UNITED STATES OF AMERICA
DEPARTMENT OF HEALTH AND HUMAN SERVICES
FOOD AND DRUG ADMINISTRATION
CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

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VACCINES AND RELATED BIOLOGICAL PRODUCTS
ADVISORY COMMITTEE

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OPEN SESSION

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WEDNESDAY,

NOVEMBER 28, 2001

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The Advisory Committee was called to order at 1:54 p.m., in the Versailles Ballrooms I and II, of the Holiday Inn-Bethesda, 8120 Wisconsin Avenue, Bethesda, Maryland by Dr. Robert S. Daum, Chairman, presiding.

PRESENT:

DR. ROBERT S. DAUM, Chairman
DR. WALTER L. FAGGETT, Member
DR. BARBARA LOE FISHER, Member
DR. JUDITH D. GOLDBERG, Member
DR. DIANE E. GRIFFIN, Member
DR. SAMUEL L. KATZ, Member
DR. KWANG SIK KIM, Member
DR. STEVE KOHL, Member
DR. PETER PALESE, Member
DR. DIXIE E. SNIDER, JR., Member
DR. DAVID S. STEPHENS, Member
DR. JUAN FELIX, Invited Participant
DR. THOMAS FLEMING, Invited Participant
DR. RALPH FREEDMAN, Invited Participant
DR. MICHAEL GREENE, Invited Participant

PRESENT: (CONT.)

DR. PAMELA MCINNES, Invited Participant
DR. MARTIN MYERS, Invited Participant
DR. DENNIS O'CONNOR, Invited Participant
DR. SONIA PAGLIUSI, Invited Participant
DR. WILLIAM REEVES, Invited Participant
DR. ELLEN SHEETS, Invited Participant
DR. ELIZABETH UNGER, Invited Participant
DR. EDWARD WILKINSON, Invited Participant

I-N-D-E-X

Vaccine for the Prevention of Human Papilloma Virus

Session IV - Open Session

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

(1:54 p.m.)

CHAIRMAN DAUM: If everybody would take their places and settle down, please. Good afternoon, and welcome to the open session. We will begin by calling on Nancy Cherry for a conflict of interest statement.

MS. CHERRY: While I am reading this, it would be a good time for those of you who are at the table and standing to turn off your cell phones, or to put your pagers on silent.

The following announcement addresses conflict of interest issues associated with the Vaccines and Related Biological Products Advisory Committee meeting on November 28th, 2001.

Should there be any voting during this session on HPV, the Director of the Center for Biologics Evaluation and Research has appointed Drs. Thomas Fleming, Pamela McInnes, Martin Myers, Dennis O'Connor, William Reeves, Ellen Sheets, Elizabeth Unger, and Edward Wilkinson, as temporary voting members for this session.

To determine whether any conflict of interests exist, the agency reviewed the submitted agenda and all financial interests reported by the

meeting participants. As a result of this review, the following disclosures are being made.

Drs. Palese, Fleming, and Freedman each have been granted a waiver in accordance with current statutes which permit them to participate fully in the discussions.

Drs. Daum, Griffin, Stephens, Fleming, Freedman, O'Connor, Sheets, and Unger have associations with firms that could be or appear to be affected by the committee discussions.

However, in accordance with current statutes, it has been determined that none of these associations is sufficient to warrant the need for a waiver or an exclusion.

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

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

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

CHAIRMAN DAUM: Thank you very much, Nancy. It is an always scintillating Conflict of Interest Report. Well, I think we will now just very briefly ask the Committee to introduce themselves to people who have not been here all day. And, David, if you wouldn't mind, we will start with you.

DR. STEPHENS: Dave Stephens, Emory University, in Atlanta.

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

DR. GRIFFIN: Diane Griffin, Johns Hopkins.

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

DR. KIM: Kwang Sik Kim, Johns Hopkins.

DR. KATZ: Samuel Katz, Duke University.

DR. PALESE: Peter Palese, Mount Sinai School of Medicine, New York.

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

DR. MCINNES: Pamela McInnes, National Institute of Virology and Infectious Diseases.

DR. O'CONNOR: Dennis O'Connor, Clinical Associates, Louisville, Kentucky.

DR. REEVES: Bill Reeves, Centers for Disease Control and Prevention.

DR. GOLDBERG: Judy Goldberg, New York University.

DR. FLEMING: Tom Fleming, University of Washington.

DR. SHEETS: Ellen Sheets, Women's Hospital, Boston.

DR. UNGER: Elizabeth Unger, Centers for Disease Control and Prevention.

DR. WILKINSON: Edward Wilkinson, University of Florida, College of Medicine.

DR. FELIX: Juan Felix, Tech School of Medicine, University of Southern California.

DR. FREEDMAN: Ralph Freedman, Indiana Cancer Center.

DR. GREENE: Mike Greene, Massachusetts General Hospital, Boston.

DR. PAGLIUSI: Sonia Pagliusi, from Vaccines and Biologicals, of the World Health Organization.

CHAIRMAN DAUM: And I am Robert Daum, from the University of Chicago. And the FDA folks at the

table.

DR. GEBER: Antonia Geber, FDA.

DR. PRATT: Douglas Pratt, FDA.

DR. GOLDENTHAL: Karen Goldenthal, FDA.

CHAIRMAN DAUM: Thank you very much. I think we will now begin with the business of the afternoon, which is an open session to look at efficacy trial endpoints for vaccines for the prevention of HPV.

And we will begin by calling on Dr. Goldenthal from FDA to give us an introduction to the session, and presentation of questions. Dr. Goldenthal.

DR. GOLDENTHAL: Thank you. Before we get started on this afternoon's open session, I would like to present the two questions to our advisory committee and consultants.

The first is please discuss and identify the most appropriate endpoints for traditional approval of HPV vaccine intended to present cervical cancer. In particular, please discuss the use of the following endpoints and clinical trials intended to demonstrate the efficacy of HPV vaccines for oncogenic types, and the indications, for example, for prevention of HPV infection that these endpoints would

support.

Incident HPV infection by oncogenic HPV types; that is, at least one positive HPV DNA test result.

Persistent HPV infection by oncogenic HPV types. Regarding this endpoint, please also discuss the appropriate number of positive virologic results in the interval between positive virologic results.

Next is LSIL cytology associated with oncogenic HPV types. The next is CIN-1 associated with oncogenic HPV types; CIN-2/3 associated with oncogenic HPV types; and cervical cancers.

The second question is please discuss the use of the accelerated approval regulations for licensure of HPV vaccines for prevention of cervical cancers; specifically, please discuss and identify possible surrogate endpoints to support accelerated approvals.

In particular, consider the following endpoints. Incident HPV infection by oncogenic HPV types; persistent HPV infection by oncogenic HPV types; LSIL cytology associated with oncogenic HPV types; and CIN-1 associated with oncogenic HPV types.

In the context of accelerated approval, please discuss and identify possible end points for

the confirmatory trials. Thank you. I think we can now proceed to the first afternoon speaker.

CHAIRMAN DAUM: That was very succinct, Dr. Goldenthal, and thank you. Dr. Unger, if we could get started with your presentation on Natural History and Virology.

DR. UNGER: Thank you very much. I feel like we have covered this topic to some extent in almost every presentation, and so I am going to emphasize those things that I think are most important, and this will be a lot of repetition.

Papilloma viruses are not only human papilloma viruses. They are widely distributed in higher vertebrates, and there is a very tight specie specificity.

They are all very similar and they are non-enveloped, double-standard DNA viruses,a nd they have a small genom that is circular. It is a DNA genom, and the viruses look very similar under electron microscopy. They are 55 nanometer spherical capsid particles.

They all have tropism for squamous epithelium. That is, they all tend to be found in squamous epithelium, and they all are associated in their specific hosts with the formation of warts and

papillomas, which are just growths of that squamous epithelium.

The genom organization is similar across all of the species. Only one of the strands is transcribed, and the open reading frames have been named in relation to the bovine papilloma virus genoms, which was one of the first species studied in-depth.

The early genes are named E-1 through E-7, but there is no E-3 in HPV, and I have pondered that for a long while, and I thought that I would get that out of the way, just so that you are not looking for it.

The late genes are called L-1 and L-2, and these genes are those that code for the major and minor capsid proteins. This is a schematic, and you will see pictures like this repeatedly of the HPV genom in its episomal form.

It could be basically any of the genoms that I am showing, and this happens to be the one for HPV 16, and it shows the circular arrangements and the little P-97 is the most widely studied promoter region, and we will come back to that.

This simple Genom means that the virus does not have near enough genetic information to copy

itself. It is very dependent on the host cell for the machinery for replication, transcription, and translation.

And it is because of that that the viral functions are very tightly linked to cellular differentiation, and the promoter region that I told you is the one that is the one that is most often studied, but there is very good evidence that there are other promoter regions that can act and that they occur, and they get turned on as a function of the differentiation state of the cell.

All of the transcripts are also very poorly understood. They are all poly-cistronic bio-transcripts. That means that they are very long and complicated, and they have multiple splice patterns, and the promoter usage as I mentioned before is very linked to differentiation.

Now to talk about each of the little regions in-turn. The HPV URRs is the upstream regulatory region. It also is called -- sometimes you will see in the literature the long controlled region, or the non-coding region.

And this is really a very important because it contains multiple transcriptional and replication regulatory elements. And this is one area

where the viral genom interacts very tightly with the cell protein machinery.

Late genes, as I mentioned before, the late genes have the greatest genetic conservation among all of the types, and the L-1 is the major capsid protein. The capsid is formed of 72 pentamers of L-1, and when L-1 is expressed in systems, the protein itself will form spontaneously into viral like particles.

