromosomal rearrangements, translocations, et cetera, so the expression is elevated after following chromosome rearrangements, similar to what Dr. Coffin was mentioning earlier on.

And tumor suppressor genes can be inactivated followed by loss of heterozygosity to eliminate the function of the other allele. But in addition, tumor suppressor genes can be mutated to induce gain of function activities. And these produce dominant oncogenes that interfere with the normal protein.

The third mechanism that is now known to be involved in transformation is through epigenetic processes. So what are these? These are changes that do not affect the DNA sequence. However, the effects need to be heritable for somatic cells. In other words, the changes that are induced in those cells need to be retained on cell division. Otherwise, the effect is lost.

So the processes are the chromatin structural alterations through histone modification, and there are a number of histone modifications to the end terminal tail of histamines, acetylation and methylation are the most common ones. The other major mechanism of epigenetic processes is through DNA methylation. And the cytosine nucleotides are methylated and predominantly at the CpG dinucleotide.

And in many cancers, global hypomethylation occurs, under-methylation, and that leads to genomic instability, as has been reported. But also, the selective hypermethylation of promoters. And these are often in tumor suppressor genes to silence those genes.

So next I want to discuss how the mechanisms of this transformation can assist in developing risk mitigation strategies. So it depends on the mechanism of transformation. For example, mutations, chromosome rearrangements, translocations, retrotranspositions, et cetera, all involve DNA. Therefore, reducing the size and amount of the DNA should mitigate this risk. And as far as epigenetic processes occurs, to our knowledge there is no known mechanism for transmitting this through vaccines or other ways. And we would be interested to hear any comments from the committee on this.

And adventitious agents, of course, depends on detection and clearance strategy during manufacture, and following Dr. Moore's talk, Arifa Khan will present more information on this.

Now I would like to just say a bit about the history of how OVRR/CBER moved forward in allowing the use of tumorigenic cells for vaccine manufacture. As Dr. Krause gave a very comprehensive history, but this is the summary of how we have allowed them to go forward. We have used extended tumorigenicity assays following recommendations, in fact, by Dr. Andrew Lewis.

And we have also involved in oncogenicity assays with both DNA and cell lysates. Dr. Khan will talk about the cell lysates, and recommending a reduction in amount and size of residual DNA.

So the extended tumorigenicity assays -- how do we assess the tumorigenic phenotype of cells? As you probably know, assessing the tumorigenicity of cells is actually required by the Code of Federal Regulations. And there it is described, the cell lines used for the manufacture of biological products shall be, quote, described with respect to cytogenetic characteristics in tumorigenicity. So therefore it is supposed to be done.

So what is a tumorigenic cell? It is actually any cell that can form a tumor in an immune-compromised animal. And usually this is a rodent. So how do we assess that? Most cell substrates derive from species other than a rodent. This means that the animal must be defective in T cells, and so allow a xenograft to survive in that animal.

There are two basic types of assays that have been used historically, single dose assays where a 10 to the seventh cells are inoculated subcutaneously in PBS. And these assays are ready to decide whether the cell is tumorigenic or it is not tumorigenic. For example, if you

are assessing the tumorigenicity of a vero cell, that you are maybe concerned that it has become tumorigenic, this is the assay that people do.

Now we have extended the time of these assays in addition to the dose-response assays to more than four months. Four to seven months, I think, is in the guidance. In addition, the second type of assay is dose-response assays. And Dr. Becky Sheets talked about that. Actually, Dr. Mayall did, too, where cells are inoculated at various doses to determine something about the degree of tumorigenicity of the cell. And tumors can be formed within days or weeks for highly tumorigenic cells. But it may take up to a year for weakly tumorigenic cells. So tumorigenic cells can be determined by dose-response assays to quantify the phenotype. And again, cells are inoculated in amounts from 10 to the seven down to 10 to the one. And they can be inoculated into adult and/or newborn nude mice. Newborn is more sensitive than the adult. And tumorigenicity can be presented as the tumor-producing dose at a 50 percent endpoint, as again, Dr. Sheets pointed out.

So what are the possible advantages of the quantitative tumorigenicity assay? Well, it provides a comparison between different cell lines. And it might reveal problems with the cell, if an aberrant or unexpected dose response relationship is observed, for example, the presence of adventitious agents, bacteria, viruses, are known to distort the dose-response relationship. So the assay can provide some useful information.

It also can provide information if, say, for example, the TPD50 was greatly lowered after some manipulation. That might also indicate some concern. However, there are limitations with tumorigenicity assays in general. Not all cells derive from tumors, establish tumors in the nude mouse or any other system.

Tumorigenic cells can be made more tumorigenic by the inclusion of Matrigel. This is a matrix derived from extra-cellular matrix of cells, and mixing tumorigenic cells with this Matrigel, commercially available, in fact lowers the TPD50 of cells. So you can make your cell more tumorigenic by a simply inclusion of this matrix.

More importantly, though, highly tumorigenic cells can be made non-tumorigenic by the ectopic expression of a single protein. Now there are many papers in the literature on this. For example, HeLa cells expressing protein zero, P0, which is a neural IgCAM, become non-tumorigenic. And expression of Connexin 43, which is involved in establishing tight junctions between cells, confers a non-tumorigenic phenotype on highly tumorigenic cells, of which HT1080 is an example.

In addition, in fact, A549 has been made less tumorigenic by the expression of a single normal protein as well. So whether a cell forms a tumor in a nude mouse, it is not the sole consideration as to its appropriateness as a cell substrate for vaccine manufacture. So I think we need to think about the limitations of this assay.

So how have we moved forward? Now I will discuss oncogenicity assays with DNA and cell lysates. So the testing of DNA and lysates from cell substrates, this was cell lysates from 10 to the seven cells are inoculated subcutaneously in newborn nude mice, newborn hamsters, newborn rats. And Dr. Khan will mention more about this.

Cell substrate DNA of more than 100 micrograms in PBS is inoculated subcutaneously into the three rodents, again. And animals are followed for more than four months for induction of tumors. And if tumors are found, then the following is recommended. The location of the tumor is noted, if not at the site of inoculation.

The species of the tumor is determined. This is particularly important for cell lysate assay, because the cells, the residual cells may be present in the lysate, and so the species of the tumor will therefore be the cell substrate species and not the rodent species.

And the presence and species of the inoculating DNA is ascertained to show that the tumor was induced by the inoculated material. And there are tumors we suggest that are retained for additional analysis should that become necessary.

So what was the rationale for recommending this? The three species detected a different spectrum of oncogenic viruses. If you peruse the literature, as Dr. Lewis did, these are the three species that detected as wide a range as possible of oncogenic viruses. And the recommendations were originally intended to detect oncogenic viruses in cell lysates. Because no standardized assays were available to test oncogenic DNA, these systems were extended to DNA testing, as members of this committee might remember, because in 2001 the introduction, as Dr. Krause talked about, of the PER.C6 line, these assays were used to determine the oncogenicity of DNA and cell lysates.

And in general the VRBPAC concurred with these recommendations. What we really don't know is, are these rodent systems appropriate? And part of our work is to try to determine that. I won't give you an answer to that today.

So now DNA. The biological activities of DNA, as Dr. Krause mentioned, has an oncogenic activity, which means it can induce tumors in animals. It has an infectious activity. In other words, if you induce, if there is a viral genome present in that cell DNA it could be inoculated and express a virus. So it could be a retroviral, pro-viral copy, or a DNA virus in the presence of that cell substrate.

First of all, I will discuss oncogenic activity. The consequence of DNA integration could be the activation of a cellular oncogene or the inactivation of a tumor suppressor gene, as I mentioned for the retroviruses earlier on, or the introduction of a dominant activated oncogene. But I wanted to dismiss the potential for insertional mutagenesis because integration of naked DNA as a contrast to a virus into the cell genome is extremely inefficient. Whether this is continued in both uptake of the DNA, traffic to the nucleus and integration, it is an extremely inefficient process. And also, in the absence of activated oncogenes in the cellular DNA, there will be little difference between the DNA of a diploid MRC-5 cell or the tumor-derived HeLa cells for integration purposes in the absence of activated oncogenes.

And plasmid DNA vaccines have been administered at levels of five milligrams of plasmid per inoculation. And these plasmid DNA vaccines have an extremely active promoter and enhancer. So therefore we think that the major oncogenic activity is really the presence of activated oncogenes.

So how do we measure this? Well I just want to go over some of the numbers that I have done on previous occasions in this meeting. How do you measure the biological activity of mammalian gene DNA? So to make the arithmetic simple, a single copy mammalian gene is somewhere between 3,000 and 30,000 base pairs in size.

Haploid mammalian genome is about three times 10^9 to the nine base pairs. So therefore a single copy gene is 10^5 to the five to 10^6 to the six, 100,000 to a million fold less abundant for the equivalent amounts of cellular DNA, as compared with a plasmid expressing that same gene.

So what does that mean? That is, the amount of mammalian genomic DNA equivalent to one microgram of plasmid DNA, say for example, one microgram of plasmid DNA elicits an effect in an experimental system, to find the same concentration of that gene in mammalian DNA is one times 10 to the five to one time 10 to the six micrograms, which is 0.1 gram to 1 gram of DNA. This should point out that the problem of understanding what the risk of DNA is when you are just considering mammalian DNA, how you measure this activity.

So how do we get to do this? Well, we wanted to establish a sensitive assay to detect the activity we are measuring, use the assay to quantify the activity to estimate the safety risks based on conservative estimates, and use the assay to quantify the reduction in DNA activity afforded by various treatments. And for the vaccine industry, where they use chemical and nuclease digestion, et cetera.

So our sensitive model system, first of all, with various consultants, one of whom is in this room, it was suggested that the human activated T24 Harvey-ras gene and the murine c-myc gene, will be appropriate. Since in mice, these two genes convert normal mouse cells into cells that can form tumors. And the statement was made, if these two genes don't work then nothing will.

So the promoter was used with the murine sarcoma virus, long-terminal repeat, and the mice were tested, newborns and adults, of immune competent mice and immune deficient mice, and cancer prone. Because we didn't know what strain of mouse would be sensitive, and so we needed to try various mice to identify a sensitive indicator mouse.

So the plasmid we are using to screen lots of strains, is this plasmid right here. This is a dual expression plasmid for the human T24-H-ras and the murine c-myc. And we made it dual because the two single plasmids that we published and found to be active were inefficient. And so we thought that if you combine the two genes on the same plasmid, one cell will take up both genes. And it turned out to be a 20-fold more oncogenic than the separate plasmids.

So this is the one, in yellow is the c-myc and in red is the T24-H-ras. And they are driven in their own cassettes by the LTRs, which are in blue, as you can see in this slide. So I am only going to mention the CD3 epsilon mouse, since this mouse was mentioned by the first presentation from industry.

This mouse carries 30 copies of the human CD3 epsilon gene, and for some reason it lacks functional T cells and NK cells. I am not sure when they made that mouse that was the intention, but that's what it turns out to have. And importantly for us, we obtained a colony of these mice, because it is more sensitive than the nude mouse in tumorigenicity assays.

So we tested it for the oncogenicity assays. And so what we did, we compared linear versus circular DNA of this dual plasmid in newborn CD3 epsilon mice. In this graph here you can see, on the Y axis is the animals with tumors, and on the X axis is the dose amount. And in blue, the DNA was inoculated as circular and in red, it was as linear.

As you can see the linear DNA was more active than the circular by about two orders of magnitude. We were quite surprised at this result at the time, but it turns out that we were just reinventing the wheel since several people had shown that linear DNA was more oncogenic for polyoma virus in mice. But importantly, this mouse can detect oncogenic activity of 800 picograms, so about a nanogram of DNA. So this is clearly the most sensitive mouse that we had identified at that time.

Importantly, can this mouse detect oncogenic activity of cellular DNA? We tested DNA from four human tumors in this mouse. Fortunately, these are the three cell lines that were discussed earlier today. In addition, we used HT-1080 because in our hands this is one of the most tumorigenic cells that we found, far more tumorigenic than the three above. So we thought if there is some correlation with the tumorigenicity of the cell and the oncogenicity of its DNA, this might be a good system to find that out. Unfortunately, DNA from the tumorigenic lines failed to induce tumors.

So again, on the Y axis is the amount of mice with tumors. And in colors, you will see HeLa cells, CEM, A549, HT-1080. And none of these DNAs induce tumors. So that is why it is blank. And we inoculated 100 micrograms of cell DNA.

So to test whether these DNAs were in fact inhibitory, along the bottom here now we have plus and minus. This is one microgram of the linear ras/myc dual expression plasmid. So clearly a negative result wouldn't be interpretable if it is just inhibiting the activity.

So as you can see, it didn't inhibit the activity, and each of those bars, HeLa cell, CEM, A549 and HT-1080, 100 percent of the animals came down with tumors in the presence of ras/myc. Zero in the absence of the ras/myc. And just to show you we did some experiments, here are the numbers up here. Fourteen out of 14 in HeLa cells with the ras/myc, and zero out of 55 without the ras/myc. Zero out of 55 for CEM without ras/myc, zero out of 53 for A549 without mix. So you get the message. So tumor DNAs, even in this very sensitive mouse strain failed to induce tumors.

We looked at HeLa cells because HeLa cells, as Dr. Sheets pointed out, we know at least the initiating events in oncogenesis for that, and it was the HPV 16, 18 and E6 and E7. So the question was, would these oncogenes score positive in this assay?

We had obtained years ago, actually from Dr. Lowy's lab, E6 and E7 from high risk and low risk HPVs. And in this experiment we can see that the ras-myc in red induces tumors in the presence of, actually in this case, calf thymus DNA as a control. But neither the low risk nor the high risk E6 and E7 induced tumors in mice.

We replicated this many times in different species, too. And these HPV E6 and E7 do not induce tumors. So the question was, is there another oncogene that can help? So we tested H-ras and c-myc and we found that yes, you can get complementation only by H-ras in blue but not in lane C, as you can see, with c-myc.

So therefore these oncogenes do not score positive in the CD3 epsilon mouse, so it is no wonder the HeLa cell DNA would not function anyway. And again, I will show you how many mice we inoculated. So then we tested the oncogenicity of HeLa cell DNA with complementing oncogenes. However, neither c-myc nor H-ras -- H-ras in A, c-myc in B and H-ras plus c-myc in C -- neither oncogene could complement the HeLa DNA for tumor reduction in a CD3 epsilon mouse. Again, the numbers.

Let me summarize that. Even in our sensitive rodent system, no cellular DNA has induced a tumor. Not all oncogenes score positive in the assays. So the ras family members are active, but other oncogenes might not be.

I haven't got time to show you the data but it turns out in the newborn rat and in the newborn hamster, in fact H-ras alone is active at lower levels, and c-myc is never active. So it is possible that these types of assays will only measure the oncogenicity of a certain group of oncogenes, and perhaps ras family members may be the only ones.

So these really do suggest a limitation with the in vivo DNA oncogenicity assays. Finally, recommending reduction in the amounts and size of DNA. I remind you that the cell substrates and WHO recommended DNA limits. In primary cells and diploid cells there are no limits for the amount of DNA in vaccines. And for continuous cell lines, as Dr. Krause mentioned, the parenteral vaccines now have less than or equal to 10 nanograms per dose. But for oral vaccines that are non-encapsulated, in other words like the rotavirus vaccine they are drunk, so they are not encapsulated and go through the stomach, the stomach is the place where the DNA is degraded, the dose is less than or equal to 100 micrograms per dose.

So we look for an assay to monitor DNA infectivity. So what we did, we had this little assay here that you transfect HIV proviral clone with a transfection facilitator into 293 cells, which are highly permissive for transfection, and then we co-culture them in these little red dots here with jurkat cells. These are cells, T cells, they are permissive for HIV replication, much like CEM cells or A3.01 cells, we heard earlier on.

And this co-culture transfers the virus made from the initial transfected material to the jurkat cells, and then in the jurkat cells they amplify. And you can detect the amplification of

the virus by RT activity or syncytia on the cell. So this turns out to be a sensitive way to detect the activity of HIV DNA.

And I just show you this figure. And on the left here, the LAI strain which is highly cytopathic, you can see doses from 10 nanograms in cyan and all the way up the chart up here to one picogram in the wretched pink. And so there is a dose-response relationship. So this assay can detect the biological activity, the infectivity of one picogram of DNA. But the slightly less cytopathic on the right, AD, you can detect three picograms of this DNA. And this is highly reproducible.

Therefore we have an assay now to measure how much, the way you can clear the DNA. And nuclease digestion, chemical treatment and irradiation are commonly used with vaccines. So I am going to show you just the DNA digestion on this slide. On the left-hand side of the slide you can see zero minutes, and then increasing time with benzonase digestion.

And along the bottom you can see, in the parentheses there, these are the fractions that did not contain any activity. So we assayed each fraction of the DNA and then tested in the assay I showed you in the previous slide. And it turns out when you get below about a median size of about 650, the activity is lost. So you can use how much DNA you have added and how much DNA you end up with and how much you can test, to calculate how much clearance, reduction activity.

So let me summarize that. We can detect one picogram of an infectious DNA -- this is 10 to the five molecules, it translates. You can also detect DNA from HIV infected cells at a level of two micrograms. And digestion of DNA with DNA is one, or treatment with BPL, I didn't show you that one. But it can reduce the activity of DNA by 100,000 fold or more. It can do more if we just let it go longer, and just measure the smaller fragments. So that is the minimum estimate we can get.

However, if you combine that with the amount of DNA, for example, less than or equal to 10 nanograms per dose, we can get safety factors of more than 10 to the seven, which have been considered adequate. So I would like to make some concluding remarks. I have told you that understanding the mechanism whereby a cell becomes neoplastically transformed can assist us in determining the risks of using that cell.

For example, activation of proto oncogenes or viral transformation, then DNA is the issue, and DNA clearance can be used to mitigate these risks. For epigenetic mechanisms, as far as we can understand there is no known mechanism for transfer of oncogenic activity. So tumorigenicity assays that I have shown you have limitations. Not all cells derived from tumors are tumorigenic in nude mice, and tumorigenic cells can be made non-tumorigenic by the expression of a single cellular protein.

So the limitations of DNA, of the in vivo DNA oncogenicity assays, not all oncogenes induce tumors. Oncogenes often become silenced by passage of cells in vitro. In fact, for HeLa cells it is well known that even though they have approximately 50 copies of the HPV 18 genome, most of those copies are silenced. And in CaSki cells, which have somewhere between 60 and 600, most of those genes are silenced, too. Therefore, if we are looking for genes, active oncogenes, they might not be able to be expressed.

Importantly, no system has detected oncogenic activity or cellular DNA. We have used cell lines derived from the ras/myc tumors, induced tumors. And so you would expect those cells and that DNA from those cells would be the most likely to induce a tumor. So far, we haven't obtained a tumor from that type of DNA.

Importantly, though, a negative result would be difficult to interpret. So if you are only measuring a subset of the oncogenes, then a negative result might give you a false sense of comfort as you see with the CEM and A549 and HeLa cells. But that may not be interpretable because of the other reasons I have mentioned above.