One thing that is very important about HPV proteins are that it is the native confirmational state that is most important and relevant for immune response.

And so the fact that this virus will allow the L-1 proteins to be formed into viral like particles has been used in studies of the immune response. L-2 is a minor capsid protein, and in functional studies it appears to be required for actually incorporating the viral DNA into the viral particle.

The early genes. I am just going to hit the high points on them, and there are many more functions that could be associated with them. But just briefly, the E-1 is essential for viral replication, and it is known to maintain that episome.

That is the small circular genom. The E-2 protein is important in transcriptional regulation, and it is a co-factor for viral replication. E-2 has been shown to have both promoter and inhibitory effects on transcription, depending on the state.

E-4 apparently disrupts cytokeratin, and it is important in the shedding of the virus. E-5 interacts with growth factor receptors; and E-6 and E-7 have been mentioned several times this morning. And they are the proteins that are characterized as transforming proteins, important in P-53 degradation and RB binding, respectively.

Now, of course, in the viral lifecycle, transformation is really not a part of the intent from a biologic point of view of the virus. So the function of E-6 and E-7 in the lifecycle of the virus appears to be one where the proteins prolong the dividing phase of the cell.

Viral replication and assembly occurs in the nucleus of the infected cell. Infection, as far as we can tell, is initiated in the basal epithelium cells. That is the renewable compartment in the epithelium.

Steady state viral replication in some

early gene transcription occurs, and this is the site of the presumed latent infection. And in animal model studies, it has been recognized that the virus can persist in this basal epithelium at very low copy number, with no phenotypic change; that is, no apparent change in the overlying epithelium.

There is some trigger, and again not very well known, which leads to high copy viral replication in late gene transcription and virion production, and this occurs only in the differentiating cells.

Now, viral integration is not a normal part of the viral lifecycle, but it is observed, and when it occurs, it occurs randomly in the host chromosome. That is, there is no one specific site in the cellular chromosomes where it occurs.

But in the virus, it occurs at a characteristic break point, and that is between E-1 and E-2. The disruption in this region means that the E-6, E-7 region, if you think back to that circular genom that I showed you, is still intact, and that the region may not be normally regulated, and it is thought that this abnormal expression of E-6, E-7 contributes to oncogenic progression.

Now, viral integration is associated with oncogenesis, but it is not absolutely required to the

best of our knowledge. The immune response to these viruses is also very complicated.

The infection as I have mentioned is a non-lytic infection. That means that the cells are not degraded in the host. The virus is released with the desquamating epithelium.

That means that the host has minimal exposure to the virions, but it is well characterized from observational studies that the immune system really does influence the outcome of HPV infection, and just as one simple example, immuno-compromised individuals have more problems with warts and persistence of warts, and with progression of cervical neoplasia.

There are both humoral and serologic responses that are identified. Interestingly, not all infected hosts -- that is, hosts in whom we have identified HPV DNA -- will have a detectable serologic response with current assays.

Now, in the human papilloma viruses, we have alluded to this many times before, there are a whole lot of them, and there is more than a hundred different types that have at least been partially characterized, and now there are more than 80 that are fully sequenced.

All of the typing is based strictly upon the nucleate assay sequence, and if there is less than 10 percent difference, it is considered part of the same type.

If it is less than two percent, and if the difference is on the order of two percent, it is considered a variant, and as -- and you can imagine that there has been a lot of investigation of sequencing of these viruses.

There is increasing consensus that the variance, that these small DNA changes could be important. The types are assigned strictly on a sequential number, which is based on the order of discovery, and this does lead to some confusion.

There is absolutely no relationship in the numbering to any kind of phylogeny, and that is just kind of the way the world evolved. HPV types can be broken down into two major phylogenic branches based on their different affinities for the site of infection.

HPV, the cutaneous types, affect the squamous epithelium. They are the ones that are responsible for the common hand and foot warts, and these cutaneous types probably are more numerous even than these mucosal types, but they have been less

studied.

And the mucosal types affect predominantly a non-keratinized squamous epithelium, and there are more than 30 of these types that are found in the anal/genital tracts. And these are further kind of broken down into what have been called low risk and high risk types.

Low risk types are those types that have been rarely or never found in cancers, and high risk types are those that have been frequently found in cancers.

Now, it is important to realize though that the high risk types are the most prevalent in the population, regardless of the disease status. And best characterized for HPV 16 and that is because HPV 16 is the most tightly linked with cervical cancer, accounting for -- depending on the population -- approximately 60 percent of the cases.

The E-6/E-7 polymorphisms could modify oncogenicity, but the variance have been cross-reactive in most ELISA assays. Now, there are some unique features about HPV that makes studying this virus difficult.

And first of all, if it has not been clear before, there is no simple in vitro culture method.

There is no such thing as culturing for the virus. Antibody methods lack sensitivity, at least in their current format.

So, therefore, diagnosing infection requires the detection of HPV genetic information, and that means that it requires a cellular sample from the site of infection, and only current infections can be identified.

Now, infection, I would like to put in quotes, because we are really not dealing with infection. We are dealing with the detection of DNA, and we are assuming that means infection.

Because we are limited by actually only monitoring DNA, our view of the disease is totally framed by the sample that is collected and by the assay that is being done to analyze it.

And this complicates the study of latent, occult persistent reoccurring infection, and it complicates comparison of different kinds of studies, and I am going to talk a little bit about the sample.

Tissue samples provide a direct correlation between observed pathology and the virus. These samples also include the basal epithelium, which is a place where occult infection might be.

The problem is that only a limited area is

sampled, and one biopsy is a very small area, and this is not a suitable method for any kind of screening or large scale study.

And therefore most studies that you are going to read about utilize exfoliated cytology samples of one form or another. This is a non-invasive approach for screening the population, but you have to realize that the sample is not specifically directed at a lesion.

It is collected to sample the area to be most representative. The quality of the sample is very dependent on the collection device that is used, and on the anatomic site that is sampled.

And a whole variety of devices are available, and the amount of yield of cells is varies with each of those devices. You must keep in mind that the basal epithelium is not usually sampled in this kind of approach.

Now, in women, the cervix is the sample that is most commonly used, and that is the site where the pathology is. And the appropriate sample in males is not at all clear.

Briefly, the estimates of HPV associated disease in the United States is that it is an extremely prevalent problem. This data really comes

from Laura Koutsky, and it is kind of a very broad extrapolation.

The bottom line is that probably 75 percent of the population has been exposed to HPV at some point in their life. Genital HPV is acquired around the time of sexual debut, and it is primarily a sexually transmitted infection.

And infection is usually transient, and it is usually not associated with symptoms. Persistent infection and the definition of that we can talk about, is the one that is most likely to be associated with the potential for neoplasia.

Now, there has been a consistent epidemiologic association of HPV with cervical cancer, and cervical cancer pre-cursor lesions. There are plausible biologic mechanisms for HPV oncogenesis, and it should be kept in mind that this oncogenesis is a rare event, with a long interval between infection and cancer.

So it is believed that infection alone is insufficient to cause cancer, and additional factors are required for neoplasia. There are certainly lots of questions about HPV infection, and one of the most common is HPV eliminated from the host.

HPV clearing again is monitored by DNA

detection and cytology samples, and negative results indicate that shedding is below the limit of detection, but the basal compartment may not be adequately sampled.

HPV can be detected in histologically normal margins surrounding growth lesions, and this is from older studies where people have done biopsies and sampled the basal epithelium.

The duration of infection is one that again we have heard discussed. I have given two studies which made an attempt to study incident infections, and then followed how long to clearing, and the median for HPV 16 in the Woodman study was 10.3 months, and for HPV 18 it was 7 months.

And in the Franco study, where they lumped the oncogenic types all together, it was 8.1 months. So how does this kind of data inform us as to what should we count as a persistent infection?

Currently, there is really no consistent on definition. What is clear is that in order to talk about a persistent infection, it requires detection of the same HPV type on more than one occasion.

And that requires a time specific assay then, and the time interval varies between 3 to 6 months, and in longer intervals people talk about

concerns about the potential for reinfection, rather than a persistent infection.

And consistent detection on each occasion is one approach, versus intermittent detection, which could then be a reflection of sampling and assay. Similarly, latent infection is a big question.

The formal definition of a latent infection is the presence of HPV DNA and the absence of virion production. But practically what it really means is a section of HPV DNA in the absence of an identifiable lesion.

And this is the situation of HPV DNA positive normal cytology. But it is also equated with occult infection. Now, the fact that there are multiple assays really complicate or multiple types really complicate HPV assays.

The sensitivity in the site type specificity of the assays all vary, and inter-assay comparisons are very difficult. The beginning of this field relied on Southern blot and dot plot and in situ, and that plot is like hybridizations.

And currently Hybrid Capture is another example of a direct hybridization. Amplification assays, such as PCR, can be either type specific or directed at a broad spectrum of the virus.

And just briefly about the hybrid capture assay. I feel like I can't ignore it. It is the current FDA approved test. In 1999, it shifted into a micro-titer format that uses liquid hybridization and it has a chemiluminescent detection.

The RNA probes react with the DNA targets, and it groups the probes into high risk and low risk, and does not have a type specific format at this point. The signal is semi-quantitative, but there is no control for the input amount of the DNA.

The probe mixes are as shown here, and this assay here really shows very good inter-laboratory comparison. It was really made to be a very vigorous clinical assay, but again the results are not type specific.

The hybrid-capture assay has been designed primarily to work with exfoliated cervical samples and the recommended collection includes the brush and the sample transport media, and the sample includes both endo-and-ectocervical cells.

And if you go through the calculations of how much the sample is put into the assay, approximately five percent of DNA from that total sample is assay for each probe group.

And again I think it is important to go

through not only how the sample is collected, and how the DNA is extracted, but in assay. HPV PCR assays target a much smaller part of the genom. It allows testing of samples with poor quality DNA.

There is some concern that small changes in the virus either from variants or integration may give false negative results, and the amount of DNA assay varies, and it limits the number of cells that can be sampled.

The type specific assays generally target the E-6, E-7 region. That's because their most type variation occurs in this region; whereas, the consensus assays generally target the L-1 region, the area where there is the most conservation.

When a consensus assay is used, the types then need to be further determined either based on hybridization, restriction, digest, or actual sequencing of the products.

This is just a schematic of one of the currently used PCR assays that relies on a consensus approach, and then a subsequent hybridization. This kind of format, using a line blot approach, allowed for the first time for investigators to look at the presence of multiple types. It wasn't a type specific single approach.

In this format beta globin is used as an endogenous positive control for amplifiable DNA, and it is in the process of PCR that the amplicons are actually incorporated into a re-agent that allows it to be detected, and then the hybridization occurs on a filter strip. And the color indicates which type is present in the assay.

Viral quantification is another concern, but at the very base the viral load is very difficult to estimate, because there is uneven tissue distribution of the virus, and there is variation in the kind of sampling.

And again because exfoliated cytology is not targeted to the lesion, the sample may or may not include more or less of cells that are actually infected.

This requires some measure of the numbers of cells that are actually put into the assay, and most quantitative PCR assays at this point are type specific. Just briefly about in situ hybridizations.

It is really the only method that permits directed visualization of the virus in a morphologic context; formalin-fixed, paraffin-embedded, and that is routine biopsy tissues can be used.

There is reasonable type specificity, but

cross-hybridizations, particularly at a high viral copy number, is almost unavoidable, and the results are very technique dependent. It is a very touchy assay. Integration status can be determined on this assay format.

Serology. Currently the most widely used is an ELISA-based format, with detection of antibodies that will react with the L-1 ELPs, and these assays have been used on both serum or mucosa samples, looking predominantly at IgG and IgA.

The assays are type specific, at least at low titers,and there is some discussion that when there are high titer present whether there is some cross-reactivity between the VLPs.

Reaction in this kind of assay format indicates a past or current infection, but longitudinal studies that have followed subjects for acquisition of DNA and then subsequently antibodies have found that 70 to 80 percent is sort of the maximum that end up to be positive, and there is a lag time of several months between the acquisition of DNA and the antibody.

The L-1 VLP assay formats vary. It can be direct or indirect, and the VLP production is not well-standardized, laboratory to laboratory. There

are different expression systems, and there are different ways to prepare this reagent and QC methods need to be developed.

In addition, there is really no good gold standard for setting the threshold for positive results, and there are relatively few inter-laboratory comparisons, although they are needed.

So basically I ended with the assays because I think assays are really are a way of understanding the virus, but I want to go back again to the sample, and reemphasize the difference that the varying sample approaches that can be made in the assay.

And the amount and how the DNA is extracted, and the amount that is actually put into the assay all will influence results.

CHAIRMAN DAUM: Thank you very much, Dr. Unger. That was a very helpful presentation. If there are a Committee question or two that specifically relate to the factual content of what Dr. Unger said, that's fine, but what I would like to do is get Dr. Wilkinson's presentation under our belt, and then begin reasoning with both of them, and have them see how they impact with the issues that the FDA wishes us to discuss. Dr. Katz.

DR. KATZ: In thinking about how a vaccine might work in relation to this virus, I am unclear about how is virus transmitted. You describe a virus that is very tightly associated with this cell.

Is it that shedded cells are transmitted to the individual, or are there free virions? What is the mechanism?

DR. UNGER: I don't have an answer. It is postulated that the cells are shed in that little packet of dried epithelium as it sheds, and then presumably the virions get out and get in, and in animal model studies, free virions, that the particles are infectious.

DR. KATZ: Thank you.

CHAIRMAN DAUM: Drs. Stephens, Pagliusi, and Freedman.

DR. STEPHENS: Can you help us understand or at least me understand the pathogenesis of HPV 16, and specifically is that a replication issue, better replication, or is it an ocogene issue? Is it a combination of both? And is there reassortment among papilloma viruses?

DR. UNGER: The last question is probably the easiest. There is no evidence of reassociation between the types. Then as far as why HPV 16 is so

different from the rest, that relies upon in vitro studies that have shown that the E-6/E-7 proteins do have different propensities to act in an oncogenic fashion in assays.

But all of the types have not been studied in great detail, and there could be other factors, such as copy number and amount of the replication.

CHAIRMAN DAUM: Thank you. Dr. Pagliusi.

DR. PAGLIUSI: I have a comment to your last slide, and so I agree that laboratory diagnostics is a very important tool, and I just wanted to say that WHO has an effort in this direction, which is in collaboration with the virion particles vaccine developers who are represented here by the NCI, Merck, and GlaxoSmithKline.

We are trying to develop some reference reagents to create the tools for the interlaboratory comparisons of results.

CHAIRMAN DAUM: Thank you. Dr. Freedman.

DR. FREEDMAN: Is there any evidence that an abnormality of the cells making up an epithelium with any degree of transformation that may preexist in the infection, may in fact increase the susceptibility to infection with HPV?

I am thinking of the work of Kurofsky a

number of years ago, which showed that malignant cells, for example, were more susceptible to infection with a number of viruses.

DR. UNGER: I don't know of any studies that would comment on that directly. There is evidence that the dysplastic state of the cell does affect its ability to be sampled in cytology. And as part of the dysplastic process, they also become discohesive, and so the greater degree of dysplasia, the easier it is to kind of scrap it off.

CHAIRMAN DAUM: Thank you again, Dr. Unger, very much. I am sure that we will be calling on you as a resource of information when we move into our discussion. Our next speaker will be Dr. Edward Wilkinson, who will talk about the clinical management and natural history of cervical dysplasia and related findings.

DR. WILKINSON: Thank you, Dr. Daum. It is a pleasure and an honor to be here at this very remarkable meeting. Our discussion here is on clinical management, natural history, and I will discuss some aspects of cytopathology, classification, and histopathology classification.

I will also show you some examples, colposcopic examples. In the screening data, you have

seen much of this, about 50,000 Paps smears a year, and about 3.5 million interpreted as normal, and about 800,000 LSILs.

And in that subset there is probably about 1,600 women who have cervical carcinoma, and about 2,500 HSIL groups, which is about at least 2,500 women in that subset that have cervical carcinoma.

Now, we recognize that cervical carcinomas primarily arise in the cervical transformation zone, which extends basically from the ectocervical margin of the original squamo-columnar epithelium to the presently identified squamo-columnar junction.

And when one reads the literature, you get sometimes confused at the squamo-columnar junction, and that that is not the transformation zone. It only marks the ectocervical edge of the transformation zone.

Now, in the transformation zone, basically what is occurring is the cervix is being remodeled. This occurs with the beginning of adolescence, and actually there is a bit of remodeling in the newborne, and in adolescents there is some major remodeling of the transformation zone.

And then after the first child, one normally has a process of reserve cell hyperplasia,

XX, and then mature epithelium. As far as the cervical intraepithelial lesions that arise within the transformation zone, the old concept was that this is a continuum, CIN-1, through 2, through 3.

And I think that concept has pretty well been disproven, and a bunch of work that I think has been demonstrated today has shown that really CIN-1 is a complex process, and many of these are transient infections.

And CIN-2 and 3 are quite a different issue, and CIN-3 for sure is a precursor as far as understanding the process. Now, from the classification from the World Health Organization, this is a classification of cervical intraepithelial neoplasia.

The WHO retains the terminology dysplasia, and so mild dysplasia, CIN-1, and this is a lesion confined to the lowest third of the epithelium. Moderate dysplasia, CIN-2 involves the lower two-thirds of the epithelium.

And severe dysplasia extends to the upper third of the epithelium, but not involving full thickness. And this is a CIN-3 lesion, and CIN-3 is also used for carcinoma in situ with full thickness changes.

Now, the Bethesda system, although it was first introduced around 1988, and modified in 1991, it has undergone recently the new Bethesda 2001 system, and these are the major issues, although there are a number of others.

The term "within normal" has been removed and replaced with "negative for intraepithelial lesion or malignancy." The term "benign cellular changes" has been eliminated. The classification has actually been eliminated.

And the interpretation of AGUS has been changed to atypical squamos cells, and this can be either atypical squamos cells of uncertain significance, or undetermined significance, and atypical squamos cells cannot exclude a high grade lesion.

And finally atypical glandular cells, and AGUS has been changed to atypical glandular cells, and this has been primarily to eliminate the confusion between AGUS and ASCUS, which many people confuse much to the negative impact of the patient.

Now, the new terminology is here in all of these slides, and I have these in your syllabus materials. So basically we have the negative category, and then we have an epithelial cell

abnormality squamos cell.

And this includes atypical squamos cells ASCUS and ASCH, and then low grade, cervical intraepithelial lesion, and high grade cervical intraepithelial lesion; and finally squamous cell carcinoma.

Now, this is an example of a low grade cytology, and atypical colas site with the distinct paranuclear halo, and the nuclear outline is somewhat irregular, and the chromatin is somewhat abnormal, and the cell is approximately three times the diameter of the normal intermediate squamous cell.