Therefore, I think because of these limitations, documenting DNA clearance below a specific level, and demonstrating that its size is reduced to below a defined size, might turn out to be the preferable approach to address concerns with DNA.

Now many people know that I have been working on these assays for years, people in this room. And I am not so sure that the DNA oncogenicity assay is really telling us what we want to know. And so therefore I think this last point here is a very important issue with respect to DNA. Thank you.

DR. DAUM: A lot of information in a very short time. Thank you very much. The presentation is open to questions or comments that clarify what we have just learned or been told. I don't know what was learned. We will find out.

DR. COFFIN: Nice presentation. You described an experiment where you tested the ability, I believe, of activated ras to boost the possible transforming activity of HeLa cell DNA, if I remember correctly. And it got a negative result in that experiment. And you also showed that activated ras could boost the oncogenicity of low risk HPV E6 and E7 if I remember correctly.

DR. PEDEN: High risk only.

DR. COFFIN: Did you actually try boosting the activity of E6 and E7 with HeLa cell DNA to ask if, even if you couldn't detect the oncogenes themselves in that, there might be a booster in there that you might be able to detect.

DR. PEDEN: We did those experiments. The strange thing about this mouse is that mammalian DNA does affect, boosts the oncogenicity of any DNA for other plasmids. H5, the ras and the myc. And it is very strange. We don't actually understand that, but it is probably the end case itself, something wrong with the NK cells. It doesn't occur in SCID mice. It doesn't occur in P53 mice. It doesn't occur in normal mice, and it doesn't occur in hamsters. So I think it is specific to the CD3 epsilon mouse, but mammalian DNA, it does stimulate the oncogenicity of those DNAs. It is not simply just the amount of DNA, because salmon sperm DNA doesn't do it, e.coli DNA -- we don't understand it exactly. But you saw all those DNAs. 100 percent of the mice came down with tumors, so yes, but it is a mammalian DNA. That's why we added calf thymus DNA, just for the aficionados. So we don't know.

If you want to know which of E6 and E7 complements ras at C7, so E6 doesn't complement. So it is just the E7 plus ras.

DR. COFFIN: Actually, now that you mention that mouse, are cells from that mouse more readily transfectable with DNA? Is there something about the ability of the cells to take up and express foreign DNA that is special in that way?

DR. PEDEN: I don't think so. I don't think there is.

DR. COFFIN: There is one of the afibecs(?) that seems to be involved in protecting against that. Or maybe there is some defect of that in that mouse.

DR. PEDEN: We haven't explored that in great detail, but in our newborn rat experiments now, that I can certainly tell you about, we can go down to about a nanogram of DNA in the newborn rat. So it seems to be equally as sensitive. The SCID mouse maybe almost as sensitive. So there are other strains that maybe are quite as sensitive.

DR. HUDGENS: I had a question about your last point, which was the importance of achieving DNA levels below a specified threshold, which I believe is 10 nanograms. And I think that calculation comes from a calculation that was done by the WHO in the 1980s. And then maybe re-visited in the 1990s. And I know there are some assumptions underneath that calculation.

And I was just wondering if that number, that threshold, is in your view very important, whether or not we should revisit that calculation. For example, I believe there is a linearity assumption underneath the calculation of that threshold, that the risk of inducing a tumor is a linear function of dose, and it looked like maybe some of your data suggested it was a linear function of log dose.

DR. PEDEN: As Dr. Krause mentioned earlier, the original WHO calculation was made, in fact, in a committee that Dr. Lowy was on in 1986, 1987, published, and that was based on the oncogenicity of polyoma virus at that time. That was the only oncogenes that had been studied in any detail. I don't think that changes it.

And so you are asking why was it changed to 10 nanograms from 100 picograms? And so that, as Phil Krause mentioned, there are a lot of considerations that went into that. But one of the considerations was that many manufacturers could not meet that criterion. It was a theoretical risk because there was no evidence that DNA was oncogenic at those levels.

And when you do the calculation from viral oncogene to cellular DNA, as I mentioned that calculation, there is already a 10 to the five, 10 to the six-fold safety margin of that. So I think there are a whole lot of calculations that went into that, or at least considerations that went into that.

I don't think these data -- if anything, they confirm that. And in fact, DNA may be even less of a concern for oncogenicity based on our studies. So I think it actually confirms the wisdom of that decision in 1996.

DR. DAUM: Thank you for our last question, Dr. Lowy.

DR. LOWY: Thank you for making me an author of the paper. I wasn't. But I just wanted to comment that I think your issue of how to interpret a negative is really an important one. On the other hand, there are a lot of transgenic animals that contain oncogenes where those transgenic animals have led to tumors.

So in principle, that is, if you will, proof of principle that those genes would potentially score in a mouse. The two caveats, I think, are first that it doesn't give you a notion of sensitivity, which obviously is very important. And the other is that there might be cell type specificity. And your assays may not be testing the appropriate cell type.

So I am not trying to suggest that you should be doing more. But I just think there are real limitations, no matter how systematically you try to go about it.

DR. PEDEN: I think the conclusion that we have is that the DNA clearance is the only way to get at that. But I want to say we have done experiments in the transgenic ras model. And of course we are using P53, heterozygous and homozygous mice to try to find that out. Yes, it is certainly true that we may not be hitting the right cell. But we haven't got much in these kind of systems. You have got the limitation of what you can do.

So I don't know. But the promoter we chose -- and in fact Steve Hughes and John Coffin were involved in recommending the MSVLTR -- was that it is a widely expressed promoter. It doesn't get shut down so easily. But yes, I am not disagreeing with you.

DR. DAUM: Dr. Peden, thank you very much for a wonderful presentation. We are going to move on and hear now from Dr. Patrick Moore, who is the Director of the Cancer Virology Program at the University of Pittsburgh Cancer Institute. His topic is use of molecular methods to discover previously unknown or undiscovered human cancer viruses, a simple, straightforward topic I am sure.

Agenda Item: Use of Molecular Models to Discover Previously Unknown or Undiscovered Human Cancer Viruses

DR. MOORE: Good morning. First I would like to thank the committee for inviting me. I am coming at this topic from a little bit different perspective. I think most of you would be very happy if you did not find a cancer virus in your vaccine. I would be very happy if you did. We are keen on finding new tumor viruses.

And so the thrust of my talk will be talking about how we try to do that, and what are some of the pitfalls and new opportunities that there are to find cancer viruses. Now also, Dr. Peden gave an excellent introduction to this topic, and I hope that my talk will be relatively short so that if you have questions we can take time to explore them.

There are at least three broad questions that I have been thinking about in preparation for this. And this is, are there unknown viruses in cell line, as has happened with the 1960 SV40 contamination of the polio virus vaccine cell lines? Can undiscovered viruses pose a human risk in vaccine development? And are there ways to discover or screen for unknown viruses?

So I won't be able to give you definitive answers to any of these questions, but at least these are some of the questions that we can begin to think about. Here are a list of these seven widely-accepted, known human cancer viruses. And this list has come together over the

last 100 years in tumor virology, since Rouse originally performed his experiments at Rockefeller University.

What we know now is that viruses together with bacteria and parasites, infection in general is responsible for one in five cancers worldwide. I think that is a very important number. At least I would like to emphasize it in this meeting, since there are a number of people here in industry, in regulatory agencies and universities, that many of these agents -- for example, EBV, KSHV, Hepatitis C virus, have no vaccine, and vaccine efforts are stuttering along, if you will, for these.

But we have seen such tremendous success with Hepatitis B vaccine and with the HPV vaccine, in dramatically changing cancer risk, that this is something I hope that all of us keep in mind. So here are the list of these seven viruses. What do they have in common? We have two large DNA tumor viruses, EBV and KSHV. We have a positively stranded RNA virus, Hepatitis C virus, a retrovirus, we have a DNA virus that thinks it is a retrovirus, that is HPV, and then we have small DNA tumor viruses such as HPV and MCV.

So from that description you can get an idea that there is not a lot of biological generality that we can put to saying this virus when we discover it will be a cancer virus, this virus when we discover it will not be a cancer virus. The important point of this list is that of the seven viruses that do cause cancer, there are obviously thousands and thousands of other viruses, many of them closely related to these viruses, that do not cause cancer.

So in trying to find out what are the common features of cancer viruses you will have to bear with me. It is a little vague. But we can say a few things. Each one of these things has a caveat, or more, a couple of caveats perhaps.

This slide classifies viral tumorigenesis into indirect carcinogenesis and direct carcinogenesis, which I think most of you are aware of from the earlier talks. This is mainly work, ideas, that came from Julie Parsonnet and Harald zur Hausen. But in particular, for direct carcinogenesis, the virus delivers and assists a viral oncogene that promotes transformation of the target cell. We don't know whether or how indirect carcinogenesis acts. At least I don't have any clue about it. So I am not going to discuss it in detail.

But the important thing about direct carcinogenesis is that we know for those tumors that are caused by a direct carcinogen, you have to have at least one viral copy in each cell, and it has to be producing one oncoprotein, presumably through an mRNA message. So you have at least one cDNA copy per cell. And I am being pedantic about this, but I hope my point will make a little bit more sense in a second.

So this is just to graphically remind you -- this is two samples of collision tumors between Merkel Cell Carcinoma and Chronic Lymphocytic Leukemia. Here the Merkel Cell Carcinoma stains for the MCVT antigen. You can see just about each and every cell is positive. So you know that every cell that you isolate from a Merkel Cell Carcinoma that is positive for the virus, that is caused by the virus, will have detectable CDNA from that virus.

Now what are some other general features? Most tumor viruses are chronic and persistent, but they are non-permissive. They generally do not generate infectious virions from the tumors themselves. And in fact, in none of the cases of the human tumor viruses are tumors the primary mode of transmission, ongoing transmission of the virus. These tumors are instead dead end events. They don't necessarily contribute to the evolution of the virus.

As Dr. Peden mentioned, host factors clearly play a role in whether or not a person who is infected with the tumor virus will go on to develop a tumor or not. And also, viral tumors tend to be associated with immune suppression.

This is a graph for HPV showing the generation of HPV through CIN, and how HPV can infect the basal layer of the skin. You see here at early stages there is lots of virus generation, virion generation. But at later stages as the target cells become more and more neoplastic and as they clone, a single clone grows out from that in general, viral production is turned off. So it is no longer transmissible from the tumor.

My explanation for why this general feature occurs is the following. As we all know, viruses generally induce a cytopathic effect in many different cells. Not all viruses induce the CPE and certainly not in all cell lines. But this is an example where corneal cells are infected with Herpes Simplex, and you can see the generation of apoptotic cells here.

This is a general innate immune response to limit virus production, and so the virus has to live in this environment in which its host cell is dying and so it is rapidly replicating DNA, generating lots of DNA ends, lots of broken intermediates, and triggering the pathways that lead to CPE.

So just to tell you a little bit about the work that we do in our laboratory, Harold Jaffe and Val Beral were among the first to identify Kaposi's sarcoma in AIDS as having a very unique epidemiology. And from the epidemiology of this tumor they were able to predict the features of the virus that would be causing it. It would not be HIV, as was then currently thought.

It should be an agent that is rare in the general population. It should be sexually transmitted but poorly transmitted through blood. And that the prevalence of this agent in different populations would have a highest prevalence in African populations, intermediate prevalence in Mediterranean populations, and lowest prevalence in North America. And that is exactly what has been found.

This is my wife, Yuan Chang. Everything that I do is co-directed by her. We work jointly in our laboratory together. And she and I took a technique from Michael Wigler's group called Representational Difference Analysis, which is a subtractive PCR-based technique. We compared a Kaposi's sarcoma tumor to healthy skin from the same patient and were able to isolate two small fragments of this virus and subsequently clone and characterize the virus. Just a few base pairs. You need base pairs of information. It is tremendously useful to us, as I hope you can see in this slide. We first described this in December of 1994. And then very shortly, within two years, I think that it was quite evident that this virus was the infectious cause for Kaposi's sarcoma. The full virus had been sequenced by that point in time. Immunoassays had been developed. So there is a tremendous movement that occurred once that small amount of unique DNA was isolated.

But RDA has a lot of disadvantages. So over the next 10 years we attempted to develop a technique called digital transcriptome subtraction in order to find new tumor viruses. Other groups obviously had the same idea and did this independently over the same time period, so we were neither the first nor the only ones to try this approach. But I think the approach has some validity.

This is work that I am going to tell you about that is the work of a very brilliant post doc in our laboratory, Huichen Feng. And in essence what we do is, we sequence cDNA libraries. The key to this technique is to develop an extremely high fidelity dataset where each and every base pair in that data set is well known, meaning it has thread scores of 20 or more. It also helps because we are doing de novo alignments if the sequence is long. So certain types of sequencing are much, much better for finding new pathogens than others. We compare them to known human databases, subtract out the known human data, and we are left with a candidate database of sequences that might belong to a virus. Then we can open up our alignments, align the candidate sequences, and try to identify a new agent.

So we did this with Merkel Cell Carcinoma, which is aggressive skin cancer. It is most common in immune-suppressed patients. And Feng, together with Masa Shuda, performed DTS on about 400,000 cDNA reads from four MCC tumors, subtracted them out -- this just shows the percentages of subtractions that we go through. We have to subtract immunoglobulin polymorphisms, for example, mitochondrial DNA polymorphisms.

And we were left with this smaller pool, and this is the key here, of candidate agents. Which we were then able to align to polyoma virus sequences at some level of stringency, and identify MCV Merkel Cell Polyoma Virus, which is a virus that is similar to SV40, has a 5.4 KB double stranded DNA genome. It encodes T antigen oncogenes. It is clonally integrated, probably randomly -- or at least we don't have specific sites of integration that are known, in about 80 percent of Merkel Cell Carcinomas.

However -- and the important point here is that most of us, if not all of us, at least most of us, are already infected with this virus. So we are carrying this virus and we are transmitting it to each other, perhaps in this meeting as we speak. And yet it causes a cancer. And the reason for that is because the virus itself is mutated.

For the sake of time, since this is not directly related to this meeting I won't go into this, but suffice it to say we believe that not only do you have to be infected but you also have to have a specific set of mutations to the virus, and it being integrated, and a certain loss of immune surveillance, before you are risk of developing Merkel Cell Carcinoma.

So again, in 2007 -- this is a cancer of no known ideology, it has a very poorly characterized pathology, has a dismal prognosis with no specific therapies. 2012, we know the cause for at least 80 percent of these tumors. The other 20 percent, at least I am beginning to believe, are an entirely different tumor. There are new molecular diagnostic tests and an immunology tests and also, various groups including ours have initiated trials for molecular targeting of the virus, and hopefully adoptive immunotherapies will be developed for this tumor. Now here is the key point of what I would like to say. When I was in medical school, I was taught there were two human polyoma viruses, JC and BK. And that was true until Dave Wang and another group in Sweden discovered two new human polyoma viruses in 2007. Shortly thereafter we found MCV. So now the list is up to five known polyoma viruses, all of them with T antigens, all of them possibly, at least theoretically, being tumor viruses.

Chris Buck's group and several other groups found four more. And then last month we are now up to 10 polyoma viruses, many of which are being carried -- I am certain that there is an example of each one of these polyoma viruses in this room, probably most of us have all of them.

So the point here is that there are a large number of viruses yet to be discovered, that we were not aware of until the development of modern molecular biologic techniques. So if we perform DTS-like analysis on tumors, and this has been done, what you can do is you can look at various tumors that are known to be caused by viruses and ask the question how abundant are viral cDNAs in these tumors.

Now obviously this will be dependent on how clean the dissection is of the tumor, whether there is a lot of infiltrating normal tissue or not. But what we can see is that the numbers up here range from roughly 9 TPM, transcripts per million, that we found in our study, up to well over 200 transcripts per million for cervical cancer that have been found through high throughput sequencing techniques.

Now we assume that there is about 200,000 molecules of RNA in a cell. This is wrong, if you care to change that on your sheet, that is obviously wrong. Five transcripts per million would be the equivalent of the abundance for a single copy of an oncogene per cell. Getting back to what I said at the beginning, if a virus is a directly transforming oncovirus, then it should have at least this level, 5 transcripts per million, of its oncogenes being expressed.

And what we can do is, we can sequence to that level, put a pause on distribution around it so that we have 95 percent confidence estimates excluding the presence of a particular viral oncogene or any identifiable viral oncogene. And so in that case we are at least at the point now where we are beginning to exclude causes of viruses for tumors.

Now a cell line is like a tumor except for it is the best possible model, it is uniform, it is clonal, and we can easily isolate the samples, the RNA and DNA from the samples. So that what we see when we look at a series of cell lines that are infected -- BCBL, KSHV. HepG2, I don't know whether that is from a Hepatitis B hepatoma or not, but that is from a hepatocellular carcinoma. EBV, two cell lines here. HPV infected HeLas, we already know. And then the Adeno and SV40 transformed 293 cells.

If you just look at this bottom row to see in this data -- and this is data that I asked Feng to download from publicly available GenBank databases for this talk -- what you can see is that there is lots of sequencing that is done up here. And when you do that, then you develop an estimate of

the viral transcripts per million that you see. And in each case it is relatively easy to detect the cDNAs for these viruses in these cell lines.

So am I arguing that every cell line that we are going to be using should go through massively processed sequencing or next generation sequencing in some way? And the answer is maybe. But there are many caveats. This is a teaching slide that I use. It is one of my favorite slides.

This is a slide that was published in 1997, I believe, in Lancet. It showed that sarcoidosis was almost certainly caused by KSHV, the virus that causes Kaposi's sarcoma. And the evidence for it that largely got this paper published, was this dendrogram. This is a phylogenetic dendrogram. Beautiful distribution of the viral sequences. This couldn't be PCR contamination because we have such a broad spectrum of sequences coming from these different sarcoid tissues.

Well it turns out in fact it actually is PCR contamination. And since Taq doesn't have proliferating capability what we are actually measuring is the evolution of a Taq product as it is being amplified over and over and over again in the laboratory, not natural distributions of sequences. But everyone knows that PCR is a problem.

Here is a case from more recently. I think most of you if not all of you know about XMRV and the different issues that came up with XMRV. It is very complicated how this data came about. And also I want to stop and say that I am not in any way making fun of the scientists who have done either the XMRV or the sarcoidosis data. These are mistakes that are not mistakes. This is part of science. And we just have to realize it has to be corrected whenever possible.

In this case, as John could probably address better, since he put the lid on the coffin so to speak, for XMRV by finding that it was an endogenous retroviral contamination, as an early investigator passaged a prostate cancer cell line through mice in order to purify the cell line. There was a recombination event that is rare, a retrovirus exogenized, jumped into the cell line, and then it was in the laboratories. And from there it is relatively easy to get PCR contamination. We shouldn't have that problem because we don't have PCR contamination with high throughput sequencing. But as the gentleman from PaxVax told the story, it was very interesting, about the bovine virus that was found because they were using a specific lot of fetal serum in their cells.

This is from a very interesting and very good paper from a group in California that has looked at papillomas that occur in the immunosuppressed patients, to try and find a new virus that causes these papillomas in these patients. They did not find a virus. And more specifically, they looked very hard to see if they could find papilloma viruses, either high risk or low risk papilloma viruses, in these tumors. And they could not.