This is a colposcopic view of a patient with a low grade lesion, and this reddish blush, you can see the lesion here, although there is no other abnormality.

Her transformation only extends from here out to the areas where we can see some of the original squamous epithelial, and here are the cysts, and so you can see all of this is the transformation zone, and this lesion is well within the transformation zone.

And with a little lugol solution one can see the intraepithelial lesion does not stain with iodine, and it does not contain glycogen, like the

normal glycogen rich ectocervial epithelium, and squamous mature metaplasia epithelium will stain with the glycogen.

So this is a CIN-1 lesion in the cervical transformation zone of at risk woman. This is the biopsy showing this parabasalar profileration with disordered epithelium in the lower third. There are also the corlow (phonetic) sites that we expect to see in the typical low grade or mild dysplasia lesion.

For the purposes of this presentation, I will use World Health Organization terminology for histology, namely CIN-1, 2, or 3 values, and Bethesda terminology for cytology; low grade, high grade, and such. So this is a CIN-1 lesion, typical HPV changes.

Now, the cytology of a high grade lesion shows larger nuclide, and usually with less cytoplasm, and in this case there is a moderate amount of cytoplasm, and the nuclear chromatin is coarse, and what has been referred to as salt and pepper type pattern outlines are somewhat irregular.

And these cells are relatively large. For example, here is a PMN in comparison. These cells reflect a higher grade lesion. Now, this is a colposcopic photo, and I hope that you can see this, but this is a very small lesion on the anterior lip of

a Paris woman.

This lesion, and you can appreciate the small size, but this slide was provided to me by Dr. Darren Ferris. I think one of the important things about high grade lesions is they can be very small.

In fact, I think many times when one looks at statistics on mild dysplasia CIN-1, the low grade Paps smears, that in fact about 15 percent are high grade harbor or reflect high grade lesions.

And I think it is this kind of a lesion. This is a high grade lesion, and it has mosaic, and it has puntation. It is on the anterior lip and it is in the transformation zone, but it is a very small lesion.

This can give you some idea. This is the cervical os here, and so this is very close magnification, and there is a very small lesion on the anterior lip of the cervix in the transformation zone.

This is the histology of a high grade lesion, and this would be classified as a CIN-2 lesion and the changes are in the upper two-thirds, but there are still nice corlosites, and usually the more severe the lesion the less probability that you will see corlosites.

There is also abnormal eidetic activity

and lots of nuclear disarray. In the very high grade lesion one will see again the chromotine features of the intraepithelium neoplastic lesion, and in this case there is very minimum cytoplasm.

These cells have marked foldings in irregularity, and are rather characteristic of a high grade lesion, and in this case, a carcinoma in situ, CIN-3 lesion.

And here is the colposcopy as such an example, but the cervical os now is here, and this is the vagina anterior, and this is a very large lesion, extending over most of the anterior transformation zone, and with the 3 percent acetic acid application, one can appreciate those extensive mosaic pattern in this lesion.

This is a very high grade lesion and very large lesion. This is a biopsy of an example of a carcinoma in situ, CIN-3 lesion, and here you will see no maturation. The cells here show total disorganization, and with actually a vertical orientation, and there is abnormal mitotic activity, and this is CIN-3 carcinoma in situ.

Now, finally, squamous carcinoma on cytology is reflected in this sort of picture, where one sees sheets and groupings of cells. The cells,

unlike the high grade CIN, do contain substantial amount of cytoplasm, and often have small nuclei, and are in groupings as you see here.

This is a rather typical picture of a squamous carcinoma of the non-keratinizing type, which is the usual variety, and this is a colposcopic examination of a patient that has an early invasive carcinoma.

Now, this patient has extensive CIN-3, with lots of mosaic pattern, in this anterior cervix. This is the cervical os, just to be oriented here, and the cervix is quite large as you can appreciate.

But this is a high grade lesion here, a CIN-3 lesion here in this location, but here we have abnormal hair pin type vessels, characteristic of early invasion, or superficial invasion, early carcinoma in a field of carcinoma or CIN-3.

Here is the biopsy showing a CIN-3 lesion, and the invasive tumor here. This tumor is relatively small actually. It was under three millimeters in depth, and less than seven millimeters in length. So it would be a Stage 1-A Sub-1.

But this is an evasive squamous cell carcinoma, and here is the invasive tumor in the stroma, with the adjacent CIN-3 lesion. Most of the

early invasion carcinomas that are seen do have adjacent high grade CIN-3 lesion.

Now, in addition to the squamous lesions, we should talk a bit about the glandular lesions. These are also HPV related. In the Bethesda system, this is considered epithelial so abnormality glandular. These can be atypical of endocervical and endometrial, or glandular, or otherwise specified.

Or they can reflect endocervical or adenocarcinoma in situ, or be obvious adenocarcinoma, or endocervical, or endometrial, or extrauterine location. Now, this is an example of atypical glandular pap test from a young woman, a 32 year old woman.

And this reflects adenocarcinoma in situ in the cervical cytology, and there the cells are crowded together, and these cells have small nuclei, but also have this feathering characteristics that are descriptive of glandular lesions.

This patient in addition had a few of these very large cells with huge prominent nuclei, and this is an example of the background of carcinoma in situ. This is the appearance of her cervix, and here her transformation zone in fact is normal from this glandular cone rejunction out and everything is

normal.

In fact, some early work, you could not recognize glandular in situ lesions. However, Dr. Cecil Wright and Michael Shire have recently published a book on recognition of adenocarcinoma in situ lesions.

They occur in the endocervical epithelium, and approximal if you would to the glandular suamo-columnar junction, and these lesions are sometimes characterized by the yellow color or red color that one encounters in the endocervical epithelium, and often quite adjacent to the columnar epithelium at the squamo-columnar junction.

Here is normal columnar mucous epithelium of the endocervix and here is the adenocarcinoma in situ, and here the cells are similar to those that we saw in the cytology. Glands are crowded together, but there is no invasion. This is a typical adenocarcinoma in situ of the endocervix.

Now, when one compares the WHO terminology for histopathology, and the Bethesda system terminology for cytology, there is correlation in the sense that a mild dysplasia or CIN-1 is LSIL, and then CIN-2 and CIN-3 are all HSIL.

So there is maybe of use when one compares

these terminologies. So when one looks for correlation, you would like to have an LSIL pap smear reflect a CIN-1 lesion on biopsy, for example.

Now, there are lots of reasons for lack of correlation between cytology and colposcopy, and this is really a partial list. Most of the problems in my opinion are related to a sampling, and either the biopsy site is not good, or the cytology sample is not what it might be, but this is of ongoing interest to cytopathologists and pathologists interested in disease of the cervix, to achieve the best possible diagnosis, both cytologically and by biopsy based on proper collection of the sample, and processing of the sample, and interpretation.

Now, let's look at some of these Bethesda classifications in cytology and in association with CIN. ASCUS is the atypical squamous cells, and average frequency the College of American Pathologist reports across the United States frequency is about 4.4 percent of all Pap tests.

Associated CIN-2/3 in these patients when one does colposcopy and biopsy, between 5 to 17 percent, and the association of ASCUS with cervical carcinoma, somewhere around a tenth to 2/10ths of one percent.

Now, the sensitivity of a single pap test for the detection of CIN-2/3 is probably not especially good. Somewhere around .67 to .76, and there is a number of studies that have looked at this, including studies from the National Cancer Institute.

Now, if you look at the ALTS trial and ASCUS cytology, looking at a review of the cytology findings, where there was concurrence by the quality assurance committee of 55 percent, and upgraded to LSIL, 11 percent; and upgraded to HSIL, 3 percent.

So about 14 percent then were considered SIL rather than ASCUS, but downgraded to negative, 31 percent, as compared to the peripheral centers, and this correlated pretty well with the HPV testing that was done in this subset also.

I think there is an important point in the Bethesda system. There is a discussion about ancillary testing for ASCUS and these are the high risk subsets that have been reported, and high risk testing does tend to focus on the 13 high risk subsites, and probably most importantly the 16, 18, 45, and 56 being the ones that are the most prevalent and associated with carcinoma.

Now, HPV testing has been looked at as adjunct to cervical cytology and cancer cervical

screening, and it has great promise as far as sensitivity goes. This is comparing HPV testing sensitivity with cervical cytology, accepting ASCUS or higher as the threshold for action, and specificity is here, but referral to a colposcopy is also included.

So this is pretty good evidence of long sensitivity to HPV testing, and this is some independent work and not NCI-based data looking, and looking at HPV positive.

And one thing to point out is that LSIL has a high frequency of HPV positivity in most studies, 69 percent or higher, and 85 percent in some series. Also, high grade SIL has high HPV frequency.

So the use of HPV testing is not recommended in adjuncts as far as LSIL or HSIL in the initial evaluation process. Normal patients here, 30 percent, were referred to as HPV positive.

Now, comparing a number of studies, and this is some work that -- this is a partial listing from work that Tom Wright and myself, and Tom Cox, and Leo Twiggs, are working on as a report. This will be the cytology report from the 2001 consensus conference sponsored by the American Society for the Study of Colposcopy.

And this work is still basically in

present embargo because this work has been submitted, but I will share this with you; that the sensitivity management of ASCUS, the sensitivity of HPV DNA testing in these four studies cited is excellent.

And when one compares it to cytology, it is certainly comparable, and in some cases better than a repeat cytology. So that the HPV testing I think has earned a place in the clinical management in dealing with patients with ASCUS.

Now, this is work again from the NCI ALTS study, and again looking at the HPV predictive value, and negative predictive value, and I think the most important thing here is this high value for the negative predictive value of hybrid capture testing in this study mode.

And this is both for CIN-3, as well as CIN-2, and the negative protective value was very high with HPV testing. The positive predictive value is relatively low. Now, ASCUS cannot exclude high grade SIL, or ASCUS-H.

In this case, the association with CIN-2 or 3, if you look at ASCUS overall, it is about 5 to 17 percent. But if you look at ASCUS to favor a high grade lesion or cannot exclude a high grade lesion, the association with CIN-2/3 is 24 to 94 percent.

And as a result, in this ASCUS favor high grade, there does not appear to be a use for HPV testing. Rather, a referral directly to a colposopy is a prudent act as far as clinical management in that setting.

Now, how is the use of HPV triage for the ASCUS U.S. pap test. This is a model that has been discussed in a number of papers. Basically, a patient has an ASCUS U.S. pap test result, and what is the next step.

One strategy would be to do HPV testing, and if the HPV test shows a high risk HPV type, the patient then could be referred to a colposcopy and evaluation; or return for repeat cytology, with follow-up on a regular basis, with the understanding that if a pap is again ASCUS or more severe, that colposcopy would occur.

The other strategy if the patient is HPV negative would be then just to return the patient to return paps smear testing in 12 months. So, HPV testing could be used in that method to reduce the reduced colposcopy and evaluation of that type.

Now, looking at management of ASCUS, U.S. acceptable options could be follow the patient with repeat cytology in 6 and 12 months; if ASCUS or more

severe lesion, refer to a colposcopy.

And the next option would be to perform HPV DNA testing for risk types. If the patient is negative, return to screening. If positive, repeat the cervical cytology in 6 to 12 months; and if more severe, then refer to colposcopy. If ASCUS or more severe, refer to colposcopy.

And there may be a use here for HPV testing and this will be discussed in the consensus conference, but it looks like these patients, if they are persistent HPV added at 12 months, that then one needs to pursue them as Dr. Schiffman discussed earlier today.

LSIL frequency in association with CIN. The mean frequency of LSIL across the U.S. is about 1.6 percent of all the pap tests that are done. Associated CIN or CIN-2/3 is around 15 to 30 percent. And as I said, many people are of the opinion that these are probably not new CIN-3s that have evolved from the CIN-1.

They rather represent a subset of CIN-2/3 lesions that in fact did not shed enough cells, or were not interpreted as CIN-2/3 on the initial evaluation.

LSIL associated with cervical carcinoma is

quite low, under .1 percent. Now, follow-up observations versus therapy, about 70 percent in a study here by a large study by the University of Florida, and 70 percent of our patients with LSIL pap had colposcopic follow-up and were found to have a lesion.

And 47 percent of these had CIN-1, and 28.4 percent had CIN-2/3. So it has generally been our practice at the University of Florida to follow patients with colposcopy that have LSIL paps, and I think that is pretty much a rule in the United States in most settings.

So the options then would be to refer directly to colposcopy, recognizing that these patients often will have something. If the biopsies fail to identify CIN, then one has the option to follow with cytology at 6 and 12 months, or to refer to a colposcopy at that point if the pap is ASCUS U.S. or more severe.

Another option would be to follow with a pap in 6 and 12 months, with referral in special circumstances, such as pregnant women, or adolescents, or patients where a colposcopy may not be necessarily needed in certain situations.

Now, HSIL frequency in association with

CIN. The mean frequency of HSIL and cervical cytology in the U.S. is about 0.45 percent, and associated with CIN-2/3 is fairly high. It is about 70 to 75 percent in most laboratories.

HSIL associated with cervical carcinoma is about 1 to 2 percent, and that is on the initial evaluation. So this is a very productive group of patients to pursue as far as finding significant disease, and finding invasive carcinoma.

Now, with HSIL recommendations are to refer directly to colposcopy, and if lesions are identified, then biopsies and appropriate endocervical evaluation, and so forth.

However, if colposcopy and biopies fail to identify CIN, then the recommendation is to review the original cytology, biopsies and colposcopy findings to figure out what exactly was identified.

If the review confirms HSIL, then a diagnostic excisional procedure, such as an electro-loop excision of the transformation zone is recommended in non-pregnant patients.

And as I said, this is a situation where there could be a significant lesion and some of these HSIL lesions are in fact quite small, and difficult to identify.

Now, HPV results by hybrid capture looking at the various subsets, and this is NCI data again, that you will notice that the ASCUS, in about half of the cases, 47 percent, were negative, and 48.9 percent positive.

In LSIL, a very high frequency of HPV testing, and NIH's ALT trial data has also shown that HPV testing in the front end of evaluation of patients with LSIL or HSIL is not of value in the assessment of a lesion.

Now, high risk HPV detection, we know that using the standard methods that are used, in the study from Dr. Wright, that women with CIN-2/3 disease, HPV will be detected in about 83.9 percent, but probably most of those lesions are HPV positive if one could study them in other ways.

Women with no disease though, about 15 percent, have HPV detected in many settings. Now, what is the risk of high grade CIN in relation to time since first exposure to HPV 16. This is very difficult information to try to come about.

Dr. Laura Koutsky has got some data, where she had some patients that actually presented within 24 months of exposure. This is Dr. Woodman's data showing that there were some that presented as early

as 6 months, and some at 12 to 18 months, and even a few presenting beyond 18 months.

But you can see that the majority of the patients did present within 6 to 18 months for certain as far if they were to develop a lesion. Now, pap test results preceding the identification of women with CIN-2/3, and I think it is interesting that when one is looking for CIN-2/3, if that is what we specifically need to find, which I think is very important, to be able to identify.

And about 31 percent in fact of the total cases are recognized initially on paps smear, or related to the paps smear test. LSIL is about 15 to 30 percent of LSIL paps harbor high grade or harbor CIN-2/3 lesions.

Interestingly, of the atypical glandular cell group, around 30 to 40 percent of those patients harbor CIN-2/3, and with the CIN-2/3 presenting cytologically as an atypical glandular cell fissure.

And among the atypical squamous cell category, or ASCUS category, about 10 percent of those patients will have high grade lesions in the general setting.

So when we look for high grade lesion, or if we are trying to find CIN-2/3, we need to look at

all four of these subsets of cytologic findings if we are going to find all the patients that in fact have CIN-2/3.

Now, let's look at the atypical glandular cell issue, and the frequency of association with CIN, and adenocarcinoma in situ are in the mutual adenocarcinoma. The mean frequency is quite low, about 0.3 percent.

Depending on the study that you look at, the frequency of CIN-1, 2, or 3 is between 9 to 54 percent in this group. So even though the cytology findings imply glandular abnormality, many of these patients in fact have a squamous abnormality.

Adenocarcinoma in situ is a fairly important lesion, up to 8 percent in some series, and with atypical glandular cells with associated carcinoma, 1 to 9 percent. And among those carcinomas are included endometrial endocarcinomas, and not just any cervical endocarcinomas.

Now, atypical glandular cells associated with CIN-2 or 3, atypical glandular cells not otherwise specified, CIN-2/3 detected was between 9 to 41 percent, given the given series.

And atypical glandular cells favor neoplasia, which is another subcategory in the new

Bethesda system, CIN-2/3 detected 27 to 96 percent. So atypical glandular cells are a very important observation and if a pathologist favors neoplasia in the interpretation, there is a very good possibility that there is either a glandular or squamous lesion in that particular patient.

I would point out that cervical adenocarcinomas are also associated with 16 and 18, and this is just one study looking at a series of 38 cases, and 60.5 percent HPV detected, and 16 detected in 23 percent, and 18, 26 percent, and in the patients that were 59 years of age or younger, 84.6 percent had detectable HPV in their adenocarcinoma.

So, adenocervical adenocarcinoma and adenocarcinoma in situ is one other neoplasia lesion of the cervix related to and associated with a human papilloma virus, especially 16 and 18.

Now, management of atypical glandular cells, this is a somewhat complex issue. We know that we need to do colposcopy because there is a lot of high grade or HSIL CIN lesions among those looking like glandular lesions.

It should include an evaluation of the adenocervix, and in symptomatic women, and women over 35 years of age, the ASCCP consensus conference, the

concept was that adenometrial samplings should also be performed, and I think other papers have supported that very well.

Now, a diagnostic cervical cone biopsy may be needed in these patients and if that is the situation where glandular lesion or a lesion cannot be identified in the face of atypical glandular cells, these patients need to have an expert clinical opinion and an experienced clinician evaluate them because of the risk of associated glandular or CIN-3 associated lesion.

Now, natural history issues are difficult to fully understand, and I think it is confused a bit because of sampling issues and other matters. But this is just a literature analysis that Dr. Andrew Ostor did. He is quite good at these sort of things.

And he did look at CIN-1, 2 and 3, and in his analysis of a little over 4,500 cases, looking at 17 studies, ranging from 12 to 1,269 cases, 57 percent regressed; and 32 percent persisted; and 11 percent progressed to a higher grade CIN or CIS.

And looking at the CIN-2 lesions, their history based on the historical review of these papers, regression, 43 percent, persistence, 35 percent, and progression to carcinoma in situ, CIN-3,

was 22 percent in that subset.

And looking at the CIS or CIN-3 subset, including severe dysplasia, he had a total range of studies from -- 21 studies, ranging from 5 to 109 patients in the study, and 32 regressed or 30 percent regressed; and 56 percent persisted, and 12 percent progressed to invasion.