But they obviously had to report any sequences that they did find that belonged to viruses. And so this is an example of one of their sequencing projects. I don't know how many sequences this was. It was many, many millions of sequences that they went through.

But if you take a look at this for starters, you see MLV sequences. This is a bacteriophage. This is a virus that was actually found in this laboratory, the same laboratory that the investigators were in. And so it is probably a laboratory contamination, possibly, again, by floating PCR products in the laboratory. But they are directly sequencing this now from tumor tissues.

I don't know how they got in there, but it is quite real that they saw the sequence. Whether the sequence is actually there or not, it is hard to know. And one of the more interesting things that I have seen in our laboratory is the presence of Mimivirus DNAs, cDNAs, in water. And it turns out that Mimiviruses, which are algal phages, are actually quite abundant in tap water.

And we are getting our reverse transcriptase from retroviral products, obviously. And so all of that means that anywhere along the chain from isolating the cells to performing the reverse transcription, to performing the high throughput sequencing, has a possibility of being contaminated with exogenous agents. Now in our case, if we are using a one transcript per cell abundance, it doesn't matter. We know that that is very unlikely to be causal for that tumor.

Let me just talk about two other quick techniques that are also widely used. One is rolling circle amplification, where you have a displacing polymerase, this Phi-139, that is able to -- it works best for very small, circular DNAs like Papillomavirus, or polyoma virus DNAs. And it can amplify. This is a technique that was used by a group at NIH to find the latest polyoma virus in the skin. So it works quite well, and a number of polyoma viruses and papillomaviruses have been found using this.

But again, this requires the virus to be circular, which means it is in a lytic replicating state, not, for example, in an integrated tumorigenic state, non-productively producing state. But once you find that DNA then you have a few base pairs, you have a test. And then also direct capsid isolation and sequencing of DNA also can be used. It has the same caveat, that you are looking at freeform virulence.

So in conclusion, at least our thoughts is, most direct carcinogens we believe that we can rule out based on sequence abundance. Certainly for any of the known tumor viruses, and possibly we can do the same for unknown tumor viruses if we get that subset of sequences small enough, the candidate sequences small enough.

Unknown tumor viruses, can we find them? Yes, depending, possibly. But the real question is, is this generally applicable outside of the case of a direct human carcinogen? Maybe, and maybe not. I am very uncertain about that. So is there a possibility that you can find a non-transforming virus as a contaminant using techniques like this? I am not certain that you can. Because in that case we have no assurance of what the actual copy number abundance would be within the target cells.

I will just stop by showing you a picture without introducing you to the people who actually do the work while I go talk about it. I do want to mention two things, that I am supported, and this is my conflict of interest, that I am a proud member of the American Cancer Society and I am funded by NIH. So thank you.

DR. DAUM: Thank you very much, Dr. Moore. This fascinating talk with insight into a problem that is just becoming simpler as we hear more and more papers -- not -- is now open for questions or discussion.

DR. COFFIN: A very nice talk, Pat. Two things. You mentioned the XMRV story. Two things came out of that, that I think are very important for this group and this topic to keep in mind. One is the really enormous prevalence of mouse DNA at levels that are easily detected, and the endogenous viruses that they are loaded with are easily detected.

And many of those are in common laboratory re-agents. For example, Taq DNA polymerase often has lots and lots of mouse DNA in it. That probably was one of the original reasons this virus was discovered in the first place.

One of the complicated things that gave rise to that. And also the frequency of virus, retrovirus in particular but other virus contaminated cell lines that get passed around. The cell line that gave rise to XMRV actually has passed around the world. It appeared in 320 papers before it was realized that this cell line was making very large, 10 to the 11th particles of the supernatant per million are the supernatant of this cell line. And so one has to be really, really cognizant of that. And I think that is one of the major lessons that came out of that story.

DR. DAUM: Thank you for your comments. Others?

DR. CHEUNG: I just want to see if you could comment more about possibly underdetection of these viruses. If some of these tumors are not clonal but rather a mixture of cells, and some have RNA virus and some don't, and when you sequenced it and came down to, if you have a few reads that are off-line, are they really real, are they a virus, or just a sequencing error? There is an issue of bioinformatics, and the kind of fidelity of the data.

DR. MOORE: That is a very good point. And the answer to that is any high throughput sequencing project is a screen. If you don't follow it up and validate it, then you don't really know what you have seen in your sequencing project. So once you actually do identify those unique sequences, it is trivial, trivial to make a PCR test, for example, to detect it, a

quantitative PCR test to actually detect how many transcripts, or DNA copies, if it is a DNA-based genome, there are in the sample that you looked at.

And more particularly, looking at a blinded and randomized set of new samples, to ensure that you are actually looking at something that is valid and generalizable. That is very important. For the purposes of looking at cell lines, I assume that most HeLa, particularly if we clone it and culture clone the HeLa and pass it around, will presumably have pretty much the same viral constituents in any laboratory that you look at.

And so we will probably be seeing the same thing. We are actually looking at two different questions, and that is why my perspective is I would love to find a new tumor virus that is truly in a tumor and truly causing a tumor. But the question of this committee is actually slightly different, and that is, can we within reagents detect viruses.

And I think the answer is, probably we are getting to the point where that candidate list within reason can be shrunk down to a very, very small number. And one could use that as a very sensitive screening approach that would then require subsequent confirmation.

DR. GRAY: A wonderful talk. I think you have certainly convinced us of the massive parallel sequencing, the value of that. And certainly for screening tumor cells initially. But you have also obviously shared with us the potential for a cell line, once it is approved, to pick up a virus through the manufacturing process.

I just wondered if you had thought about perhaps a recommendation for the FDA with respect to the periodicity of employing these new techniques and examining manufacturing processes. Obviously your technique is going to get better over time, as you get more data. Have you given that any thought? Sort of an algorithm.

DR. MOORE: That is a good question. I don't know whether I would feel comfortable giving you recommendations of how often you would have to check, and so forth, or whether sequencing is the way to approach this problem. That is certainly a valid question. But it is trivial to do it now. A few thousand dollars, one can get a very high density, either cDNA or DNA sequence from the sample. And you can use that to monitor any changes in the genetic properties of that sample over time.

So it is not too much to ask if someone is using that as a central reagent for manufacturing something very important that is going to be given to lots and lots of people at that kind of surveillance, being asked around that certain stages at least and perhaps periodically.

DR. DAUM: That is a great question, though. Other questions and comments?

DR. PIEDRA: I was wondering what happens after benzonase treatment and one uses deep sequencing technique on that material? Are you still able to identify the same, let's say, constituents that were there? Or have they been eliminated through benzonase treatment?

DR. MOORE: In our case they would be eliminated. In our case we are looking at unencapsidated cDNAs. So we are essentially doing gene hunting in tumors, because our assumption is, the virus, if it encapsidates, is being transmitted, is going to initiate an innate immune response within that cell that is likely to kill it, which is a basis for oncolytic therapies, for example, vaccine therapies.

However, your point is right on the button. Here is the capsid isolation techniques, and both the rolling circle and in direct capsid isolation techniques generally use some nuclease -- benzonase or some other nuclease -- in order to get rid of the background human DNA, which you don't care about, the unencapsidated DNA.

You are looking at the specific physical properties that that DNA is now protected, or RNA, if you will, that is protected by capsid. And hence you are using that as a physical means of searching for viruses. It works, if you have got encapsidated virus present.

DR. DAUM: I have a question. If you were sitting on VRBPAC today instead of presenting to it, what would be your recommendation for a new vaccine that was grown on the tumorigenic cell line such as we heard about this morning, for testing for unknown or undiscovered viruses, if you would have one? If you were counseling the agency, what would you say?

DR. MOORE: Well I haven't given a lot of thought to it, so I don't want to say something that would be off. But clearly what I would like to see before I received, or someone in my family, or anyone I knew, in fact, received a vaccine, was that it was from the cell line that was extremely well characterized, such as a clonal strain, a clonal culture of a cell line.

And that it was under some level of genetic surveillance. And certainly the level in terms of testing for in *vero* cells or whatever for transmissible viruses is wonderful, and the other tests that go on. The other question that I would have is that I think it is better to have a human cell line rather than a non-human cell line, from the perspective of having an unknown virus.

For a couple of reasons -- one, the genome is much, much, much more well-known in terms of polymorphism. So you can do this kind of surveillance. You know what level of variation there is. Two, we are awfully familiar with each other. Every time we kiss or fornicate or whatever we do, we are transmitting some level of DNA among each other. Let alone generating children. So I do believe that we have, if you will, the most protected genetics compared to being introduced to some other species.

The reason why I bring this up is because in the 1990s I was asked this question about baboon heart transplants in immunosuppressed patients. And I thought that was a terrible idea, because we have no idea of these very rare, odd animals that we don't typically over our historic and prehistoric time, have a lot of contact with, are suddenly being introduced into an immune-suppressed person. That would be problematic. Or at least that would be the place where it would be most likely to be problematic.

DR. DAUM: Thank you. We may ask you to revisit some of these comments this afternoon. I don't know the committee's pleasure. But are there other comments? Dr. Gellin?

DR. GELLIN: So Pat, since Bob has already turned the highlight onto you, I thought your questions that framed your talk were really very helpful. But I want to build on this question, and maybe it is one that we will talk about later. And I would have ordered them a little bit differently. So you were asking about are there ways to discover a screen for unknown viruses. The key, then, is can undiscovered viruses pose a human risk in vaccine development? And I think that is really the bottom line here.

I think, Pat, on your slide, the infectious disease people would like to see that 20 percent of cancers have an infectious ideology. I guess over time that might grow, so we have some hope that we can prevent those cancers. But you also said there are a lot of related viruses to these that don't cause cancer.

So I think we are in this gray area where you may find things that may be related to things that you know are bad, but then you don't know where you are as far as the health risk. So I wonder if you might speculate on that question, recognizing that you said you didn't have definitive answers to any of them.

DR. MOORE: Again, Bruce, that is a really good question. What we are using is sort of the theoretical baseline, again, of this mantra of one CDNA per cell for a direct carcinogen. Because we can't rule out pathogenesis from these viruses, that may be causing some other infectious disease.

But I think that we can rule out a direct carcinogenic effect if the abundance of a transcript is so low that it is unlikely to be present in most tumor cells. And that is, again, easy to check with an assay, that you can just simply take that sequence and do RTPCR, or PCR on the sample.

So good case in point are the algal viruses, or the bacteria phage viruses. Do they cause human disease? I don't know. We are loaded up with them. They very well might. Do they cause cancers? So far we have never seen one at an abundance in a tumor that would make us think that it is likely. But we certainly see them in every project that we do. So that is our filter, if you will, for how we look at them, just purely through drug transformation. Other diseases, I don't know whether I can say anything intelligent about them.

DR. DAUM: Thank you very much, Dr. Moore, for a nice contribution to our deliberations. I appreciate it very much. For our last presentation, certainly not least, before lunch, we will call on Dr. Khan of FDA, a senior investigator, to talk about the evaluation of vaccine cell substrates for adventitious agents. Welcome, Dr. Khan.

Agenda Item: Evaluation of Vaccine Cell Substrates for Adventitious Agents

DR. KHAN: Thank you. In my talk I would like to describe the adventitious virus concerns in human tumor derived cell substrates, strategies to mitigate adventitious viruses in vaccines, recommended assays for adventitious virus testing of cell substrates, and additional assays for novel cell substrates with a discussion of emerging virus detection methods.

Although you have heard a lot about adventitious agents and viruses, I would like to start my talk, again, with the definition of an adventitious agent, which is a micro organism that is not intended to be present in a biological product. And this includes a long list of agents indicated here.

However, the focus of my presentation will be on adventitious viruses, since these are a particular concern in tumor cell substrates. In fact, adventitious viruses are a major safety concern in all cell substrates. Adventitious viruses include exogenously introduced viruses that can replicate, resulting in a productive or lytic infection, as well as latent viruses, or occult viruses, that can result in a persistent or latent infection such as some RNA viruses, oncogenic DNA viruses, as you have heard from Dr. Moore, as well as retroviruses.

Additionally, adventitious viruses may arise from genetically inherited and germ-line transmitted sequences, such as endogenous retroviruses, which are highly stable elements present in multiple copies as a normal part of the DNA in all species. Such sequences may reside as inactive sequences, latent sequences, or be active. And they may be expressed as proteins or particles which may be either defective or infectious. And it should be noted that there has been no infectious, endogenous retrovirus recorded in humans.

Adventitious viruses are a particular concern in tumor cells due to the potential presence of viruses that may be involved in the tumor development, or viruses that may have been acquired by the cells due to increased exposure or susceptibility of the cells to virus infection during cell line derivation and establishment, which may have involved extended passage in vitro or through a rodent species, as Dr. Coffin alluded to earlier.

Additionally, activation of endogenous viruses may have occurred during tumor development. The next slide outlines OVR's current approaches to mitigate the risk of adventitious viruses in vaccines. A multi-pronged strategy is used, that involves identification of potential safety concerns to enable development of a comprehensive testing plan and risk mitigation strategy. And this is based upon characterization and history of the cell substrate, cell banking and use of qualified raw materials that includes development of well-characterized cell banks and the use of certified or tested animal-derived biological materials such as serum trips in antibodies and other reagents that may be used in the banking or cell propagation. Also on the incorporation of steps during manufacturing for viral clearance and purity that can be used for process validation for clearance which includes both inactivation and removal of potential viral contaminants in the final product as well as steps that can be used to reduce the levels of residual cellular materials which can be DNA, RNA or proteins.

Additionally, testing is important for mitigating the risk of adventitious viruses in vaccines, and this includes extensive testing for known and unknown agents in the starting materials such as the cell substrates, the virus seeds, the vector virus preparations, and adventitious agent testing at different stages in the manufacturing process, and at steps with the greatest potential for contamination and with the use of various sensitive and broad detection assays.

The details of the strategy will be presented in the next few slides. Characterization and history of the cell substrate can identify safety concerns for development of a comprehensive testing plan for implementation of a risk mitigation strategy.

This includes evaluating the tumorigenic phenotype of the cells to determine whether it is non-tumorigenic versus tumorigenic, and knowledge of prior exposure of

adventitious agents due to the viruses in the donor species of the source materials that may be either naturally occurring viruses or due to specific exposure. Or, viruses that may have been acquired during the cell culture passage history, due to the propagation in different labs, or due to biological reagents that have been used.

The ability of cells to be banked allows extensive characterization and testing of the cell substrate. This cannot be done in the case of primary cells and tissues where testing and safety relies mainly on the donor history, donor testing, the use of specific pathogen-free donors when possible -- for example, in the case of eggs -- and extensive testing of the control cells.

However, in the case of diploid cell lines and continuous cell lines, cell banks can be established and generally include the Master Cell Bank, where there is generally a one-time extensive characterization and testing, Manufacturer's Working Cell Bank, where there is limited testing when the Master Cell Bank has been extensively tested. And End of Production cells, which in some cases may be banked where there can be limited testing. And additional testing may be done at this stage.

Using qualified or tested raw materials in the generation of the cell banks can reduce the risk of introduction of adventitious viruses in the preparation of the virus seed or the vector virus preparation, and in the upstream vaccine manufacturing process. So steps during the manufacturing process may also afford reduction and removal of residual cellular materials, including DNA, RNA and proteins. And clearance of viruses by virus removal and inactivation. Process validation using different model viruses in spiking studies to estimate robustness of the process for inactivation and removal of potential unknown viruses. The total viral reduction, or the log-clearance value, is based upon reduction afforded by each orthogonal step in the manufacturing process.

And it should provide sufficient viral clearance to assure that the product is free of virus contamination. And the details of the clearance, and the clearance studies, can be found in the reference, as indicated here, the ICH Q5A(R1) document.

Strategies to mitigate the risk of adventitious viruses in the case of some inactivated vaccines and sub-unit vaccines can rely on clearance studies that can contribute to viral safety assessment. However, these can assist in the quantification of the risk but do not by themselves prove the absence of risk. And therefore, testing is needed for vaccines. In the case of live viral vaccines, product safety relies on a well-defined process, the use of qualified biological reagents and extensive testing. And in some cases there may be steps included that can afford purification of the product to reduce residual cellular material in the final product.

The next few slides will describe the adventitious agent testing recommendations by OVR for characterization and qualification of cell substrates and other biological starting materials used in the production of viral vaccines. And, as Dr. Krause had mentioned, this is described in the 2010 guidance document.

The routine cell substrate testing includes identity, sterility, tumorigenicity assay using the adult nude mouse, and adventitious agent testing. The adventitious agent testing includes testing for both non-viral agents as well as viruses. The non-viral agents include bacteria and fungi, mycoplasma, spiroplasma in the case of insect cells, and mycobacteria.

The adventitious virus testing includes both general detection assays as well as species-specific assays. The general detection assays include in vitro cell culture tests in cell lines of three species, which includes the cells from the species of the cell substrate, a monkey cell line and a human cell line. The in vivo assays generally include testing in adult mice, suckling mice, and embryonated hens' eggs.

Transmission electron microscopy can be used as a general method, although it is not sensitive for detection of high level of virus production, if it is present. And reverse transcriptase assays that are currently recommended for retrovirus detection is the PERT assay, which has replaced the traditional, less sensitive, reverse transcriptase assay.

The species-specific assays include tests for animal viruses. For example, for bovine and porcine viruses, if there is the use of serum or trypsin in the development of the cells, for propagation of the cells, or development of the cell banks or at any step throughout the manufacturing.

And the testing is described in the 9CFR. Antibody production assays, if there is exposure to rodent materials, and assays for known viruses that can be based upon the cell substrate species, the information about viruses that may be present in the species, as well as any exposure that the cells may have gone through in their passage.

So the routine assays have generally been effective for mitigating the risk of adventitious viruses in vaccines, although they have some limitations. And the next few slides, I will just describe briefly some of these trends, and limitations of the current assays. The in vitro and in vivo assays can be useful for detection of known and unknown viruses. However, the viruses need to be replicating for detection. And they can detect a broad range of viruses that are pathogenic for humans. The tissue culture assays can test a large volume of sample.

As I mentioned, for detection in these assays, there is a need for the contaminant to replicate in the selective cell lines that are used for the detection, or in the in vivo species used for the assays. And in the case of the in vitro cell culture assays, the viruses need to be able to produce CPE, hemagglutination or hemaadsorption, as these are the read-outs for these assays, or animal death in the case of the in vivo assays.

And in some cases, there may be difficulty in accomplishing neutralization of the vaccine virus in conducting these assays. The 9CFR testing for bovine and porcine viruses is based on immunofluorescence assays for detection of specified animal virus pathogens. And some viruses cannot be detected in the tests, for example, bovine polyoma virus.

There are other infectivity assays that may be used, and these assays are sensitive for virus detection. However, the results are based upon the selection of the target cells. The molecular and biochemical assays can be highly sensitive and quantitative as well as rapid. The PERT assay is highly useful since it can detect all retroviruses, because it is based on the detection of reverse transcriptase that is present in all retroviruses that can replicate, as well as in defective viruses in some cases.

However, there is a need to follow up a positive result to determine the biological relevance, to determine the risk of an infectious, intact virus. And in most cases only a small amount of sample can be tested. There is a need to develop individual PCR assays for newly-discovered viruses, and there is a caution in interpretation of the results of the PERT assay, since some cellular DNA polymerases can be detected in this assay. And the PCR results for the PCR assays need confirmation by sequencing, to distinguish viral from cellular origin.

As I have mentioned, the currently recommended assays have been generally effective in demonstrating the absence of adventitious viruses in traditional vaccine cell substrates for vaccine production. However, the guidance document does allow for updating of the assays as need develops, such as the availability of new methods for sensitive virus detection, new virus discoveries and use of novel cell substrates that pose new issues, for example, tumorigenic or tumor-derived cells, which aren't being discussed today.

The recommended testing has been updated over time. For example, there has been introduction of the PERT assay, agent-specific PCR assays. And you have also heard oncogenicity assays in the case of tumorigenic cells as well as chemical induction assays in the case of novel cell substrates.

So the challenges for the use of tumor-derived cell substrates are the presence of unknown viruses, unexpected viruses, as well as known viruses, which may include tumor-inducing viruses, latent viruses, occult viruses and novel viruses.

So in the next few slides I will go into some of the assays that may be considered for evaluating adventitious viruses in tumor cell substrates. These include the currently

recommended, the oncogenicity assays for detection of tumor-inducing viruses, chemical induction assays for evaluation of the presence of inducible endogenous retroviruses.

And for the presence of latent DNA viruses that may become reactivated, as well as potentially new virus detection technologies such as microarrays, broad range PCR with mass spectrometry, as well as massively parallel or deep sequences, as you have already been introduced to. So the in vivo detection of oncogenic viruses in cell lysates was mentioned by Dr. Peden earlier, and this approach was used in the discovery of the first avian retrovirus, the Rous Sarcoma virus, by injecting filtered extracts of tumor cells into chickens.

This was actually in 1911. However, it does demonstrate the usefulness of the assay in the discovery of a tumorigenic virus. Additionally, other tumorigenic retroviruses have been discovered by injection of tumor tissue extracts such as gross leukemia virus as well as a polyoma virus by injection of cell free material from an AK mouse leukemia cell extract by injecting into newborn C3H mice.

As well, there have been various pathogenic virus discoveries by extracts from a variety of different transplantable sarcomas and carcinomas injected into newborn mice, such as Moloney Murine Leukemia Virus and Friendly Leukemia viruses. Additionally, cell line extracts were used by Bernice Eddy to demonstrate induction of tumors into newborn hamsters due to SV40, that was present in primary rhesus monkey kidney cells.

So as also was mentioned before, these assays were discussed at the 2001 VRBPAC meeting. And although they were not standardized and lacked positive control, the assays were deemed to be useful for evaluating the presence of potential oncogenic agents in novel continuous cell line substrates.

And here I just briefly describe the assay. It consists of inoculation of cell lysates from 10 to the seventh cell equivalents into less than four day old mice. Three species are used, and the assay is similar to that of the DNA oncogenicity assay. Newborn hamsters, newborn nude mice, and newborn rats. The observation period for tumor formation is four to seven months. There needs to be follow up, of course, in the case of a positive result.

In addition to what doctor Peden had described for a follow up for the DNA oncogenicity assay, it is also important to identify the origin of the virus to determine the virus identity and to characterize the biological properties of the virus to assess the potential risk. As I mentioned, there is an absence of positive controls for assay standardization currently.

Next I will describe the chemical induction assays for latent virus detection. These assays have been used since the early seventies. They were used for discovery of several endogenous retroviruses using either IUdR or azacytidine. And they have been successful in cells from a variety of different species, including avian and mammalian cells.

Latent DNA viruses have been well studied using chemicals such as TPA and sodium butyrate and can induce various latent DNA viruses, for example, herpes viruses. We have used mouse cells, and the BC3 cell line, which contains latent HHV8 or Kaposi's sarcoma virus DNA as positive controls to develop a strategy for determining optimized conditions for various activation using these four drugs.

And an overview of the strategy is shown here. The details of the induction strategy is published in these references indicated here for activation of endogenous retroviruses as well as for the latent Herpes virus. So the overall strategy consists of using four different inducers. And these inducers provide different mechanisms of activation of, as I mentioned, endogenous retroviruses and latent DNA viruses.

After the cells are exposed to the drug, then there is a need to use various detection assays that can detect both known and novel viruses. And of course once a signal is detected, either using the PERT assay for retroviruses or using PCR assays for DNA viruses, it is important to distinguish the origin of the signal, viral versus cellular, since the drug treatment in most cases does result in some cell lysis.

So then it is important to evaluate for the presence of the induced virus, both novel and known. And the effectiveness of the induction can be evaluated based upon activation of

RNase in the cell. However, the real concern for the potential induced viruses are associated with particles in the supernatant.

And then the induced viruses need to be characterized molecularly, physically, and biochemically, and furthermore to investigate the potential risk biologically using infectivity and co-culture studies, with various target cells including non-human and human cell lines. There is still a need to develop positive controls for the assay that are relevant to novel cell substrates, and also a need for methods to detect novel viruses.

So induced endogenous retroviruses have been detected and characterized using some of the available broad virus detection technologies such as I have listed here -- microarrays, broad range PCR with mass spec or PLEX-ID, and massively parallel sequencing, MPS.

The results show that these methods are important research tools. Additionally, these methods have been used as investigative tools for evaluating the presence of virus contaminants in a variety of biological materials. And the remainder of my talk will describe some of the details of these methods.

Virus microarrays consist of high density arrays of virus-specific oligos that are designed based upon known and related virus sequences. And the use of long primers allows for some mismatch. The technology uses direct application of nucleic acid to the arrays, or a random PCR step prior to the application.

Some of the examples that I will show for each of the technologies demonstrates the usefulness and potential application of the technologies. The examples of virus discovery with the microarrays is the SARS virus, as well as detection of metapneumoviruses, gammaretrovirus that was referred to earlier, the XMRV, as well as porcine circovirus sequences in a rotavirus vaccine.

However, there is a need to update the assay following novel virus discoveries and update of databases. The PLEX ID includes long PCR primers that are specific for virus families. The amplicons are detected and sized by mass spectrometry. The mass of the amplicons are compared with a database to identify the organisms.

And examples of use of the technology includes strength specific identification of bacteria and viruses, for example, the 2009 H1N1 pandemic virus, and the novel variant of the blue tongue virus. And there is also a need to update this assay following novel virus discoveries and update of databases.

So next I will describe some of the features of the MPS, although the usefulness and the applications have been described by some of the previous speakers. This approach of sequencing is without prior knowledge of the sequences for detection of both known and novel viruses. However, there are several high throughput sequencing platforms currently available, and some are emerging.

And although the platforms differ in their engineering configurations and sequencing chemistries, hundreds of mega to gigabases of data is routinely obtained from sequencing DNA or cDNA in a massively parallel manner. And these different platforms have different characteristics for the amount of sequences that is generated.

The read length, the accuracy and the speed, as well as differences in application suitability, needs to be considered. For example, transcriptome versus whole genome, de novo assembly for novel viruses versus using a reference virus. And therefore, there is consideration that a hybrid approach, using a combination of the technologies as well as a combination of the bioinformatic analysis of the data generated from the technologies, may fill the gaps in the different platforms.

The examples for viruses detected by this technology is the identification of PCV1 in sequences in a rotavirus vaccine that was referred to earlier, virus discovery in a variety of live organisms in the environment, in cell culture and raw materials. I think if you just put this into Google you will get a long list of literature that you can read on your own time. However, there is a need to reanalyze the data as databases are updated with new virus sequences.

So I think Dr. Moore described some of the challenges with the MPS, and which I think is relevant to all of these methods that I have described. I think a critical one is asset standardization and validation, the need to develop relevant and appropriate controls to determine assay sensitivity and specificity.

Additionally, the bioinformatic analysis also needs to be considered. Bioinformatic tools and experts are required for data analysis and interpretation. Advanced equipment and experienced personnel are needed for data transfer, storage and management, especially in the case of the MPS.

And standardization of methods for data analysis, since there are no current guidelines for acceptable quality of reads. Parameters for short read assemblies, as well as there are challenges to define approaches to identify a novel virus that has minimal nucleic acid sequence homology to known viruses. And I think a discussion was initiated after Dr. Moore's talk about this.

However, it should also be noted that in the case of the MPS, the full length sequences may not be likely to be acquired unless the virus that is present in high titer in the cell's line, and also if there is no reference sequence available and de novo assembly needs to be done for novel viruses, the data may not cover the entire genome due to insufficient depth of coverage.

So although these emerging methods are powerful research tools for virus discovery as well as investigative tools in a range of biological and environmental samples, there are challenges that need to be addressed for consideration for application to the safety of biological.

And one further detail that I would like to point out is the need for a follow up strategy to evaluate the biological relevance and significance of a positive result. I think these are nucleic acid based technologies. I think one would expect some type of a signal, if they are working. And therefore I think that it is very important that there be a follow up strategy in place, in the event of a positive signal.

The next slide outlines a path for follow up of a positive signal to determine its biological relevance. The results initially need to be confirmed by PCR or another assay. It needs to be determined if a complete viral genome is present. And then are particles present? Are the particles infectious? Is there a replication competent virus? And can the nucleic acid particles be quantified to assess potential risk?

In the next few slides I will end my talk with describing the general approaches to mitigate the potential risk of adventitious agents. These include the use of well-characterized and qualified biological raw materials for production, which are based upon characterization of the cell substrate, qualification of cell banks, virus seeds and animal derived raw materials, development of a comprehensive testing plan for quality control during manufacture. And designing an efficient manufacturing process with robust viral clearance capabilities.

In summary, I would like to conclude that the potential presence of adventitious viruses in traditional cell substrates for vaccine production is routinely evaluated using assays as described in CBER's cell substrate guidance document.

Identification of potential adventitious viruses associated with tumor-derived cell substrates requires development of a comprehensive testing plan and risk mitigation strategy, including use of current assays, and may include use of novel technologies. Thank you.

DR. DAUM: Dr. Khan, we thank you. That was a very excellent downloading of current agency policy and will give us a good basis for starting our discussion remarks this afternoon. I would like to ask the committee if they have questions or comments or clarifying questions for Dr. Khan now.

DR. AIR: When you are using deep sequencing, it is obviously easy to identify a known virus. But for an unknown virus are you looking for sequences that don't belong in the human genome? Or are you looking for characteristics of a virus?

DR. KHAN: I think a virus is probably something that doesn't belong in the human genome. So I think you have to look for that and you have to possibly include in the analysis some characteristics to identify a novel virus.

DR. AIR: And do you know what those are?

DR. KHAN: Well, I think I can speak for, for example, retroviruses. For retroviruses, there are characteristics in terms of what is the full length viral genome. You have certain genes that are present, for example, gag, pol, env, LTR, terminated by terminal repeats in that context.

In terms of retroviruses and I think in other viruses as well, there are regions that are highly conserved. For example, the polymerase gene, that can be used as a starting point. But then I think you need to extend to see if those sequences are in the context of the gene, which is in the context of the adjacent genes that are in the structure of the virus that are associated with the promoter elements at the end of the virus. I think these are things that need to be considered in developing a strategy for looking at novel viruses.

Again, one point I didn't indicate here is that I think it really has to be a very cooperative, collaborative effort between the buyer informatician and the virologists. And I think that is also, I think, a critical aspect of looking at the virology of the system and identifying viruses.

DR. DAUM: Thank you. Dr. Kester, please?

DR. KESTER: Thanks for a very interesting talk. I guess a lot of these types of analyses and assays really are indirect in the sense that we are measuring genomic material and sequences and things like that, and yet we have other approaches, as we have heard, where we are looking at really functional readouts. That is, are tumors produced?

Earlier we heard, in the assessment of cellular inoculation studies, that observation may take as long as a year for weekly tumorigenic cells to show an effect. And for lysate studies, as you outlined the observation period may be as short as four to seven months. I wonder if you can comment on, especially as we broach this concept of some novel approaches to vaccine development perhaps, do we need to re-look at our observation periods? Is that relevant? Or are we confident that that is a reasonable approach?

DR. KHAN: I have to mention that this was a great challenge to come up with the observation period, especially for these additional assays for novel cell substrates. The original tumorigenicity assay is a three-week -- no, I'm sorry, I think it is a three-month period. So I think based upon what was in the literature, and based upon the rodent system, it was felt that a period of four to seven months may give you some accurate results.

I think when you get close to the one year period, some animals may spontaneously come down with tumors. So I think then you are dealing with false positive results. So it is a challenging system. And I think most of us, when we have animal studies -- and unfortunately in the protocol we write down the time that we are going to terminate the animals.

And as the time nears then you feel like maybe we let them go a little bit longer and see if something comes up. So this is a range that we felt based upon the literature.

DR. GRAY: A nice summary. You mentioned that you were considering in some situations a hybrid approach of using one or more massively parallel systems to detect adventitious agents. Could you give us an example of why you think that is appropriate, and how you would do that?

DR. KHAN: I just want to clarify that we have not recommended these assays. These assays are under consideration because they need to be further data-generated in terms of the application of the assays as well as in terms of the bioinformatic analysis. The hybrid approach that I mentioned, it is from the literature and from current thinking of these technologies from people that are working on these technologies.

We are also investigating these technologies but don't have an extensive experience with the use of the different platforms, also due to funding restrictions. They are expensive technologies. So it is felt that -- and I didn't include that slide here, but each of the platforms for the MPS technology has some deficiencies, some gaps, in terms of providing data

that will generate a complete genome, a complete virus genome, based upon the read length, based upon if you are looking at transcriptome versus whole genome.

So the thinking is that an approach that will combine the use of different technologies as well as the data generated from the different technologies may fill the gap in terms of providing a complete genome.

DR. DAUM: Thank you. We may need to revisit some of the issues behind that question this afternoon. The final question of the morning goes to Dr. Gellin.

DR. GELLIN: As though the current, the present, isn't complicated enough, your slide about selected new technologies left some space at the bottom, which tells us there are more things that are going to come. But in looking to the challenge of emerging technologies, both for the assays and the bioinformatics, you talk about standardization. Where does that go on? And how does that happen?

DR. KHAN: I think because -- and again, I didn't present the details of starting from the sample preparation to the actual generation of the data -- I think the quality of the data needs to be assessed based upon each step that is used to generate the final results, starting from the sample preparation.

There was discussion earlier about if you use DNase treatment can you use these technologies. Well, the effectiveness of the DNA treatment, that needs to be controlled and demonstrated. So you may need some relevant control for that step itself. If you are looking at particle associated sequences, viral sequences, then you need to demonstrate that you are able to, if you are using a centrifugation step to pellet the virus, you need to be able to demonstrate that the conditions that you are using will pellet sufficiently all the particles that are present in the sample.

So I think each step needs to be controlled and demonstrated for effectiveness. And overall the sensitivity of the assays and the specificity of the assays then need to be standardized for validation of the total technology. But each step will need, I think, to be controlled to demonstrate effectiveness to get reliable and accurate data generated from the technology.

DR. DAUM: When we come back this afternoon we will have an open public hearing, of which there are currently two speakers signed up to present briefly to the committee. And then we will have a discussion focused on the questions that the FDA has posed to the committee for us to consider.

In the meantime, there is lunch. At this moment I will turn the floor over to Don, who wants to make some announcements about lunch.

MR. JEHN: (Housekeeping details)

DR. DAUM: I would like to thank the presenters this morning for really an outstanding overview of this topic, and getting even those who confessed to limited expertise thoroughly immersed in the issues that the agency is confronting. And also setting the stage for what I hope will be a fruitful and lively discussion this afternoon. Please, if you did not contribute this morning to the discussion, my style is that I will call on you. And I would like to hear everybody's opinion at the table about issues that have been raised. Many thanks, and see you after lunch. We will reconvene at 1:30 sharp.
(Whereupon, a luncheon recess was taken.)

AFTERNOON SESSION (1:30 p.m.)

Agenda Item: Open Public Hearing

DR. DAUM: It's time to call the meeting to order.

As part of the FDA Advisory Committee, we are required to hold an open public hearing for those members of the public who are not on the agenda and would like to make a statement concerning matters pending before the Committee. We have three requests to speak.

First, we have to talk about the philosophy of open public hearings.

Both the FDA and the public believe in a transparent process for information gathering and decision making. To ensure such transparency at the open public hearing session of

the Advisory Committee meeting, FDA believes that it is important to understand the context of an individual's presentation. For this reason, FDA encourages the open public speakers, at the beginning of your written or oral presentation, to advise the committee of any financial relationship that you might have with a sponsor, its product, and, if known, with its direct competitors. For example, this financial information may include the sponsor's payment of your travel, your lodging, or other expenses in connection with your attendance at the meeting. Likewise, FDA encourages you at the beginning of your statement to advise the Committee if you do not have such financial relationships. If you choose not to address this issue of a financial relationship at the beginning of your statement, it will not preclude you from speaking. I would first like to call on Dan Shawler, senior vice president of operations of NovaRx Corporation, who apparently needs five minutes for his presentation.

Welcome, and we're looking forward to your five-minute presentation.

MR. SHAWLER: Thank you, Dr. Daum.

I'm Don Shawler, vice president of NovaRx.

Earlier this morning, Dr. Daum asked a question: Can these tumor cells be used as vaccines for cancer patients? That's what my five minutes will be about.

NovaRx is developing allogeneic therapeutic tumor cell vaccines for the treatment of cancer. The work that goes into these -- the process to develop them, the testing that's done -- is very similar to what we were discussing this morning. In fact, we quite frequently make use of the different guidances from this group to help us design our product safety testing.

Our two lead products are trade name Lucanix, which is for non-small cell lung cancer -- and it's composed of four cell lines. We have completed enrollment in the Phase III trial. We're now waiting for the data to mature in this blinded trial so we can look at our overall survival endpoint.

The other lead product is trade name Glionix. That's for the treatment of glioma. It's composed of three cell lines. We're currently writing the clinical protocol.

Concentrating on Lucanix, or belagenpumatucel-L, it's an allogeneic whole tumor cell vaccine. As I said, it's composed of four non-small cell lung cancer cell lines. In these cell lines we have blocked TGF-beta by antisense gene modification. The cells are then sublethally irradiated to prevent replication and are cryopreserved, and patients receive monthly intradermal injections. The histology slides shown here are showing infiltration by activated T cells, CD45RO-positive cells, in the tumor bed before and after vaccination.

We started with the observation that cancer patients are immunosuppressed. Why would you expect a vaccine to work in an immunosuppressed patient? As we looked into this, we discovered that tumors secrete TGF-beta to evade the immune system. TGF-beta shuts down multiple parts of the immune system, T and B cell activation. It inhibits dendritic cell activation, NK cells, and it also induces immunosuppressive FoxP3 Treg cells. So by blocking TGF-beta, we are generating a vaccine that leads to activated T cells. These T cells are refractory to TGF-beta inhibition. They can therefore transit through the body and target the tumor *in situ*. The various issues that we have to deal with are very similar to what you have in your Advisory Committee. Biological systems have a large variance, and we have to let the biology dictate the process. That means that we need to allow testing and validation to fit the biology and don't force the biology to fit the testing.