Now, I must say that I think that when one looks at some of these studies, it is really hard to understand regression in CIN-3, and I think that maybe there is need of some further discussion about that, but I think there is reason to seriously question if a true carcinoma in situ CIN-3 lesion will ever regress. So I think there are some issues that need to be looked at there.

Overall then looking at persistence at CIN-1, CIN-2, and CIN-3, progression to higher grade CIN, low in CIN-1, 22 percent CIN-2; and progression to invasion, the studies would support that CIN-3 is the biggest issue, but some CIN-2 lesions are also reported.

They are rare in CIN-1, and this may in fact represent sampling issues that we don't fully understand. Now, when one looks at the cytology issues, and this is in studies done by Dr. Melnikow,

looking at the -- this is a meda analysis looking at regression in cytology issues, and looking at the range, ASCUS, you can see the fairly common regression of ASCUS cytology, and low grade SIL cytology regression from 40 to 60 percent roughly.

ASCUS with low grade is similar, and then high grade SIL, some regression here, 20 to a little over 50 percent in cytology regression. But of course cytology regression, one is always facing issues of sampling also.

Progression, ASCUS, about a little over 10 percent progression, and this is interesting comparing six months follow-up to 24 months follow-up. So you had 6 months follow-up fairly low, but there are some cases here having progression in the ASCUS subset; and in low grade lesions, the range is quite variable.

You can see from about 7 percent and all the way up to almost 35 percent of progression in these low grade subset of cytology. We would expect in any low grade subset that we would see about 15 percent with CIN-2/3 eventually being manifest.

And then in looking at high grade cases, there was some recognition of regression by cytology in these cases also, or in progression, and you can see the progression rate here again, the 3 month

progression rate versus 24 months, and there is quite a significant difference.

And then finally invasive carcinoma, which is a fairly typical marker, you are looking at ASCUS, and this is 6 months and 24 months follow-up, and you can see some invasive carcinoma cases are found in the ASCUS subset or cytology, and basic carcinoma infrequent finding in low grade lesions, .5 percent here.

ASCUS low grade SIL, again low, and high grade SIL is up to 4 percent here, and one of the high rates in this subset. Now, management of CIN-1, some of the issues that we need to consider and these have been summarized by Dr. Howard Jones very nicely in this study, basic cancer may already exist in the case of CIN-1.

We know that CIN-2 or 3 can exist in some cases; and invasive cancer develops between follow-up visits; or patients are lost to follow-up and develops invasive cancer.

And so we choose to follow CIN-1 lesions, and we have to recognize that situation. Here is a study from Dr. Shafi, from the British Journal of OB-GYN, looking at a LSIL atypious situation, and immediate LLETZ for 24 months.

And of those that had immediate LLETZ, he found CIN-2/3 in 23 percent, and what is interesting is that those that he followed for 24 months and then evaluated, he had 24 percent.

And this observation of CIN-3 in these follow-up studies has also been supported in the ALTS trial, where you see the CIN-2/3 subset emerging from cases initially interpreted as CIN-1. So this probably represents a small subset, maybe a quarter of those cases where we don't recognize the CIN-3 lesion or CIN-2 lesion in the face of a apparent CIN-1 case.

Now, these are some ACOG committee opinions that have been stated and published, and there are authors who have contributed, such as Drs. Gold and Ferris, for example.

But here is some observational follow-up of CIN-1 that has been recommended to clinicians, and if the patient has a follow-up paps smear that is within normal or benign cellular changes, repeat follow-up at 4 to 6 month intervals should continue.

If the smears remain normal, or benign, a patient may return to annual screening after four consecutive normal or unremarkable pap tests. On the other hand, if any of these return as ASCUS or LSIL, or HSIL, the patient should then be referred to

colposcopy, with directed biopsies and this is very important in the evaluation of these patients, recognizing the possibility of underlying CIN-2/3 or invasive tumor.

Now, the treatment or decision on such patients is dependent upon pathologic findings. Patients that have CIN-2/3 require appropriate treatment for cervical endometrial neoplasia. However, patients with CIN-1 may have observational follow-up if that is acceptable to the physician and the patient.

Treatment versus observation. Grossly visible lesions of the cervix require a biopsy and this is very important. Cytology can miss even if we see a lesion. Grossly visible CIN-2 or 3 lesions may be associated with invasive carcinoma, and usually a micro invasion or rarely adenocarcinoma in situ or invasive adenocarcinoma.

And importantly the approach using electro-loop excision of any visible lesion is generally not recommended due to common treatment of non-CIN lesions over treatment basically, but biopsy of course is necessary for appropriate diagnosis.

Outcomes. Systemic review of controlled and randomized trials in connection with CIN-1, and

CIN-2, and outcomes as far as recurrence of CIN-1 or occurrence of CIN or non-recurrence of CIN between cone biopsy, cryotherapy, laser ablation, electro-loop excision, demonstrated no substantial differences in outcome as described by Dr. Norvelle in a med-analysis of treatment outcomes.

Methods of treatment. These are the most common used in the United States. I would say that electro-loop excision is probably the most common right now used for low grade lesions that are treated in the outpatient setting.

Laser ablation is of less common use, and cryosurgery is still used for low grade lesions in some settings. You can see that depending on the method of treatment the complication rate related to hemorrhage is highly variable, but electro-loop excision is generally well tolerated in most settings.

Residual CIN after loop excision is an issue, and if margins are involved, or if the ECC contains CIN, there can be persistent disease in as man as 48 to 59 percent of the cases, even though they undergo LEEP incisions.

So doing electro-loop excision is not the whole answer. We have to recognize that in the management of CIN lesions that ablative therapy is not

recommended for squamo-columnar injunction when limits of lesions are not identified.

A negative ECC prior to ablative therapy is suggested by most experts. ECC at the time of loop excision may indicate an increased risk of residual disease, but may not influence post-LEEP management, and a CIN-3 approach for low-grade lesions tends to result in excessive types of surgery and results in negative histology.

And with that, I will stop, and we will open it up for questions. Thank you.

CHAIRMAN DAUM: Thank you very much, Dr. Wilkinson. What I would like to do again is ask the committee members for questions of clarifying data or issues directly related to the factual content of Dr. Wilkinson's presentation.

Well, I have one that I would like to ask actually. Can you put in perspective maybe in just a few clarifying comments, and I know it was all in that talk, but the difference between CIN-2 and 3?

I have seen data today where they are lumped, and data today where they are separated, and could you just comment on that?

DR. WILKINSON: Well, the WHO has defined this as a CIN-2 lesion, and the abnormalities may

extend up to the two-thirds edge of the epithelium. If you would grade the epithelium as one-third, two-thirds, and top, and so a CIN-2 lesion could be called -- the adenoepithelial cell abnormalities could extend all the way to the two-thirds edge, but not beyond that.

With the CIN-3 lesion, the abnormalities, the cellular abnormalities would extend beyond that. Now, this is sometimes a bit arbitrary, because you have cordocytotic changes and other features, but I think for most pathologists the grading of CIN-2 and CIN-3 is fairly consistent.

In fact, the higher the grade of the CIN, the more reliable the grading becomes.

CHAIRMAN DAUM: So in your mind, most of the time you are a splitter, and that there are two distinct pathologic entities here?

DR. WILKINSON: Yes. I think most -- there is a trend among pathologists to try to lump them together, and I think that -- for example, most pathologists would prefer not to call carcinoma in situ because it has certain implications, but there are cases that clearly fit the definition of carcinoma in situ as I showed you here.

We have full thickness change without any

surface maturation. But I think in general that is in a poll across the United States, but I think that most pathologists still separate out CIN-1, 2, and 3.

CHAIRMAN DAUM: I have one more question actually, and that is that official bodies that impinge or set a standard if you will for clinical practice, and I am trying to choose my words carefully -- like ACOG, for example.

DR. WILKINSON: American College of OB-GYN, yes.

CHAIRMAN DAUM: I gather from your comments that they have now established clinical definitions of persistence of HPV infection, and if that is the case, can you just say exactly what they are?

DR. WILKINSON: I am not certain. The American College of OB-GYN has done that. Dr. Zinberg and his committee, clinical practice committee, have been looking at that very carefully, and also looking at the issues of HPV testing, and its applications.

And I think they are still considering that situation very carefully, but I don't think they have made a formal statement that I am aware of about what the definition of persistent HPV is.

CHAIRMAN DAUM: Are they about to or do

you know?

DR. WILKINSON: I think they are very interested in it, the clinical practice committee is, and you could probably contact Dr. Zinberg and he would be glad to discuss -- Dr. Stanley Zinberg at the ACOG, and I am sure that he would be glad to discuss that with you.

CHAIRMAN DAUM: Well, it may have an impact on some of the things that we are deliberating, and that's why I am sort of curious to hear your opinion.

DR. SNIDER: If I could just follow up on the first question you asked. I understand that from a particular specimen that there are -- that there would be a reasonable expectation that you could differentiate CIN-2 from CIN-3.

I wonder though how that really represents what is going on in the patient, because you showed us, for example, a patient who had invasive cancer, but then at another site had -- I guess it would be CIN-3.

DR. WILKINSON: CIN-3, right.

DR. SNIDER: So would the same kind of thing be expected to occur in a fairly high proportion of patients, where at one site you might get CIN-2,

and at another CIN-3?

DR. WILKINSON: I think that may occur, and as a matter of fact, the lesions that occur closer to the squamo-columnar junction tend to be the higher grade lesions.

And skilled colposcopists have learned to identify these more significant lesions in the field of CIN on colposcopy findings. But we are as pathologists extremely dependent upon the clinician's ability to biopsy a specific area or areas, and that is one of the potential variables in the correlation between cytology and biopsy findings.