I like to tell people that we keep the part of the process that you throw away and you throw away the part that we keep. We just grow the cells in a very similar process to what you're doing here. Then at the end, we don't have any filtration steps, so we have to maintain septic processing throughout and be able to vial these cells in a sterile environment.

Thank you.

DR. DAUM: Thank you very much, Dr. Shawler. That's very enlightening.

Does the Committee have any brief questions, clarifying questions to ask of Dr.

Shawler?

(No response)

If not, I would like to move on to the second presentation, the second of three, which is from Dr. Ursula Fritsch, who is the vice president of regulatory affairs for a company called Jennerex Incorporated. Dr. Fritsch has also requested a five-minute presentation.

Dr. Fritsch, welcome.

DR. FRITSCH: Thank you very much. I don't think I'll need five minutes --

DR. DAUM: All the better.

DR. FRITSCH: Okay.

I work for a company Jennerex, J-e-n-n-e-r-e-x. We are located in San Francisco. I echo the previous speaker. While therapeutic vaccines are not really being discussed here today, our issues and questions run parallel to a lot of what is being discussed. Just briefly, our product, JX-594, is an oncolytic vaccinia virus which is engineered to express GM-CSF and has a thymidine kinase disruption. It's designed to replicate in cancer cells selectively. By definition, these viruses are not able to replicate efficiently in normal cells, including proliferating normal cells.

After much research around different cell lines, the cell line that was selected for our product was the HeLa cell line. We felt that HeLa was necessary because it's well defined. That's why we chose it. It's also a well-known cell line, with defined oncogenes. It's also a human cell line, which is required for our product, because it helps with the replication efficiency, the pharmacokinetics, the pharmacodynamics. Namely, the human HeLa cell allows for human-specific, species-specific anticomplement protein protection for IV therapy. As discussed this morning several times, we feel it's important to consider the theoretical risk of carcinogenicity resulting from products using a HeLa cell line in manufacturing compared to the benefits it could bring to critically ill patients. Oncolytic viruses will only be approved if they increase survival duration in patients who have cancer and short life-term expectancies.

Also please consider that these patient populations are almost uniformly exposed to proven carcinogens, such as chemotherapy and radiation and numerous radiologic CT scans.

In summary, I would just like to say that oncolytic viruses represent a promising emerging therapeutic class, with novel mechanisms of action. The human cancer cell lines such as HeLa are necessary for efficient manufacturing. We believe that the risk-benefit ratio in advanced terminal cancer populations favors the use of oncolytic viruses manufactured in cancer cell lines.

Just to sort of echo what was discussed this morning, the use of cell lines derived from human tumors and substrates for the production of preventive viral vaccines should be differentiated from oncolytic viruses used as therapeutic vaccines for the treatment of cancer.

Thank you very much, and I really enjoyed all the discussion this morning.

DR. DAUM: Thank you, Dr. Fritsch.

Does anyone have any quick clarifying questions for Dr. Fritsch?

(No response)

If not, I would like to call on the third and final open public hearing speaker, someone from PaxVax -- I don't know who is going to speak -- that wanted to clarify something that came up this morning.

DR. ONIONS: Thank you, Mr. Chairman. Just a slight clarification. My name is David Onions. I'm the former chief scientific officer of BioReliance. I'm appearing today as an independent paid consultant to both PaxVax and BioReliance.

I'm going to be very brief. If I may, my colleague John Kolman from BioReliance will also just add a little bit more information about the NPS approach that was used in the PaxVax data you heard today, because I think it would clarify a few issues.

I want to make two very simple points. The practical applicability of this technology, I believe, is now. It's not a replacement technology. It's an adjunct to what we already do in well-characterized cell systems. I want to illustrate that by two points. One is to pick up on the issue that was mentioned today. When we looked in the supernatant media of

PaxVax cells, we were able to find a new virus. It's a new dependovirus, a member of the parvovirus family. This virus would not have been detected by any of the conventional techniques that are currently used in either cell-line characterization or looking at fermenter samples, for instance.

We were then able to make very specific PCRs for that virus and exclude that virus presence in the cell line and demonstrate that it was purely confined to the fetal calf serum used in the medium.

I also want to illustrate a second method that we have already published on. I want to allude now to the other form of analysis, which is the transcriptome analysis. We have been able to show, in looking at an insect cell line that was mentioned by Dr. Krause this morning, High Five cells -- we were able to show in those cells that we can detect a nodavirus contaminant, which is, we know, there at about 1 in 100 cells. Not only can we detect that contaminant, but we can completely reconstruct the bipartite genome of that virus and completely reassemble that virus.

I think comments were made today that maybe you just get bits of fragments. That is not the case. We can actually reconstruct the whole genomes of these viruses when we find them.

The second point I want to make about that is that even if we knew nothing about nodaviruses of insects -- these are called alpha nodaviruses -- we would still have detected that virus because we have done that exercise where we have taken those out of our database and then looked for matches from much more distantly related viruses. We still get hits against these much, much more distant beta nodaviruses. We can still reconstruct the whole genome based on that information.

Thank you.

DR. DAUM: Thank you.

If there's another gentleman from the same company that wishes to speak, you will still have to finish within the five minutes.

DR. KOLMAN: I'm afraid I could probably speak that fast, but I'll try not to.

My name is John Kolman. I'm also from BioReliance. I run the genomics unit that executed the assays for PaxVax.

This is really meant to engender further discussion. I have listened to many of the questions that have been asked about MPS. They are excellent, and the presentations have been outstanding. There are a few issues, though, that I think I can answer directly, or at least give another insight into, by virtue of our experience.

I would like to begin by discussing a couple of issues regarding contamination signals, systematic signals that might occur as the result of sequences that are present in enzymes, and other such matters. I would like to just begin by pointing out that we don't see a single identification as a contamination. A single read which matches something needs to be understood in the context of what you are looking for.

To begin with, the suite to which we perform MPS at BioReliance is a GMP. We have wipe-down procedures. We have cleaning procedures that support our very high-sensitivity qPCRs, and they prevent us from having spurious contaminations from one sample to the next.

Enzymatic contaminations do occur. These things are signatures that you are going to see systematically. They are going to be in every run, and they can be identified as such. So one of the ways that you can start making catalogues of things which could well be red herrings and need to be thought of as possibly a red herring -- that's the kind of information that accumulates as you do more and more of these kinds of runs.

It's important to remember that the sequence reads help you understand the risk. At the end of the day, the risk has to be evaluated very, very specifically.

On this slide, error rates, how to find viruses, and the virus list. Where does it come from? Here is an example of looking for signatures in serum, ten different vials of serum. As you can see, the BPVs are quite pronounced. The AAV that was described by A5-4 is shown there in the bottom.

Also there are a large number of fungal signatures in each one of these bottles. Should these bottles be thrown away because they are contaminated with fungus? Absolutely not. It's a handful of signatures that represent a tiny fraction of a fungal genome, and for that reason, is really nothing that needs to be worried about. It is likely a systematic or low-level nucleic acid signature that's not relevant to an infection.

On this slide --

DR. DAUM: Excuse me, how much more do you have, sir?

DR. KOLMAN: I have about six slides.

DR. DAUM: Can we cut that down?

DR. KOLMAN: We sure can.

I would just like to point out that 4-5-4 sequencing on this slide allows you a great deal of tolerance. This is a sequence which has been degenerated sequentially down to, in the red box, 66 percent identity. Standard NCBI BLAST can still detect this signature relative to a wild-type control. Therefore, distant viruses can be easily detected. Error rates that might arise by virtue of MPS can be easily overcome by virtue of these kinds of matches.

The other thing that I would like to have the Committee consider is that MPS, as you choose to do it, is a matter of sample complexity. Dr. Moore gave us a beautiful exposition on how to find a virus within a highly complex nucleic acid substrate, the full transcriptome. That's important work. The insensitivity of those tests is very, very important. But if one rids the system of the cellular material and looks only at cell-free material, the ability to detect signatures becomes much, much easier. The signal-to-noise increases dramatically.

Here's the important bit. Once you have signatures, what do you do with the them at the end of the day? I put this flow chart up here to show, first of all, that the algorithm can be built. It is a mathematical process. I won't go into the details, except to point out two things fundamentally. One, an efficient algorithm is very similar to the process that Dr. Moore described, and that is, look at what you have. Is it cellular? Is it not? If it is not, is it a virus or is it something entirely undetectable by the current BLAST processes? That's, in essence, what we do. This is a process which is completely arithmetic at this point and which we are going to validate shortly.

On this slide, I simply point out here that at the end of the day, signatures which are not cellular still need to be evaluated in a very concrete fashion. In this demonstration we tried to balance the degree of genome coverage with the degree of redistribution to evaluate the importance of a set of identifications. A genome that is hit by many reads over a vast extent of a genome is something that should be of great concern. Reads that localize only to a short region of a known virus are typically a red herring. We don't know that every time, but typically that is going to be an instance where a client was using, for example, a promoter from a virus as part of an expression system as opposed to seeing a real infection. Those kinds of things can be called at the end of the day, and if you are to look at the slide with a few more moments' time, you will notice that a lot of the passes that we provide when we provide this as a GLP study are really conditional, that orthogonal assays, as Dr. Khan pointed out extensively, are required to confirm an identification.

On the next slide --

DR. DAUM: Is this the last slide?

DR. KOLMAN: Yes, this is the last slide.

We know GMP is important, as has been pointed out. We also know how difficult this is. This is not like making GMP a simple qPCR. There are a large number of modules. It's extremely onerous. We are near completion. We expect to have a GMP-like process by December. The key here for us was to have individual modules tested and assessed for robustness, as well as the entire process in its entirety.

This is the laboratory work. The same is going to have to happen for the algorithm, whether we build it or the FDA builds it or somebody else builds it. It's going to have to be validated and processes are going to need to be in place for all of us to understand how to use these things and standardize them.

When it comes to updates to databases, some which Dr. Khan brought up, which is very important, when new viruses are new detected, if you have done MPS, it's a very simple matter for us to rerun the algorithm with the new database. When one chooses a different approach -- for example, arrays or other things -- it may actually require that the sample be revisited entirely -- again, one of the things that I think is a benefit of MPS.

These notes are just meant to expand the conversation, because we, as you, have been thinking long and hard about this.

Thank you very much for your attention.

DR. DAUM: Thank you very much.

I would like to call on Dr. Krause now to set the stage for our afternoon deliberations. He will reiterate the questions that the Committee is being asked to address and make a few other extemporaneous comments as well. Dr. Krause.

Agenda Item: Committee Discussion and Recommendations

DR. KRAUSE: Sure. Just to remind you, one of the questions, the second one, discusses the current approaches. This is the approach as I summarized it this morning to cells. Obviously, completely removing cells during manufacture, to DNA, as Dr. Peden described, the extended tumorigenicity testing, cell DNA, animal oncogenicity testing, and fragmentation removal during manufacture, having been the things that we have done so far when we have been faced with thinking about tumorigenic cells. For adventitious agents, the main summary, then, is testing, which up until now has been cell lysate oncogenicity testing in *in vitro* virus-induction studies. But obviously the use of these new technologies is on everybody's mind, as well as inactivation and purification of vaccines during manufacture, where that's possible, and cell banking and use of prequalified reagents.

I mentioned earlier that I think that, for the purpose of this discussion, to start off thinking about what we would want to see for a parenterally administered vaccine is probably more helpful than trying to initially separate these out.

I did want to make a couple of comments that are related a little bit to what we just heard in the open public hearing, as well as some of the questions which came up earlier in terms of the scope of the discussion.

We are talking about the use of human tumor cell-derived cell substrates for use in vaccine production. We really should keep the discussion focused on that. The new technologies have many other potential uses in dealing with manufacturing vaccines, as well as other biological products. But the key question, I think, to the extent that we are going to think about these new technologies, is, what role might they have in the context of thinking of about introducing newer cell substrates? For instance, the idea of using them to look at the manufacturing process for newly introduced adventitious agents or things like that -- that may be something that can be done, but that's not really something that is necessarily so different from the manufacture of any vaccine. That's not on the table here. We are really just talking about the investigation of this new cell substrate.

The other comment is one about standardization and validation. That's something that has come up a couple of times. That's something that we are going to have to deal with the manufacturers on. We are not going to ask you for advice on how to standardize and validate new assays.

The real question that you can help us get to the root of is, what do you want the assays to show? If you are not satisfied with the current assays, what would you want a new assay to demonstrate? We also have the situation where the technologies keep changing. You have heard of a broad range of technologies. The purpose of presenting these technologies is not for you to say, oh, I like this technology and I don't like that technology. It's more to give you a flavor of what the current technologies are capable of, which then gives you a basis for saying what it is you would like to know about the cells. Then we can work out with the manufacturers and everybody else how to adapt these technologies to actually show that, assuming that that is feasible.

With that in mind, here are the questions for the committee: Please discuss safety concerns

associated with the use of human tumor-derived cell lines for production of preventive and therapeutic vaccines for infectious diseases.

There we are really asking, what could be the components of these cells that would give rise to concern? Is it the cells, the DNA, adventitious agents? Are there other things that you are concerned about that need to be addressed?

The second one: Please discuss approaches that should be used to address any concerns that have been identified in that first question. Please consider the role of the manufacturing process, any steps in the manufacturing process that you think could make a difference in this assessment, including live versus inactivated vaccines, the adequacy of those current approaches to mitigate potential risks that I have described that we have used so far for vaccines that are produced in tumorigenic cells, and then, finally, the potential use of new technologies.

Thank you, and I really look forward to hearing your comments.

DR. DAUM: Thank you very much, Dr. Krause.

I must say that, in many years of serving this Committee, this is a particular challenging discussion and list of concerns. I call on the Committee's wisdom to help clarify them. Let's begin with the first one: Please discuss safety concerns associated with these human tumor cell line vaccines for production of both preventive and therapeutic vaccines for infectious diseases.

Who would like to begin that discussion? Dr. Coffin.

DR. COFFIN: We have clearly heard from a number of the presenters what the major issues are about the possibility of introducing potentially oncogenic DNA, which Dr. Peden did a very nice presentation on. He has done some very elegant work on a subject that has been hanging around for a long, long time, to finally come to quantitative grips with it.

Another issue that comes to mind -- and one that actually can be sort of discarded, but hasn't been raised -- is that the history of many human tumor cell lines can be quite muddy. In particular, thinking back to the XMRV story, the problem there was that this is a cell line that, like many tumor cell lines, was originally passed as a xenotransplant through nude mice. That introduces all kinds of additional possibilities for these cell lines to have been infected by endogenous viruses of that mouse, but also by adventitious exogenous agents that might have been infecting the same animal.

So one has to take extra-special precaution, I think, with cell lines that might have had that kind of history. I don't think that's true of any of the ones that are under consideration right now, but it's a caution that comes to mind.

Another one that came to my mind as the discussion was going on is that having human tumor cell lines gives people a much wider range of cell lines to choose from and also cell lines that themselves are already known to exhibit a much greater genetic plasticity than diploid cell strains or whatever. That gives the opportunity to select for the cell lines that will be the best ones to grow the viruses of interest. Almost by definition, that means you are also selecting for cell lines that have the greatest potential to be contaminated with other viruses because they are most likely to have lost innate immune factors, interferon response pathway genes, and so on and so forth, that might have prevented infection by wild-type cells.

Those are a couple of additional considerations that come to my mind as we discuss these. The two main concerns are, of course, adventitious agents that might come in this way and the oncogenic DNA considerations.

It seems to me that one of the issues -- to go to question 2 -- if we are not recommending a complete genomic sequence of everything that's used, at least understanding the nature of the known oncogene and known, say, Ras mutations and things like that that you can easily assay for I would think would be something to know for any cell line that you are using for this.

DR. DAUM: I guess the question to come back to you with is, you have read the briefing document and read the guidance document and heard the presentations this morning. The

question that I would think the agency would be looking for is, what do we think should be done differently or additionally or an altered approach because the vaccine was grown on a tumor cell line? Would you want to comment on that before you give up the microphone?

DR. COFFIN: I raised a couple. One is that it may be more susceptible to contamination by adventitious agents. There's certainly something in it that makes it oncogenic. Often that's some kind of modified proto-oncogene -- BCR-ABL fusion, perhaps, in the case of the CEM-derived cells and so on. Those things are ones that increase the potential, if there is any potential, for oncogenic DNA coming from those cells. Obviously, if there are altered proto-oncogenes in those cells, particularly Ras mutations, they increase any risk that there might be relative to cell lines that don't have that.

DR. DAUM: Thank you very much. I hope I didn't push too hard. Dr. Lowy.

DR. LOWY: I agree with what Dr. Coffin has been saying. My sense is that we have traditionally relied on bioassays for a certain amount of detection, either of DNA or infectious agents. I think the presentations this morning really were very high-quality. Relying on bioassays is fine when you get a positive result. The problem is when you have a negative one. The biggest technological advance, it seems to me, in addition to doing various kinds of molecular screens for known adventitious agents, is something analogous to extensive sequencing or massive parallel sequencing. This really is able to distinguish between the cellular DNA or cellular nucleic acids and the non-cellular nucleic acids.

In the situation with HeLa cells, as Dr. Sheets presented, we already know that there are important viral sequences, but we can ask whether there are other adventitious sequences beyond those of the papillomavirus E6, E7, and the regulatory region.

So I think this is actually a very important advance. Dr. Khan talked with us a little bit about what happens if you find something. I think that developing appropriate algorithms to analyze whether they are biologically relevant is something that could be certainly considered.

Finally, I think that in terms of identifying a particular DNA that you think is mutated or something like that, as Dr. Coffin said, this enables you also to follow it in terms of the manufacturing process and whether it is appropriately cleared.

DR. DAUM: Thank you very much.

Other comments and opinions? Dr. Cook.

DR. COOK: When I was listening to the presentation about HeLa cells by Dr. Sheets, I was thinking about the completely other side of the equation in these vaccine preparations, which is the 500-pound gorilla. It's all the virus, not the contaminating bits that might be left behind, which have been discussed in exhaustive detail today -- how much you can get rid of and how much you can't. I begin to think about the story in adenovirus. The model that I'm talking about is, virus replicates in mammalian cells -- in this case, in the proposed human tumor cell lines -- and the virus replication, at least in the context of adenovirus replication, can involve various types of recombination that are important to talk about in detail, but can acquire things from that host cell. In the context of adenovirus, if you have a defective virus that's missing a gene in the early region that's required for replication, when those viruses are grown in cells that provide that gene in *trans*, analogous to HeLa cells containing HPV E6 and E7, there are rare events of recombination that allow the virus now to acquire the genes from those mammalian cells in which they are grown and change the nature of the virus. They now become replication-competent because they have acquired that bit from the cell.

That's a huge selection pressure for the virus. You can find them because you know how to look for them. I'm not sure exactly how you would find these others, but it is known, for example -- just thinking about the adenovirus HeLa cell model for a minute -- that you can replace certain adenovirus gene functions with certain early region functions from HPV E6 and E7 and other kinds of assays.

So it's not completely out of the question that there could be some acquisition of something from that cell, especially in the context of HeLa, by the replicating virus that could be carried along into the vaccine. Now, the frequency of this would probably be very low. I think in

the adenovirus world this thing that I described of adenovirus becoming replication-competition by acquiring sequence from the cell is something like less than 1 in 10^8 or 10^{10} or something, so a very small number of vaccine products would contain that.

But what I was thinking, in listening to the presentation, was that this is something that isn't being discussed at all. What is changed about the vaccine by growing the virus, by growing in human tumor cells, as opposed to growing in something else like a primary diploid cell?

DR. DAUM: Not to plant a response in your mind, but would some measure of sequencing help in that regard or would you advocate some other approach?

DR. COOK: I suppose. It depends on the sequencing mechanism. There's no reason theoretically that you couldn't -- the experiment in the adenovirus story is, you put the virus on a non-permissive cell, you pick the plaque, and if it turns out to be the one that will acquire the gene, you can find it. In this case, if you don't have a selection pressure to find it, you would have to hope you could grow up enough virus and do sequencing that was sensitive enough that it could detect that bit of whatever was acquired from the cell -- that you could get it without swamping out the system.

So the answer is, possibly yes. You probably could construct an experiment like that to find out how it would work.

I would just encourage thinking about this. I'm not sure there is much that needs to be done about it -- but the question of virus modification as a result of growing in one cell type versus another and the interaction with that cell's DNA.

DR. DAUM: It sounds like a very important point. I guess I'm just asking back, would sequencing solve addressing the issue for you or is there something else that should be done to address the issue of whether the virus has changed.

DR. COOK: Well, I don't know what the phenotype would be. You would have to say this virus, which is intended to immunize the recipient against something, now does that and brings along something else. Does it change the virus phenotypically? There's no reason to think it would change the antigenicity unless it changed some of the structural proteins. So a sequencing would work. But I think just trying to develop a checklist of what phenotypic changes might occur in the vaccine virus strain would be worthwhile. I can't give you a very long list, other than just the functional acquisition.

DR. DAUM: Several people took me aside at lunch and commented on the revolution in biology and the role for sequencing as part of this review process. I wonder if any of those people would like to make any comment at this point.

DR. CHEUNG: Just listening to this morning's presentations, a lot of them, as you know, present whole transcriptome analysis looking for transcribed RNA. But I would submit that, besides the RNA sequencing, we also need to do DNA sequencing, because RNA sequencing would miss all the latent viruses that are in there. They are not transcribed. They are just sitting in there and waiting for another opportunity to replicate. I think, in order to make sure these cell lines are completely safe over the long term, in terms of vaccine production, we have to do whole genomic sequencing, as well as the RNA-seq, which is the whole transcriptome analysis.

DR. DAUM: Other comments about this or any related topic?

DR. COFFIN: I would completely agree with that last point. I think transcriptome sequencing has a major role to play, but I think also understanding what's in the genome of the cells that are being used for this, particularly in the case of these tumor cells, is very important.

DR. DAUM: I think what we're talking about here is really the changing technology and availability of different kinds of tests and assessments that might not have been apparent in the not-so-distant past. I'm sure FDA has heard this before, but I think one of the central messages we can share today is the need to keep up with technology in terms of revising these guidances and guidelines.

Which brings me to a question that I have sort of been wondering about. Can you tell us what a guidance is and how it differs from regulation? What is the process at FDA by which a guidance gets updated?

DR. KRAUSE: I can take a stab at that. A regulation is actually a law. Under the regulatory authority that the FDA has, the regulations that are promulgated describe things that, if the manufacturers don't do them, they are breaking the law and are subject to penalties and so forth.

A guidance is FDA's advice on how one could follow the law, in essence. It's not prescriptive. It's not necessarily the only way in which the things that are in the regulation can be accomplished. But it may describe ways in which manufacturers have done it in the past or may describe ways in which the FDA believes a manufacturer can completely solve an issue that is raised by a regulation.

Guidances, then, are created by the FDA, normally by teams of people who sit down and really try to put together their experience and their scientific knowledge to come up with those kinds of explanations and that kind of advice. But it is advice. It's not a requirement.

That then undergoes the typical process that one finds in any government bureaucracy, which requires some additional vetting and examination to make sure that everybody agrees that the advice is good advice. Then it ultimately gets published as a guidance.

DR. DAUM: Can you talk to us, Phil, a little bit about how it's revised and updated? As new technologies come online and you say, oh, that should be in the guidance, how do you get it in there?

DR. KRAUSE: In some cases we will actually issue a letter to manufacturers. For instance, when the PERT assay came out, which you heard about earlier today, CBER looked at this and said, this is an assay which could help improve viral safety analysis, by helping to improve the identification of retroviruses. So a letter was sent to manufacturers saying this is something that should be done. It was, of course, also publicized and put on -- I don't know if it was put on the website, because I'm not sure if we had a website then, but it would be now. That information is made available to supplement that which is in the guidance.

So there are ways of updating this kind of advice that is sort of between revisions of a guidance. If things have changed enough, then a guidance can be updated as well or one can write additions to a guidance.

In some cases -- and I think for the issues associated with introducing new cell substrates -- one of the best resources for the manufacturers -- and you can ask those who are in the room -- have been the transcripts of these Advisory Committee meetings. This is where these issues have been very comprehensively discussed.

Also, of course, we welcome conversations with manufacturers who are contemplating doing things which are unusual, and we will give them advice on their specific set of circumstances as well.

DR. DAUM: Yes, Dr. Khan.

DR. KHAN: Because the 2010 guidance document took so long to update the 1993 document, we did conscientiously lay out the philosophy for development of a customized testing plan based upon the issues for the manufacturers. It does allow for introduction of new assays and technologies, if they are equivalent or more sensitive and relevant for safety.

DR. DAUM: Dr. McInnes.

DR. MC INNES: I have a question for our experts, for their opinion on surveillance of the cell line over time and how that might bear on safety. You have mentioned that the virus may not be the same virus grown in different cells and how that might ultimately impact potentially on efficacy of the product. Just sort of philosophically, what might one be looking out for? Say you ran the gauntlet and you demonstrated what we think we need to know right now to actually license a vaccine. With time, what would one want to have in place for surveillance of that cell line to understand its stability -- I may not be using the right word -- how much change is going on in that cell line as it may speak to both safety and efficacy?

DR. DAUM: Does one of the experts want to take on that question? Dr. Lowy?

DR. LOWY: I'm not sure that I'm an expert on addressing this issue. But it seems to me that, first, when you develop a master cell bank, you then freeze down multiple vials, so there should be similarity when you bring each one out of the liquid nitrogen. I don't see changes in that respect being something that you would anticipate. Presumably, as part of a qualification of a line, you go through the manufacturing process to see what happens. I think that's pretty well set.

However, as we have heard, frequently there are biological products that are used in the propagation of the lines, and they need to be appropriately qualified. If they are appropriately qualified, then presumably the process that would maintain the integrity of the process -- maybe Dr. Tsai might want to comment on it, since he manufactures vaccines for a living.

DR. TSAI: I'm not involved in the technical operations of vaccine production. But I would agree, I think the principle of establishing a master cell bank is precisely as you said.

The points of discussion about using newer technologies to detect adventitious agents -- I wonder whether those would be applied to the reagents, as well as the cell lines, in order to intercept the unintended introduction of adventitious agents to the manufacturing process.

DR. LOWY: I have a question for our FDA representatives. I assume that if there are substantial modifications in the manufacturing process, then you need to go through some kind of fairly rigorous requalification?

DR. GRUBER: That would be correct. It depends on the manufacturing changes that you would introduce in the process. And depending on that change, there are actually different categories. But usually if the process is licensed, the manufacturer would have to come back to the FDA, make a submission, we would look at the new data to really determine what the change is all about, and we would have to approve that change before it could be implemented.

DR. DAUM: I would like to return to one comment, Dr. Coffin, that I think you made, and that is that if the vaccine used human tumor-derived cell lines for production, you would like to have the nature of the cancer defined in advance. I guess my question is, how would you interpret that information and how would you use it? What would differ?

DR. COFFIN: I think, in general, it's going to become feasible and important to have complete genome on all cell lines that are used in production of vaccines. I can't imagine why we would say we wouldn't be doing that at some point in the future. But the question comes around the issue, specifically, that Dr. Peden raised. That has to do with the potential transfer of oncogenic DNA and the observation that certain mutated oncogenes have, at least based on animal studies of plasma DNAs, a greater potential of causing these kinds of problems than others. While it probably wouldn't greatly change the stringency with which one assays products for DNA contamination, I think it would be still useful to know whether there was a potential for there being a mutated Ras oncogene, for example, in any DNA that might have gotten through in that product. It could give you something very specific to look for, as I think Dr. Lowy mentioned, with a very specific PCR assay at the end. You could actually look and see if there was any of that surviving the process. So it could give you a specific target to actually assay for, conceivably. It has to be looked at on a case-by-case basis, but it will give you that kind of information.

DR. DAUM: Thank you. Dr. Lowy. Then I would like to hear from some people that haven't made comments yet about their safety concerns.

DR. LOWY: I think the HeLa cell line, for example, could be an example. Most cervical cancers have amplification of a region of chromosome 3 that includes PI3 kinase. You have more copies of PI3 kinase than of other genes in the cell. Along the lines of what Dr. Coffin is suggesting, you could, in principle, follow that, because it's presumably pathogenically involved in maintaining the transformed phenotype of the cell.

DR. DAUM: Great. Dr. Cook.

DR. COOK: I think another thing that's missing from the defined risk assessment, thinking about safety concerns -- and I guess the way I would think of this is as something being

more safe than less safe that hasn't been included in the analysis -- the obvious point that whatever is coming through in the vaccine is not being put into an *in vitro* transformation assay. It's not like a 3T3 immortalization focus-formation assay. It's not like a nude mouse being injected with Myc and Ras. This is usually a normal immunocompetent host that's receiving a vaccination that is eliciting a brisk inflammatory response at the site of the vaccination. So it's not a passive immortalization transformation -- probably not a conducive environment for little bits of things getting through to have a very good time of trying to cause an initial immortalization event, because it's an initial inflammatory response.

We think about that all the time. There's a lot that goes on at that site that may be worth at least acknowledging in the context of perceived safety versus risk. Without that inflammatory response, things might happen that might not happen in the context of infiltration of inflammatory cells, cytokines, amplification of the response, that might be undesirable if you are trying to establish that initial cellular event.

DR. DAUM: Other comments? We'll just maybe go around. Dr. Schoolnik, what are your concerns?

DR. SCHOOLNIK: The only thing I would emphasize beyond the near-term application of next-gen sequencing, both of the transcriptome and of the genome, would be to have modern, forward-thinking bioinformatics support. I think one might open the question as to what might constitute adequate bioinformatics to get the most out of those data.

DR. DAUM: Can you be more specific about what kind of support that would support?

DR. SCHOOLNIK: I think one question for the FDA is whether they are envisioning having this capacity in-house, not only the sequencing capacity -- maybe you already do -- but also something like a bioinformatics core that would be oriented toward addressing these particular issues. There are lots of different kinds of bioinformatics. Not all are relevant to this particular question. I think here the bioinformatics would be those that are based on a deeper understanding of sequences and their derivation, their phylogeny, and their particular relevance, given the questions that we have been considering.

I would like to hear from the FDA about what their current vision is for bringing next-gen sequencing or whether they feel that this belongs in the domain of the manufacturers, as well as the bioinformatics to support it.

DR. DAUM: Thank you very much for raising that. It sounds very important.

Dr. Wilson of FDA is at the microphone, ready to respond.

DR. WILSON: I do want to address that, because we have recognized at CBER how critically important this new technology is, not just to this, but a lot of our efforts. We do want to be able to have in-house expertise and ability to understand these technologies. As you can see from the presentations, there are all kinds of complexities associated with the application of these technologies, and if we aren't using them ourselves, we can't fully appreciate them and we can't address them either.

We actually, last year, invested in Illumina technology. We have a HiSeq and a core facility, which is starting to work with our scientists to provide sequence information. We also have several MiSeqs in various offices.

To support that effort, we also recognize the critical nature of the bioinformatics piece, and so we have actually hired a staff of about 11 people who are developing a Web-based portal, taking advantage -- CDRH has a high-performance computing server, a supercomputer. We are leveraging that capability for the computational aspect. We're developing cloud computing for the data storage and so on.

We're not quite there, but we're getting close. We are going to have a pre-production pilot rolling out within the next month or two, hopefully being fully operational within the next six months. So we're working in that direction.

DR. DAUM: Thank you very much.

DR. SCHOOLNIK: That sounds really very forward-looking. That's great.

DR. DAUM: It sounds wonderful.

Dr. McInnes.

DR. MC INNES: Just following up on Gary's comment, obviously the agency has to have capacity, but so does each sponsor. If you are going to employ this type of sequencing, the analytical and computational ability will need to be there to support the data they are submitting to the agency. It's going to have to be multicenter and accessible. We will admit that bioinformatics capability is actually pretty stretched at the moment. I think that's going to be a challenge.

DR. CHEUNG: One of the things I'm thinking during this morning's presentation -- and I would like to address that to the FDA -- are you happy with the state of the animal model that you have presently, about the ability of some of these vaccines or cell lines to cause tumor? I notice that you have the rat, you have the hamster, and you have the nude mice. Do you think those are reasonably accurate to reflect what's going on? Do you have a plan to develop other animal models, such as transgenic, that might more mimic human conditions?

DR. DAUM: Before I ask them to respond, can we ask you if you think they are reasonable?

DR. CHEUNG: I personally believe there is.

DR. DAUM: Does anybody from FDA want to respond to that?

DR. PEDEN: As you probably gathered from my talk, we are trying to evaluate these rodent models to find out whether they are appropriate. We recommended them in 2001, but it's really only now that we can start to assess whether these particular recommendations are sensible. We need to determine whether they are detecting oncogenic activity of DNA, which genes they are detecting, and what the sensitivity is of these. If they are not sensitive enough to detect DNA, whether it's amplified, as Dr. Lowy said, or not, then it doesn't make any sense to use them.

To your other question about what transgenic mice to look at, I did mention that we have actually looked at some other models, such as transgenic mice expressing activated and non-activated H-Ras, without success in terms of cell DNA and also the fact that they are not very sensitive for oncogenes in plasmids.

My own view is, at this stage, I'm not optimistic that we're going to find animal models to assess oncogenicity of DNA. That's why I'm feeling that maybe it's the clearance aspect that we have to deal with, with respect to DNA.

I just want to come back to some things Dr. Lowy and Dr. Coffin were discussing. Yes, it is nice to know that there is activated H-Ras in these various cell substrates, but what do you do with that information, apart from clearance of the DNA? If they have amplified three or four times, or even ten times, you can still clear those molecules. So I'm not clear what that information would gain us. I understand that it's nice to know what the transforming event were in those cells, but I still think it will come down to DNA removal in some way, or digestion, in activation. Am I wrong?

DR. COFFIN: In the best of all worlds, you would have a variety of cell lines to choose from, and you could choose the ones that had the -- that probably is almost never going to happen. In some cases that might be possible, to use that information to select cell lines that are actually going to be used, all other things being equal. I don't know that that will ever arise in practice, but it's not inconceivable that it might sometime down the road in the future, if you know this information for a wide range of cell lines and you have some choice about what you're going to use for your cell substrate.

DR. PEDEN: That's usually a sponsor decision. They will come to us with the particular cell line. I don't disagree that it would be helpful for a manufacturer to make that decision.

DR. COFFIN: The other general point is, of course, you will get that information in the course of

doing a sequencing project, looking for viruses and so on. Once you get it, it should be catalogued and retained for future reference, in any case.

DR. LOWY: I was referring to the clearance. I think the data you showed about the DNA with different times of exposure, et cetera, are very useful, but it seemed to me that, in addition to that, if there was a known DNA that was contributing to the oncogenic phenotype -- for example, in the HeLa cells, if it was E6 and E7 -- just showing that that was degraded specifically, along with the others, was simply an additional form of reassurance. If you think that this is redundant, I think one could make an argument in that way.

DR. PEDEN: You're right. In fact, manufacturers have done that. With products that were made in HeLa cells they looked for E6 and E7. I remember in 2001, in the first E6, they made sure there was no E1a and no E1b in the adenovirus that was grown in E6. So you're right, they have done that.

DR. COFFIN: But they needed to know first which oncogenes to look for in order to be able to do that. It brings us back to the other point of knowing what's there before you can actually do assays like that intelligently.

DR. KRAUSE: Can I ask you, Dr. Coffin, to amplify there? You can certainly get the entire sequence of the genome of a cancer cell, say A549 or whatever, but unless there's something really obvious there, where you see some mutation that you know has previously been associated with activating an oncogene, you may have a lot of data and not know how to interpret it. Are you saying that you would be uncomfortable using cells unless you were in a position where you actually knew the mechanism, which, it seems to me, is a substantial additional research project that goes beyond simply sequencing the DNA?

I'm just trying to drill down on whether this is a need-to-have or a nice-to-have.

DR. COFFIN: We have catalogues of probably over 1,000 oncogene mutations that are found in association with cancers. I would be very surprised, actually, if one or more of those didn't show up in almost every cell line that you looked at, once you looked deeply enough.

I can't say for sure that seeing a specific one would or would not make me more nervous about the risk of a vaccine that came from that cell line, but I still think we should know what's there and what the potential of the potential of the potential might, in fact, be in the worst-case scenario.

Can I ask a question --

DR. DAUM: We have several people in line that want to ask questions. I'm happy to have you ask -- can you hold it for a few minutes?

DR. COFFIN: Go ahead.

DR. DAUM: Dr. Kester is next, then Dr. Wharton.

DR. KESTER: I was struck by the comment just made that we may, depending on the approaches, generate a lot of data that we don't really know what to do with. Certainly, as one envisions more advanced and complex methods of sequencing, that may, in fact, be the case. I think I heard before mention of relooking at perhaps better animal models. I go to the point that I made in my question before at the earlier session, that we also try to consider optimizing, improving, or enhancing our functional assessments of these, whether it's observational periods, different animal models, or different approaches to these functional assessments, in addition to the genetic sequencing and other types of analyses that are either being done or being entertained.

DR. WHARTON: Thanks to the very deliberative process that FDA has engaged in over the years, I think there's a really good foundation for this discussion today, which is good, because I think it's sort of a difficult and complex discussion to have.

But I haven't heard anything today or seen anything in the background material that makes me see anything specific that we actually are concerned about in terms of something that would pose a real safety hazard, beyond the issues that already have been identified and addressed in the approaches that have been taken -- intact cells, residual DNA, and adventitious agents. There have been very thoughtful approaches taken to all of those. The question really is, what's different about this that requires doing things differently?

I have been struck by some of the things that seemed historically like they were a conservative way to do it -- like to have a preference for primary cell lines -- that now don't look like a conservative way to do it, because, in fact, we can't characterize them so well and there may be less consistency, as I understand the discussion today.