And not only cytologic sampling, but also biopsy sampling. But this is true in all fields of biopsy as you know, and I think that has been one of the issues with the interest in the electro-loop excision device, because with that they can excise the entire transformation zone, and not have to deal with that problem.

The trouble is that the poor patient ends up with a lot of cervix being removed that doesn't need to be removed. But that is a very important issue; accurate biopsies or multi-biopsies.

And I would just add that some physicians are under the misunderstanding that a patient is

charged for every biopsy separately, but by pathology standards of billing, if the biopsies are all placed in the same container, because it is a contiguous site, it is only one bill.

But if they make an effort to clearly define different sites, there will be a different bill for every site.

CHAIRMAN DAUM: Are there other committee questions of Dr. Wilkinson? Okay. The next item that I guess -- and thank you very much, Dr. Wilkinson. That was very helpful. Dr. Goldenthal, do you want to say some words now to us about -- you are on the program here for reintroduction of questions.

DR. GOLDENTHAL: Well, this is when I was going to give a 30 minute presentation on endpoints.

CHAIRMAN DAUM: That would be wonderful. Would you now do that. Thank you. I couldn't tell for sure.

(Brief Pause.)

CHAIRMAN DAUM: I am going to yield from dissent from the committee here. Dr. Goldenthal needs a few minutes to set up, and I apologize for this. We will take a short break. We will break until 3:30, and then Dr. Goldenthal will begin her presentation at 3:30.

(Whereupon, the conference was recessed at 3:20 p.m., and resumed at 3:32 p.m.)

CHAIRMAN DAUM: Could we have everybody settle down and take their seats, please. During the break people have come up to me to ask about this business of ACOG's view of persistent infection, and I have tried to persuade some of them to say a word in public here, and so I will call on Dr. O'Connor briefly, and then Dr. Freedman to say one or two sentences about clarifying the question that I asked. Dr. O'Connor.

DR. O'CONNOR: One or two sentences, and the best thing that I can say is guidelines are forthcoming. I am not purporting to speak as a spokesperson for ACOG, but much of what has been presented here is information that has happened very recently.

You are talking about just within the past year, and changes in the cytology reporting with the Bethesda system, and then the American Society for Colposcopy and Cervical Pathology Conference in September, all of these things are to be published, and you can expect from that that parent societies such as ACOG, and the American Academy of Family Practice, will come out with endorsements, guidelines

that would reflect the information that is presently to be published.

CHAIRMAN DAUM: Thank you very much. Dr. Freedman.

DR. FREEDMAN: The only thing that I can add is that it is obvious when you see the data that the lack of standardization of approaches to some of these lesions, like ASCUS and CIN, really is demanded some type of standardization.

And that is what is forthcoming, and I think it should be helpful at least in perhaps having fewer people undergo a procedure that they don't really need.

CHAIRMAN DAUM: Okay. Thank you very much, both of you, for your clarification, and we will try one more time to get Dr. Goldenthal's talk under way. Dr. Goldenthal.

DR. GOLDENTHAL: Thank you. I would like to take this opportunity to give you FDA's perspective on HPV preventive vaccine endpoints. But first I would like to acknowledge some FDA staff who have served as clinical primary reviewers on the various HPV vaccine files, as well as staff who assisted in the preparation of the two briefing documents.

Worldwide, cervical cancer is the third

most common cause of cancer in women. In developing countries, it is the second most common cause, and in developed countries, it is the sixth most common cause of cancer in women.

Worldwide, there are an estimated 400,000 to 500,000 new cases per year, and of interest is that there has been a disturbing trend in one developed country, which is where an increase in actually instances of cervical cancer has been observed for women under 55 years of age.

And worldwide for cervical cancer there are approximately 190,000 deaths per year, and 78 percent of which occur in developing countries. Now, looking at it from the U.S. perspective, in the 1930s, cervical cancer was the most common cause of cancer deaths in U.S. women.

Now, the incidents of mortality rates for cervical cancer declined dramatically following paps screening and intervention, and for 2001, approximately 12,900 new cases of cervical cancer, and approximately 4,400 deaths due to cervical cancer have been projected for the U.S.

A woman's lifetime risk of developing cervical cancer in the U.S. is currently estimated to be 0.85 percent; and the risk of dying from this

disease has been estimated at 0.3 percent.

This slide depicts the histological precursor lesions of carcinoma of the cervix, and on the left side you can see the normal epithelium, with orderly maturation.

And as you proceed over to the right, you find increasing progression of the abnormalities, and going from CIN-1 to CIN-3, and ultimately over to severe dysplasia, and then when a full thickness of epithelium is involved, carcinoma in situ.

Now, just briefly, I wanted to show you one interesting example where squamos carcinoma in situ, where normal epithelium on the right is just as posed to a full thickness or carcinoma in situ on the left.

And of course this case is carcinoma in situ because the basement membrane is intact. This slide depicts the -- if you will, the pyramid of abnormal cervical findings in the U.S., with an ASCUS finding of approximately 2 million cases per year, and then carcinoma of the cervix is much less common, with approximately 12,900 cases per year.

I am not going to -- we have already covered the natural history, and I am not going to do that, and I did want to make a comment about a study,

a worldwide survey of over a thousand invasive cervical cancers, where 93 percent were found to be HPV positive by PCR.

The five most common types found in the cancers were -- and I listed them on this slide, but the two most common were Types 16 and 18. Subsequently, the negative samples from the study were retested, and 97 percent were found to be HPV positive, with more sensitive primers.

If one look at the author to find adequate specimens for this retesting, then the overall rate of positivity was 99.7 percent. I wanted to make a few comments about the differences between squamos cell carcinoma and adenocarcinoma of the cervix.

As shown in these two studies, HPV type 16 is more common in squamos cell carcinoma of the cervix than is Type HPV 18. Now, this slide shows adenocarcinoma of the cervix, and for adenocarcinoma in a variety of studies, HPV Type 18 is as common or more common than Type 16.

And this slide also illustrates another important distinction between squamos cell carcinoma and adenocarcinoma of the cervix. Specifically, cytology screening has had far less impact on the incidents of adenocarcinoma of the cervix, compared to

squamos cell carcinoma of the cervix.

In the U.S. review of the SEER database has shown a clear decrease in the incidents of squamos cell carcinoma and invasive cervical cancer overall. However, these data have also shown an increase in the rate of adenocarcinoma of the cervix.

And interestingly a similar finding has been observed in six Scandinavian countries. I now wanted to cover to longitudinal studies that evaluated the relationship of specific types of HPV to the development of CIN-2 or 3.

In this first study, 241 women presenting for STD evaluation with negative cervical cytology were followed very frequently every 4 months, with an average follow-up of 25 months.

And for this follow-up, women had cytology, colposcopy, HPV DNA, as well as testing for STDs. And in this particular study it was found that if the subject was positive for HPV Type 16 or 18, there was an adjusted relative risk of 11 for developing CIN-2 or 3, compared to those without HPV.

This is another longitudinal study that was published more recently, and in this study a little over a thousand women with normal cytology and who were HPV negative at baseline were followed every

six months with paps smear and HPV DNA.

And the median follow-up in this study was 26 months. If someone had abnormal cytology, or any abnormality cytology, they were referred to colposcopy and biopsy.

Now, in this study there was also found to be an adjusted relative risk of 8.5 for the development of CIN-2 or 3 if one was HPV Type 16 positive at baseline, compared to those who were HPV negative.

Now, I want to go on to a specific discussion of various theoretical endpoints that one might consider for an HPV Types 16 and 18 vaccine. Virology is a potential endpoint, using either any incident infection, or persistent infection, with various definitions, and of course persistent here would also be an incident persistent infection.

LSIL cytology or worse with virology is another potential endpoint. CIN-1 histology, adenocarcinoma in situ, or worse with virology, is yet another potential endpoint.

And I throw in adenocarcinoma in situ because I think at least theoretically that you have got to consider glandular lesions in your endpoint, although I doubt that many, if any, will be observed

in most of the trials that we would see.

Another potential endpoint is CIN-2/3 histology, adenocarcinoma in situ, or worse with virology; and finally invasive cervical cancer, with or without virology.

Now, looking at the virology endpoint, one of the advantages is definitely feasibility, and this could be conducted with a smaller trial, and there are certainly populations in many countries with sufficient incidents of HPV infection.

For example, here I give the rate of incident infections for Type 16. For example, in this one study there was a 10.5 percent cumulative incidence over 3 years, and in another study there was a 7 percent cumulative for two years, and so on and so forth.

One of the advantages of a virology endpoint is that HPV has been strongly associated with HSIL cytology, carcinoma in situ, and cervical cancer in a variety of studies, some of which are shown here. And certainly you would be able to make an assignment as to whether a case if you will was vaccine versus non-vaccine type.

You also might be able to determine an immune correlate of protection if you did the

appropriate follow-up post-vaccination serology. However, there are some disadvantages. At least now HPV infection is not a clinical disease.

Most HPV infection resolves, and there would be important questions about the durability of protection, especially if one had let's say a trial for several years with virology, and one would wonder about the change in lifetime risk for HSIL, histology, or cancer.

Virology is not as approximal to cancer as other endpoints, and it is possible that one may want definitive high grade clinical endpoint data prior to extensive deployment of a new HPV vaccine.

Some other disadvantages are listed on this slide. There is uncertainty in the existence of or detection of latent infections in the cervix, and some of that could be questions about sampling, and you could have HPV theoretically in basal cells, and you might not detect it with a cervical sample.