In thinking about these particular cell lines, I guess what I would like to have is a very good understanding of the cell line, as others have mentioned. I agree with getting the best understanding possible of the mechanism of oncogenesis -- not that it's easy to describe what one would do with that, but it seems to me that it's part of understanding the cell line that we're using and making sure that there is nothing new or novel that is not understood, that it's fitting into one of the existing paradigms, so that we think, by handling this the way we handle other cell lines, we have addressed whatever safety issues might be present.

I'm strongly in support of using some of the newer technologies to make sure that we don't have more in the cell line than we think we do, acknowledging that doing that, given particularly where we are with it now, we are likely to find out things we don't understand. But I think it's better to do it and then to try to figure out what it means than to not do because we're not sure what we will do with the information.

I'm one of the people who has to talk about these things after it's all done. When I'm talking about a vaccine, I would like to have a really good understanding of where the cell line came from that it was grown in and what exactly is in that cell line and how it works, in order that I can have a good understanding of what it is that ends up finally in the vial.

DR. DAUM: Before I call on our next person with a hand up -- now I have two -- let me ask you, what's different, in your -- I'm also one of those people that has to talk about this after the meeting is over. The question is, what is different, in your mind, with respect to your comments, about these vaccines that come from human tumor cell lines from, generally, vaccine development?

DR. WHARTON: It seems like the risk-mitigation strategies are the same, but I think there is a level of concern that comes with using a human tumor cell line that is not necessarily based on science, that's based on the fact that it just seems like there could be something there that maybe we don't understand. That's why I think understanding everything about this that we can is really important.

DR. DAUM: Thank you very much. I have Dr. Gellin and Dr. Piedra.

DR. GELLIN: I'm going to build on what Melinda started. To me, this is a metaphor for **what FDA does all the time. They have to look at how to evaluate important products and to, to the degree that they can, ensure that they are not only effective, but safe. And you are never going to have 100 percent of the information. They do this in drugs, they do this in foods, and we're seeing it here as well. With new technology, we're just going to see more of this.**

Phil's presentation this morning highlighted some past events. There was this SV40 and, more recently, the porcine circovirus, where, because of technology, things showed up and then there had to be an assessment of what it meant when products had already been in use.

I just want to emphasize the latter part of this. I think you have to do everything possible to try to ensure that you can make this assessment. But ultimately you're not going to know 100 percent. Therefore, emphasize the need to continue to have systems in place to continue to look to see whether or not something has turned up. We saw that clearly with SV40 long after. So you can at least make an assessment of what might have been a latent problem more quickly than you otherwise would have. We have a similar conversation with the porcine circovirus.

So just remember that there is so much that can happen pre-licensure, but there need to be some mechanism in place to continue to look because of the kinds of questions that will come up because of the nature of the cell line.

Then I'll just put this as a question for a placeholder, maybe for Phil or FDA: Bob earlier raised the question of how this is being regulated with other products. I guess the question is whether or not there is a similar conversation going on with veterinary vaccines. If so, is that going to be informative for what we're trying to do?

DR. DAUM: Thank you very much, Bruce.

Dr. Piedra.

DR. PIEDRA: A lot of emphasis has been placed on oncogenes and understanding the mechanism behind the cells. One area that I have not heard too much has been that these cells have come from -- I won't say a muddled background, but we don't know exactly the pathway, how they came to be. They may have been contaminated with fetal bovine serum or other materials. With one cell line in particular -- but this would apply to others -- the issue of prion came up. One of the questions that I did not hear well in the evaluation for the cell line is how well the animal studies that one does or the testing that one does do to evaluate for the potential for prion disease.

DR. DAUM: I'm very glad you raised that question, because I was concerned that it might get buried in the cracks. I guess the question for you first is, what do you think should be done? These are the safety concerns we have.

DR. PIEDRA: I don't believe the mouse is an appropriate model for prion, but people who are experts in this area could talk further there. But understanding how best to evaluate -- from what I have seen here, the sensitivity didn't seem very satisfying. One would need to think about sensitive models, and they take time. I don't think they are four to seven months. They may be one to two years.

DR. DAUM: I don't know if any of you noticed, but what I'm doing is, people who stick their hands up get called on right away, and I'm gradually working my way around to make sure that everybody says something. You know who you are.

Dr. Cheung is next.

DR. CHEUNG: I think our discussion kind of focused on what Bruce just said. There are a lot of things to be done in post-licensure. The field of the BSE prion is really, in terms of sensitivity, of detection, is pretty poor. I think we really need to keep on following the field. I think that's a major concern, in addition to the issues of DNA sequencing and bioinformatics and so on.

But I do like this morning's presentation of the DTS, which is the digital transcription subtraction technology. I thought that was pretty neat, that you can actually sequence filtering all these things out and came down to two viral genomes that they can get out of 10,000 samples. I think we need to keep track of this technology to be able to really fit into the goals of what we are looking at.

DR. DAUM: And I am mindful of the fact that you have to leave in 13 minutes, so you'll get priority if you stick your hand up again in the meantime.

Dr. Brady.

DR. BRADY: I have two questions or comments. We previously talked about surveillance of the cell lines for safety and efficacy. I was just wondering how it's determined, the length for observing those animal models. How is the four to seven months determined versus one or two years, which kind of dovetails onto some of the other disease states that we may or may not be currently studying?

The second comment or question, as consumer representative and as a clinician: How is this going to be able to be accepted by the consumers as far as -- I think it goes back some of what you were saying earlier. As soon as you hear "a tumor-derived cell line," how do you explain that, put the public at ease?

We have an example with

DR. DAUM: I was waiting for a break in the action to bring that issue up. Can we deal with your first question first?

I guess the question for you is, what do you think should be done with respect to question number 1, which is safety concerns with respect to these vaccines. **Are they watching**

these animals long enough? Should it be longer? Should the manufacturers be doing more extended studies?

DR. BRADY: I guess the question would be, is there an industry standard for -- is four to seven months kind of the standard for other vaccines that have been developed and things like that?

DR. DAUM: Dr. Peden wants to comment on that question.

DR. PEDEN: No, I don't, really --

DR. DAUM: Well, you indicated a desire to comment.

DR. PEDEN: It was really a compromise. The original assay is maybe a three-week assay. It was clear that not all tumors came down in a tumorigenicity assay in that. So then we extended it for cells that take longer to form tumors. But there's a limit to what you can do. The lifetime of a nude mouse is probably two years, at the very outside. But then you are asking sponsors to keep these animals on for many years. The cost may not be commensurate with the reward here. I think, if they don't come down soon, then keeping them on for much longer is not going to help.

the Vero cells that did sometimes take a year or even longer -- eight months to a year or more -- to come down with tumors.

Does that make any sense, to do these assays when we're not even sure what this assay is telling us? Is it relevant to safety that a cell forms a tumor after a year, a year and a half? I think that is open to some discussion.

The four to seven months was really a compromise between people not wanting to spend the money looking after the animals for long and getting an answer where you can think that's the window when those tumors are going to come down.

For the oncogenicity experiments, we know that almost all the tumors that we look at come down within eight weeks. The Ras-Myc and the complementing E6 and E7 plus Ras -- with the DNA, they come down very quickly. They are going to come down. So leaving those animals for a long time may not be useful.

I think, again, it's this compromise. If you look at the WHO document, it's about the same amount of time, four months or so. That's how those times were decided.

DR. KRAUSE: Just one more thing to add there. There is also, especially with the nude mouse, a rate at which tumors will spontaneously form. The longer you keep the animals, the more likely you are to get results that are very difficult to interpret. Part of this is the fact that the rate of spontaneous tumor generation goes up with age. But if you were to hold the animal that long, then you would be obligated to investigate those tumors, for most of which you might not be able to come up with a clear idea of why they occurred because of that background tumor rate.

DR. COOK: I would just follow up on what Dr. Peden said about the animal studies and several of the questions that have been asked. I would personally divide the animal studies into two things. One is, testing of tumor cells for tumorigenicity is a distinct thing from all the experiments that Dr. Peden talked about that were very interesting in terms of testing DNA or spiked Myc-Ras plasmid mixed with DNA, that sort of thing. If you divide those up, I think it's quite reasonable to test nude mice for tumor induction, as long as you have some kind of standard. I don't think you have to test the mice for a year. If they are going to get a tumor with an inoculum of a high dose of an immortalized cell, they will usually get a tumor in six months. Beyond that, they start getting skin disease. They are very expensive to maintain. They have spontaneous tumors. Lots of things happen that make it impractical. So there's a limit to just watching them forever, just in case you pick up that last tumor. Dr. Peden's point about "so what" -- if you get out there at a year and an animal pops up with a tumor, you probably will spend \$10,000 trying to find out that it was a spontaneous tumor.

So then you say, all right, we're going to do nude mice for tumor testing just of find out if this tumor cell line or immortalized cell line or whatever it is will form tumors or not, yes or no. In that is buried the other question: **Are all nude mice created equal? We and, I'm**

sure, Dr. Peden and I know Dr. Lewis have had the experience that if you get nude mice from different sources, you can get different results. Not everybody in the room knows that not all nude mice are the same nude mouse background.

So there needs to be some kind of standard, something like an index cell line, where you say that the TPD₅₀, the 50 percent endpoint, in a nude mouse of a Swiss Webster background or whatever you choose to be the standard that everybody uses, is 10^{4.5}, and if it varies by more than a log one way or the other beyond that, these nude mice are not suitable for FDA purposes, for regulatory reasons. There's something different about those mice, and then you are going to go off in a different direction.

I think knowing who the vendor is, knowing what the background of the nude mice is, coming up with a standard length of observation and some kind of measure about a cell line -- some characterized cell line, I don't care whether it's HeLa or A549, or whatever it is -- that will induce tumors at a given rate makes some sense.

The other side of the coin, the testing of DNA for tumorigenicity in animals, is problematic as well. If it's going to be the CD3 epsilon transgenic mouse, those are not so trivial to maintain or to breed or to afford. So settling on something -- and Dr. Peden and colleagues are well along the way in trying to find something -- it would be nice for people to say, okay, if we use this mouse with spiked Myc-Ras DNA or whatever the combination turns out to be, that's okay. But it can't just be newborn this and newborn that and three different species. I think eventually you're going to get so much information, you'll go blind. It would be good to have some kind of agreement on what everybody should do.

DR. DAUM: So you are appealing for more rigid standardization of the testing that is done. Dr. Coffin.

DR. COFFIN: Just a very quick comment to that. Actually, it's even worse than that, because with at least two of the major nude mouse colonies, the animals you get are deliberately outbred. So even two mice that you buy at the same time will have some genetic differences between them.

DR. DAUM: So you're appealing for tighter standardization of the tests that are done also.

No one so far has addressed -- if you look at the questions and listen to the discussion that we have had so far -- do you want to say anything before you go?

PARTICIPANT: (Off-mic)

DR. DAUM: Okay. Then we'll continue the discussion, I think, without the summary, if that's okay.

Nobody yet has said anything about how your views on these safety concerns would differ in live versus inactivated vaccines. We heard some things this morning that would indicate that there might be different approaches to these two different situations.

I see Dr. Air's hand up. She hasn't spoken about this afternoon's issues yet, so I'm glad to call on her.

DR. AIR: I was waiting to get to this point. To me, there's an enormous difference between live versus inactivated. Inactivated -- it's just a biochemical problem to clean it up, and you really should get rid of all the cellular DNA without too much trouble. The live is an enormous problem. We all know you can't clean it up in the same way. But on the other hand, what we have going for us is that the live is usually, hopefully, given orders of magnitude less actual virus than the inactivated or subunit vaccine, and so the amount of contaminant is going to be a lot less.

In the end, there probably isn't too much difference, maybe, but I do think that every effort should be made in the inactivated and subunit vaccines to make sure that they are as clean as they can possibly be.

DR. DAUM: So let me push you, in gratitude for raising your hand. How would your safety concerns differ, and how would you translate that into manufacturing approaches that would differ, between the two types of vaccines?

DR. AIR: It's all to do with the level of cellular products that are still in the manufactured product. I would set those levels differently for the inactivated vaccines to the live vaccines. I think they have to be.

DR. DAUM: Other comments on this live versus inactivated issue?

DR. COFFIN: Actually, another level is intact virus versus subunit vaccines, even among inactivated ones. In subunit vaccines, you are breaking things apart much better. In an intact virus vaccine, whether it's live or killed, the DNA that's in there is much more likely to be in chromatins, sub-nuclear fragments, and structures that may be very resistant, in fact, to treatments like Benzonase and that kind of thing as well. So that does, in fact, incur this additional level of difficulty perhaps.

DR. DAUM: Do you have different safety concerns for these different situations? That's the question.

DR. COFFIN: I think one at least needs to investigate that issue in these contexts. That actually gets to the question that I was going to ask Dr. Peden before. We have very, very sensitive assays for the level of DNA contamination in a final product. Assays for the size of the DNA fragments, as far as I know right now, are extremely insensitive. Can you conceive of much more sensitive assays that would actually allow us to address this issue more directly?

DR. PEDEN: People have been using PCR primers of different sizes. But, of course, once you get to a certain size, a PCR assay is not that useful. The answer is, yes, I think there will be some improvements in that. What they do, I think, is maybe 1,700 or 2,000 base pairs, above which you don't get a signal, and then people say that's good because that means that all the fragments are below that. But the sensitivity goes down a bit, so I think it is a problem of actually quantifying how much of the DNA is above a certain size and how much is below. People are using capillary electrophoresis with various detection systems. That's more sensitive than gels, but it still doesn't really get at the -- if you have very small quantities of DNA in your vaccine, you can't use that assay either for that.

Does that help?

DR. COFFIN: Would you agree that that's an issue that does merit at least more investigation to try to get something --

DR. PEDEN: It might, but a lot of people do it on the box where the DNA -- before they dilute them and distribute them and things, where the DNA is at high concentration. I think if you do that, then you have a higher sensitivity.

But I'm not disagreeing that improved techniques would be useful.

DR. MARCUSE: I have been quiet in part because when I first saw these questions, it harked me back to my final exam time the last semester in college, when I had cut a few too many classes and couldn't respond directly to the questions asked.

Today has been a real education for me on these issues. As someone who is involved a lot in issues about public perceptions of safety and risk, there is just a giant null-hypothesis problem here, in the sense that you can't prove it's safe. I guess the hardest thing -- I heard repeatedly that negative results are not necessarily reassuring, which makes it more complex yet.

The key point to me is the gap between this science and the knowledge base of most clinicians and the need to begin to bridge that gap long before licensure and, in fact, create a basis for understanding. There's just a desperate need for a *Scientific American* type of article that summarizes what we have heard today that is made available to clinicians who will use these products.

I guess there are two other things that have come to my mind. I would to hear a similar session as today's on TSE agents. That kept being alluded to, and I got a sense that there was not nearly so robust an approach. That is concerning to me.

The last thing that I wonder about is, how have other regulatory agencies in other countries approached this? Are there different philosophical approaches that have been expressed in other

forums?

DR. DAUM: As usual, I find the points that you raise very compelling. I think one question I would have that I would like the agency to respond to is whether there could be some integration across at least US government lines -- but also I take your point about the international community as well, and perhaps the veterinary community as well -- in terms of pooling resources about these ideas that we have bandied about today. Other people are making vaccines. Other people either have used tumorigenic lines or will. I wonder if there should be more sharing in an open public forum of some of these ideas and concepts.

The obvious thing that occurred to me in what we heard this morning and also in open public hearing is that the cancer vaccine developers have to be wrestling with these same issues. I would really like a chance to share with them a lot of the knowledge that has been talked about at this table today.

So I would actually push for either a joint meeting or some kind of conference within the governmental agencies to pool ideas and resources. I'm sure they have ideas we haven't thought of and perhaps vice versa as well. That's a suggestion, based on Edgar's comments. I think the point is well-taken.

Other comments? Dr. Hudgens.

DR. HUDGENS: I don't have any particularly insightful comments. I agree with most of the things that have been said.

DR. DAUM: You can make some non-insightful comments.

DR. HUDGENS: Yes, these will be very un-insightful.

I guess the first one is with regard to the MPS technology. I thought that was very exciting. In these high-throughput situations, these sort of multiple-comparison types of problems come up. It's an interesting statistical problem. Usually one worries about false positive rate or false discovery rate when you look at all of these different sequences. To address that in this case, the suggestion is to think of the results from MPS as a screen, and then there would be some sort of confirmatory test -- a PCR assay or something of that nature. That sounds quite reasonable to me.

But it strikes me that the false-negative issue occurs here, maybe more so than in other settings. What sorts of assurances do you have, when you declare that a sequence is not matching, that that's the correct conclusion?

I would echo the sentiment that strong bioinformatics support is going to be needed in that room. Just thinking about the science, I think some of the statistical issues, the analysis issues, might be a little different than in other settings, other high-throughput settings.

In terms of safety concerns, there are sort of two sub-questions for the Committee that I haven't heard discussed. One is, if a vaccine is developed using a human tumor cell line and it's allowed to go into Phase I study in humans, what would be the endpoints of that trial, and would those safety endpoints be any different than any other Phase I vaccine trial?

The second was --

DR. DAUM: What do you think they should be?

DR. HUDGENS: I don't know. As a statistician, when I have helped design Phase I vaccine trials, I always ask the investigators what the endpoints are. You try to push the investigators to be very specific about those endpoints. I feel like we have been upstream all day and we haven't talked at all about how you would actually design the Phase I trial if we got through all the other issues we have been discussing. Would you change the safety endpoints? Would you add endpoints, in addition to the usual suite of safety endpoints?

The other part is whether or not you would do anything different in looking at a preventive vaccine versus a therapeutic vaccine. I don't think we have had much discussion about that.

DR. DAUM: I think the first question sort of boomerangs back to you. Should the endpoints be any different if we're using one of these vaccines versus another, in terms of safety or in terms of effectiveness?

DR. HUDGENS: Typically we don't look at efficacy in a Phase I trial. The primary endpoint would be safety and maybe immunogenicity.

DR. DAUM: Fair enough. You can answer any part of the question you like.

DR. HUDGENS: The experts should weigh in here, but I'm guessing the safety concerns we have are ones that you typically wouldn't observe in humans on the time scale that a Phase I trial takes place on. We are worried about these vaccines causing cancer in people many years down the road, well beyond the conclusion of the Phase I study. I don't know if there would just be added surveillance and long-term follow-up of anybody who participates in these studies, more so than there would be in a Phase I study that used a cell line that wasn't based on tumors.

DR. DAUM: Can I push you one more time? What would you add? What would you survey? We need to advise the agency. We can't sort of say we're worried about this.

DR. HUDGENS: I'm asking you guys these questions. What would somebody who studies cancer and sees cancer patients -- what sort of early endpoints would they be looking for?

DR. DAUM: Dr. Cook, do you want to comment on this?

DR. COOK: I'll say that I have no idea early, but the one thing that would be worth considering is to maybe go to school a little bit on the concerns about SV40 contamination of polio vaccines. For those of us who were polio pioneers back in the 1950s, who got vaccinated, there was an analysis. Now, it took a long time and not much came out of it and it led to a lot of controversy. **But certainly, if you are going to address this question about tumor risk of vaccines made in tumor cell lines, it's going to have to be a decade's question,** and it should at least acknowledge all the work that went into trying to analyze that problem, because that's exactly the question that was asked.

DR. DAUM: And to coin a term from Dr. Hudgens, it probably can't be a Phase I question.

DR. HUDGENS: Just to be clear, there wouldn't be surrogate measures available that would tell us early on that somebody appears to be at an elevated risk of developing cancer or developing a tumor.

DR. DAUM: If there are any.

I think we're getting near our ending point here. Dr. Marcuse?

DR. MARCUSE: I just want to make the observation again that it's perception of risk-benefit. I think it will be much more difficult if the first tumor cell-derived vaccines were intended for newborns as opposed to older children or adults.

DR. DAUM: What are your concerns there?

DR. MARCUSE: My concerns are that if you are giving a vaccine on the first day of life, you are dealing with a different immune system, in addition to the obvious issue of length of time that the individual will survive with the agent. I think that's something that one just has to be cognizant of.

DR. DAUM: I would like to ask Dr. Hudgens for his comment first and then ask the agency a question.

DR. HUDGENS: In terms of risk-benefit and public perception, I would think that the first-in-humans would be better received if it was in a therapeutic setting.

DR. DAUM: Dr. Krause and Dr. Gruber and Dr. Peden and everybody else -- Dr. Khan, Dr. Wilson -- are you getting what you want from this discussion or do we need to go into something we haven't touched yet? I would like to ask the Committee to comment on a couple

other areas, if you are satisfied with where we are.

DR. GRUBER: From my perspective, this has been a very interesting discussion. What I would like to hear a little bit -- some of the Committee members did speak out loud and clear -- I would like to hear a little bit more of the discussion in terms of the adequacy of current approaches to mitigate potential risk. There were some Committee members who spoke out that perhaps the issues with human tumor-derived cell lines and other cell lines that have been discussed here and previously in Committee meetings are not that different and the current approaches are adequate.

Does the agency have to take it that, okay, we keep with the current approaches and we look into the feasibility of using the new technologies in discussions with manufacturers? Or do we look at them as an adjunct technology at this point?

There were some people who, I think, did say that. But I would like to have a little bit more discussion on that. That is, current approaches -- is it the way to go with this type of cell line or do we have to take a closer look at the new technologies?

DR. DAUM: You are talking about risk to the patient here?

DR. GRUBER: Well, the risk to the patient, I'm sorry, no. I think that's a different question. Do you want me to comment on what Dr. Hudgens commented on?

DR. DAUM: Whatever you like, sure.

DR. GRUBER: It is my perspective -- and I would like to see what my colleagues say -- I listened to your concern with great interest. But from my point of view, we took today to the Committee the question -- we are having human tumor-derived cell lines. How do we have to characterize them? How do they need to be tested so that we can use them as substrates to manufacture vaccines? If in the end of all the testing the assessment and evaluation of that cell substrate using current approaches or combined with new technologies -- if at the end of all that characterization, there is still concern about a vaccine produced in such cell substrate having something integral to it that it may induce a tumor or cancer in a human subject -- if, after all the characterization, that concern is sufficiently out there and significant, I don't think I would want to use that cell substrate for the production of that vaccine in the first place. The goal is really to be reasonably convinced that after all the characterization that is done, I don't have that concern about that product or that cell substrate for inducing some cancer in humans when I use it to produce a vaccine in it.

I also don't see the feasibility in Phase I studies to really get at that concern. I think that's just not feasible. You are looking at the duration of the trials. You are looking at the endpoints. You are looking at the number of subjects you would enroll. That, I think, is not doable.

If we entertain using these cell substrates, I think we have to be reasonably confident to say that the testing that we are doing on the cell substrate is sufficient and adequate so that we put these worries to rest by the time we go into clinical trials.

DR. DAUM: Marion, we're going to have trouble giving you a definitive answer to that question, because it's going to depend on, first, what the risk is from the disease we are trying to treat and prevent. Secondly, it's going to depend on what we believe the adequacy of the testing will lead us to. I think we have heard over and over again today that the testing is extensive. I think FDA is to be congratulated for the extent that they do it and present it in the documents we have reviewed. Nevertheless, we're always left with, well, maybe if we had a little more technology, maybe if we did something else, we might find something.

I think the best we're going to be able to do is tell the agency that the risk appears to be very, very low, and secondly, that you are obviously on top of this and doing the right kind of approach and the right kind of testing and, perhaps more importantly, revising it as new technology becomes available.

So I'm not sure that we can give a certainty -- there's no risk, don't worry about this. It's

sort of a brave new world. We're all doing it together. But I think you are doing a beautiful job.

I don't know if that's helpful or not.

DR. GRUBER: It is helpful. I didn't really want 100 percent certainty. I think what I was expressing is a perspective that I thought about as I heard this discussion here. No, I don't expect 100 percent certainty here. I think what I have heard today was a good discussion. I think it was very helpful. We had a lot of good comments and suggestions about what should be looked for and looked at. Thank you.

DR. DAUM: Dr. Lowy, then Dr. Cook.

DR. LOWY: My perspective is, what do you want to do that is qualitatively the same as looking at other substrates and what do you want to do that might be qualitatively distinct from looking at other substrates?

In terms of looking for adventitious agents, it seems to me that the technology has evolved so that, irrespective of whether the substrate is from a tumor line or some other cell line, you want to use the state-of-the-art technology in order to rule out the presence of adventitious agents.

What I think is qualitatively different about the tumor cell lines is the fact that they can cause tumors.

So demonstrating, as you are already describing, how many cells you need to put into a test animal to give rise to a tumor and then, when you go through the manufacturing process, how many orders of magnitude of protection you have so that, under some system where you go to a much higher level, you don't see the development of tumors -- from a biological point of view, it seems to me that's something qualitatively different that you want to do and then, superimposed on that, to look at the issue of clearance, perhaps with oncogenicity more in mind than you would have for a continuous cell line that was not inherently oncogenic.

DR. DAUM: Thank you very much. I think that was really well put.

Dr. Peden, then Dr. Cook.

DR. PEDEN: So, Doug, the issue of tumorigenicity is an interesting one. These cell lines are no more, and perhaps less, tumorigenic than maybe the tumorigenic MDCK cell line that was used before.

I agree with you, by the way, completely about trying to make the calculation for how much per dose you are giving them. In fact, we are trying to do that, but it's not so easy because we don't always know the dose of vaccine made in a cell line. So I think that's important, what we think.

What I'm still not hearing -- and maybe you told me and I just didn't hear it -- is, is there something qualitatively different about a cell line derived from a human tumor and something like MDCK cells, which can be highly tumorigenic, but are made through spontaneous passage --

DR. LOWY: I was trying to make a distinction between non-tumorigenic lines versus tumorigenic. I'm not sure that I would make a qualitative distinction between MDCK and the others.

I see, for example, an advantage, say, with A549, that you have mutant K-Ras, which you could follow directly, whereas with MDCK, it seems to me that there's not a clear oncogene that you could follow. Otherwise, it seems to me that the issues are really qualitatively similar.

DR. PEDEN: Is that true for Dr. Coffin and Dr. Cook? Do you think there is anything different about these cells or a tumorigenic -- spontaneously arising tumor?

DR. COFFIN: Yes, I would agree with Dr. Lowy. The one thing that I raised before about predicting some of these tumor cell lines is that their history is much longer and much more -- one has to be much more alert to the possibility that something untoward is happening in terms of the appearance of latent viruses and so on in these than might have been true with cell lines that have been around for a much shorter time and were originally derived in a laboratory setting that is fairly well defined. HeLa is now over 60 years old. Lots of things could have happened.

But, in general, I think the standards of approaching the safety issues and how you could assay for them shouldn't be qualitatively different in the two types of cell lines.

DR. DAUM: Staying on the same subject, but bringing back comments that Dr. Brady and Dr. Marcuse made, one of the differences that has to be put on the table, because it has been in the room, but nobody has said it directly -- a few people have said it directly -- is the scientific community's perception, including the practicing medical community, and also the lay public. They are going to hear that we are recommending or that you are doing or that the manufacturers are making vaccines with tumorigenic cell lines and say, oh, my God, even if there's no scientific basis to say, oh, my God.

I think we're better off heading that discussion off at the pass and starting some of the ideas that Dr. Marcuse said, which is a *Scientific American* type of article informing practitioners -- and I had some lunch conversations with other people that talked about some of the vaccine-consuming community that might perceive a very great difference even if scientifically there isn't one.

I think those issues are going to have to be addressed, because they are better headed off at the pass than dealt with in a reactionary way.

DR. COFFIN: I have thought for a long time that we are extraordinarily lucky that HIV was not present in the monkey kidneys that were used to grow polio vaccine.

DR. DAUM: We are indeed.

DR. COFFIN: Had we been using HeLa cells at that time -- which we could have been, actually, I think -- to make vaccine, we would have been much safer than, in fact, we were.

DR. MARCUSE: Framing a discussion, as we have learned, is everything. This discussion has to be framed by what the potential for new vaccines that require human cells is. You have to start with the positive here, which is what impact we can have with new tools, and then get into how we will mitigate theoretical risks.

DR. DAUM: I'm going to try to bring this to a close at 3:30, but we're scheduled until 4:00, and if there are enough comments, we'll go longer.

I have Drs. Cook, McInnes, and Piedra.

DR. COOK: Your comments are perfect, because that's what I was going to go to. I think the FDA ought to develop a project that's called "Think like a Patient." Think from that perspective. The last patient I saw in clinic Monday evening before I was getting organized to come here was an elderly couple who wanted to get flu vaccine. If they knew all of this, they would still have had the same question, which is, which one should I get? Is it safe? What do you recommend?

This is like PSA antigen testing right now, it's like breast cancer screening, and a lot of things that people are confused by because the data is confusing. But they are going to eventually come back to Dr. Marcuse's point. **You need to have people who are going to be in the clinics every week talking to their patients or to the parents of their patients, explaining to them why they think this is a good idea. And they are going to have to be convinced.**

But they are going to have to understand the patient's perspective, which is, is it safe and will this help me? They are not going to care a whole lot, I guarantee you, about what it was made in, as long as their physicians, who they have trusted over the course of many years, tell them, I've thought about this carefully and this is what I think you should do.

If you start thinking like a patient instead of like what we all are, which is virologists and immunologists and biochemists and statisticians -- and we're trying to figure out how much of this, that, or the other is left -- you have to think about, what's in the end product? If you want to focus more testing, I would say, go out and get some vials full of the stuff that's made that is the vaccine and test it to death and then say this stuff is okay and then convince the physicians who are going to be prescribing it to convey that to their patients.

DR. DAUM: Dr. Krause.

DR. KRAUSE: I guess I'm struggling a little bit with this part of the discussion because it's

a discussion of how one communicates these issues and how the public will perceive them. But I'm not completely sure that we have a complete answer on the fundamental scientific question. So how can you communicate a scientific consensus that the product is safe unless we're sure that you, the experts we are asking to advise us, are convinced that it's safe?

I guess part one of that is -- I'll just ask it very directly -- do you have scientific concerns about aspects of these vaccines beyond the DNA and the adventitious agents? I think that's a critical question first, because if there's something that we are missing -- if the only concern about things that might not be DNA, which, as Dr. Peden points out, can be dealt with by adequate levels of clearance, and adventitious agents, which, if one uses the best available testing procedures -- then we get into the situation that Dr. Gellin pointed out that we are in where the FDA does have to make decisions, and you cannot have 100 percent certainty that something is safe, but you are sure that you evaluated something to within the best limits that one can and the limits of the current technology.

Is that enough to give you a scientific sense that these products are okay?

I think one can look at this in the context of the specific three products and the specific antigens that are being looked at. But one could argue that, for at least one of them, we're talking about a vectored vaccine, where, if this is successful, there may be the potential to create other vaccines, whereas if it's not successful, that potential might not exist. You have, I'm sure, for everyone in this room who represents a company, someone from another company who is watching this meeting on the Web and is trying to decide whether this is a viable approach for thinking about making new vaccines or not.

I have heard a lot of suggestions for things which might be done -- for instance, additional sequencing of the DNA or better assays for DNA size or standardization of animal testing and things like that. Those are things we can take under consideration in the context of this. But the critical question is really the scientific one: Are we addressing the real issues here?

DR. DAUM: I'm going to begin an answer to your question, but I'm going to encourage anybody at the table who thinks I don't have it right to disagree with me.

I think we have said to you -- although it's not one of your questions per se -- as clearly as we can that we think there's nothing *a priori* that puts a red flag in front of using a tumor cell line to develop a new vaccine. We want a careful search done for the adventitious agents that might be present in it, but you do that anyway. We want to hear about the nature of the cancer in the cell line and whether it can be followed and useful as a marker to pursue. You would probably do that anyway. We want more sequencing of any vaccine product that's coming down the line, but you would probably do that anyway.

But the answer to your question is that there's nothing *a priori* that would red-flag, from this Committee's perspective, a vaccine that's developed in a cell line like this. In case I don't have it right, I'm going to ask my colleagues to disagree with me and chime in. But I think we're saying that to you as clearly as we can.

I think there's a brave-new-world aspect to this that we have to deal with. I think we have to tell providers about it in a way that they get it. I think we have to tell the public about it in a way that they get it. But I'm convinced after hearing the data today and the discussion today that these cell lines are important in continued development of vaccines.

I'm a vaccine guy. They are wonderful to prevent infectious diseases, and they may turn out to be wonderful to treat infectious diseases. That remains to be seen, in my opinion. I hope I'm speaking for everybody when I say that that's the answer to your question. If not, please chime in now.

DR. KRAUSE: I think it does help very much to hear that explicit comment from you.

DR. DAUM: I think it's representative of our opinion.

I again want to commend the agency. I think you have been all over this. The guidance document, the briefing document, and the presentations today make me think that the

issues that we would think of and many issues we wouldn't have thought of have been addressed by the agency. I think it's superb work. And I don't say that lightly, as you know.

Dr. Piedra.

DR. PIEDRA: This is much downstream, but it goes with the issue of public perception. At the end of the day, information will need to be included in the vaccine safety information and the package insert. When one does animal testing and you see that tumors were induced at such-and-such with the cells -- let's say at 10⁴ -- will that type of information then be translated into there being a potential risk for induction of tumor, in the package insert or vaccine safety information?

DR. KRAUSE: I think we would have to work that out, depending on whether or not we believe there is a risk for potential induction of tumor. Listening to Dr. Gruber's earlier comment, I think we would be very reluctant, and I'm sure manufacturers would be very reluctant, to proceed if they really thought there was a significant risk of a potential induction of a tumor. Normally the package inserts describe ingredients in products which are safety factors, that have some role in assessing the safety of the product. So I can't answer your question directly, because it will depend on that final assessment. But the hope would be that if one could proceed with these cells, one would have addressed those issues as well as one possibly could.

DR. GRUBER: I would like add to this -- it goes back to the earlier point I made -- I think the minute that we think that we have to address any of these concerns in the clinic, we would not be using -- we would be very reluctant for a cell substrate such as these cells to be used for vaccine production, if we are not reasonably assured that the characterization done, as we have discussed today, is adequate. The minute you describe something in the package insert in terms of potential clinical safety concerns, I think that really precludes using these cell substrates.

That I don't think we would really elaborate on in a package insert, because these things should be thoroughly tested and evaluated in establishing the manufacturing process. In our review of the data, we will have to have come to the conclusion that these cells are safe for use for vaccine production.

DR. DAUM: Thank you, Dr. Gruber. Dr. Gellin.

DR. GELLIN: To Marion's last comment, this is a microcosm of what you do all the time. You go through your process. When it gets through the licensure process, you then recognize that it has been in X number of people, and there is post-licensure monitoring. We have an elaborate safety system that goes looking for things, because you want to continue to look -- not that you are expecting them, but you have a system in place to look. So it doesn't seem to be any different than your normal approach to bringing things to licensure and then follow-up.

DR. DAUM: Thank you. Dr. Cook.

DR. COOK: I'm not a regulator, so I will say just what I think in reaction to the comment about putting comments in a package insert about tumor cells making tumors in which vaccines were made. I would say, from a personal opinion, that you would have to be very careful about the degrees of separation between where the stuff comes from and what you are putting in the vial that the package insert is describing. It's not at all related, in a certain sense. I'm not 100 percent convinced that whether or not a cell makes a tumor in a nude mouse has anything to do with the safety of a vaccine that ends up getting made. There are concerns we have. It's a really interesting intellectual discussion. When it gets right down to what's in the vial and what the patient is going to ask me about, whether it's safe, I'm not going to back and say, well, you know, HeLa cells kill nude mice. It's not a very good analogy, but think about what we do in terms of making bacterial toxins or preparations related to that. You don't want to go to somebody and say, "You know, botulinum kills people. It can paralyze you. It's really dangerous," or, "Anthrax kills

people,” when you are trying to make some kind of an antibody against protective antigen or lethal factor.

The degrees of separation between what is being used to make it and what the product is in the vial have to be considered when you are talking to the lay person, or they are going to get completely confused and refuse to use anything. They do that already. They come in with a PDR. You can imagine how it is trying to tell them what to do.

So I would be very careful about that.

DR. DAUM: To come back to the agency’s question of whether this Committee believes it’s correct scientifically to go forward with the development of these vaccines, our answer is yes. I think we need to work out some of these communication issues and education issues. It’s, to some extent, a whole new discussion. I’m not sure we can resolve it easily today.

Dr. Hudgens.

DR. HUDGENS: Just a minor follow-up on Dr. Cook’s comment. It seems like you want to do experiments in animals with the cell line and also experiments in animals with the vaccine, and you want to distinguish those two. In the package insert you could say that and say that the vaccine itself did not cause tumors in the animals, and make that very clear.

DR. DAUM: I don’t know that our charge is to micromanage the package insert today. As I said, I think that’s a new discussion, with lots of issues that we haven’t really aired completely.

We’re getting very close to the end. We’ll call on Dr. McInnes.

DR. MC INNES: Bob, thank you for your summary. I feel like I need to leave with a clear statement about what I think are the guiding principles. I think for all vaccines and the way FDA regulates them, we have always embraced every practical technique to minimize the risk of transferring infectious factors in vaccines. Now we’re sort of expanding that to minimize oncogenic factors by vaccines. The practical technique is important because there are lots of theoretical things that might not be feasible.

But I think you take pragmatically what we know and we build on that as time goes by. In the intervening 14 years since your first consultation, we have embraced new technologies, refined animal models, refined schemas.

So it seems that in 2(c), the potential use of new technologies, even though there are challenges to using the new technologies, they have to be embraced and we have to continue to try to learn from them and struggle through that learning curve.

But I think the guiding principle remains that we need to do everything we can to minimize the risk of transferring infectious and oncogenic factors by vaccines. That is the best that I feel I can contribute.

DR. DAUM: Since you thanked me for my summary, I will now thank you for your summary.

Unless there are further issues or things that haven’t been said, I would like to adjourn the meeting. Seeing none, I declare it adjourned.

(Whereupon, at 3:40 p.m., the meeting was adjourned.)

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