And you may not have it, and there are questions about whether a vaccine might -- a vaccine-induced immune might make HPV DNA more difficult to detect, albeit that is a theoretical.

There is also uncertainty in distinguishing new HPV infection from reinfection,

although this problem would tend to negatively bias efficacy estimates.

And there is also questions about the appropriate definition for persistent infection, and in fact that it has not been clearly validated in prospective longitudinal studies if you will with incident infections, and that a particular definition has not been validated in that sense.

It is also possible that the use of virology only may not allow the identification of unanticipated vaccine associated problems. For example, if there was to be enhanced disease for some reason, you might not detect that in a trial designed to look at virology.

Another disadvantage is depending on one's viewpoint is a smaller efficacy trial, because a smaller efficacy trial would provide less well controlled safety data, although that could be addressed with supplemental trials.

Now, I wanted to move on to LSIL cytology, or worse. Again, LSIL cytology would have the advantage of feasibility in a smaller trial, and certainly LSIL leads to many clinical workups in developed countries.

Disadvantages are shown here. There might

be questions about clinical relevance, and LSIL cytology by itself does not represent a definitive diagnosis, and a particular one would need histologic diagnosis usually for therapy.

Another disadvantage, and again that it is not as approximal to cancer as other endpoints, and again one may want to have definitive high grade clinical endpoint data before extensive deployment of a new HPV vaccine.

And it may be easier to detect or identify unanticipated vaccine associated problems with a high grade disease endpoint, and I have already mentioned the small efficacy trial issue.

I am moving on to CIN-1 histology or worse with virology. I would make certain assumptions about a trial like this, and that is, first, virology would be used to classify the CIN-1 cases as vaccine type or not, just like they would have in the other endpoints.

However, one would need to pre-specify whether HPV testing on cervical samples, versus the histology, would be used to classify cases. Cases would be identified mostly from colposcopic workup of atypical squamos cells findings, and LSIL cytology.

Obviously, the plan or algorithm for colposcopy will effect the number of endpoints that

one attains, and I do want to emphasize that one cannot use economics as the basis for U.S. licensure. It has got to be based on risk benefit.

Advantages of CIN-1 histology are worse with virology as an endpoint, and to include feasibility. Certainly there are populations in many countries with sufficient incident of CIN-1.

CIN-1 certainly would necessitate a definite workup, and so you would have a definitive diagnosis as your endpoint, and certainly there are data available on the natural history of CIN-1 from an initial diagnosis.

Now, CIN-1 histology or worse with virology has certain disadvantages, and at least half of CIN-1 resolves without therapy, and also it is not as approximal to cancer as other endpoints.

And one may want definitive high grade clinical endpoint data before extensive deployment of a new HPV vaccine. Again, we have mentioned that it may be easier to identify unanticipated vaccines associated problems with high grade disease endpoints.

Now, moving on to CIN-2/3 or worse with virology, I put some assumptions on this slide for such a trial, and again one would need to pre-specify whether it is HPV testing on cervical samples, versus

on histology specimens, would be used to classify the cases.

Now, many, if not most, CIN-2/3 would be actually identified from colposcopic workup of ASC and LSIL paps smears; and of course the plan or algorithm for colposcopy would be critical to the number of endpoints, and probably even more critical here in a way than in the CIN-1 trial.

And I would assume that many, if not most, of the CIN-2/3 cases would be those found at the first workup of abnormal cytology because if there was CIN-1 previously, and if it occurred, there might tend to be treatment.

Advantages of CIN-2/3 histology or worse with virology is that it is more approximal to cervical cancer, and it would provide a definite high grade clinical endpoint data before widespread public health use.

I think that there is good supporting natural history data, and it prevents lesions that clearly would need therapy by the U.S. standard of care.

With this endpoint, it may be easier to identify unanticipated vaccine associated problems, and I think you would be getting into a larger

efficacy trial, which would have if you will positive implications for your randomized safety database.

The disadvantages of CIN-2/3 as an endpoint are given on this slide, and one of them is feasibility. It may be that the subject numbers may not be that different from most vaccine efficacy trials. As many of you in the audience, we have vaccine efficacy trials that range from 10 to 40,000 and that is not terribly uncommon.

However, the thing that is different here is that the type of follow-up per participant is likely to be more resource intensive than perhaps a typical preventive vaccine efficacy trial.

There certainly is uncertainty with regard to trial size and duration of the trial. There is really little natural history data to estimate a trial size if one looks for a longitudinal study and very frequent follow-ups, especially in women with a negative baseline HPV normal cytology.

I made a preliminary estimate from a trial that was very recently published by Dr. Woodman, and actually I have already requested some additional information from Dr. Woodman to further refine this.

And I believe so as I discovered is one of the sponsors, but I have estimated that for an HPV

16/18 vaccine that one would need 12,000 women in a trial, but this would only rule out the lower bound of zero.

If you wanted to rule out a lower bound that was higher, you would need to enroll more subjects. And this assumes a vaccine efficacy greater than or equal to 80 percent, which I think is quite conservative if we allowed a sponsor to start counting cases, and let's say after the primary immunization series.

It might even be that it would be reasonable to use 85 percent for that estimate. And it would take I would assume 3 years on trial, and at least 6 months for enrollment for a very roughly 3-1/2 year trial.

But again I have requested some more information and perhaps we will have a revision of that estimate. Now, I wanted to move on to the example of cervical cancer with or without virology as an endpoint.

I don't know if anybody would actually propose doing that. There was one publication by a Scandinavian investigator, Dr. Lightman, who is actually looking at the possibility of cancer. He kind of had a trial that was -- it didn't enroll many

people, and it went on for like 15 or 20 years.

But I did want to mention though some of the assumptions that one might have if looking at a cervical cancer endpoint in a developed country setting.

I assumed that one would randomize subjects, and that there would need to be an infrastructure in place for routine follow-up and a method of capturing all diagnoses for an area.

And perhaps a cancer registry and death certificates, and actually in the Scandinavian countries there are comprehensive cancer registries in at least some of the countries that could fulfill that.

One critical decision would be HPV testing. I am assuming for all of the previous endpoints that I have mentioned that you would definitely know what your baseline status for HPV was, as well as do HPV testing during the trial.

Perhaps given the size of whatever for a cervical cancer trial, that may not be the case, but obviously the presence or absence of baseline testing before randomization, as well as cervical sample histology testing, would affect the efficacy assessment.

And of interest based on at least some data or some studies that I have looked at, I wouldn't be surprised if many or most of the cervical cancer cases were identified from colposcopic work of ASC, AGC, and LSIL.

Now, here is some advantages of cervical cancer as an endpoint. Clearly the major concern is cervical cancer. This would be viewed as very, very definitive data, and it may be easier to identify any unanticipated vaccine associated problems.

Another advantage of cervical cancer as an endpoint is that I thought it would give a better understanding of the impact of the vaccine on adenocarcinoma, which has been increasing as I mentioned in incidents.

And again you would have a larger efficacy trial, which would be good, although I suspect that with this sort of trial that you might not have detailed information on the participants.

The major disadvantage of cervical cancer in an endpoint is feasibility, and uncertainty with regard to trial size, duration, population selection, and there may be issues in looking at countries without screening program, and so on and so forth.

I did want to make a couple of other

comments about endpoints for HPV vaccines. Obviously the protocol would need to specify the handling of mixed infections, especially vaccine versus non-vaccine types prospectively in the primary endpoint, and I believe that there would need to be evidence of overall benefit from all endpoints, including ones attributed to non-vaccine high risk HPV types, and are included in the analysis.

In other words, we may well -- and I suspect that we would allow a sponsor to use vaccine types of HPV for their primary analysis, and that would mean the non-vaccine types would not be included in the primary analysis.

However, again we would also like to see the impact on the disease as a whole. I wanted to briefly go over accelerated approvals since it has been raised several times today.

The accelerated approval regulations kind of apply to new biological products, and also to drugs, and they are intended for biological products for serious or life-threatening illnesses. And these should be products that provide meaningful therapeutic benefit to patients over existing treatments.

The FDA may grant marketing approval for a biological product on the basis of adequate and well

controlled clinical trials establishing that a biological product has an effect on a surrogate endpoint that is reasonably likely based on epidemiologic, therapeutic, pathophysiologic, or other evidence to predict clinical benefit from the basis of an effect on a clinical endpoint other than survival or irreversible morbidity.

That is a real mouthful I know, and it may take a minute or two to digest it, but that is the guidance from the regulation. And the purpose of the accelerated approval regulations is that they are intended to make available promising therapies while definitive, confirmatory efficacy trials or trial is being completed.

And the confirmatory post-marketing study is usually well underway at the time of accelerated approval. It is like the trial of the surrogate, the trial for the confirmatory endpoint must be adequate and well controlled, and it must be carried out with due diligence.

The original and current purpose of accelerated approval is to serve the best interests of the public, and I did want to note that presented vaccines have not been previously approved using accelerated approval regulations.

So I did want to bring that to your attention. Most of the products that have been approved using accelerated approval regulations has been AIDS therapies and cancer therapies.

Now, in concluding, I would like to re-review the FDA questions for you to think about before our discussion in more detail tomorrow; and that is -- and again I have got two questions.

One is please discuss and identify the most appropriate endpoints for traditional approval of HPV vaccines intended to prevent cervical cancer. And in particular please discuss the use of the following endpoints in clinical trials intended to demonstrate the efficacy of HPV vaccines for oncogenic types in the indications, such as prevention of HPV infection that these endpoints would support.

And listed here are incident HPV infection by oncogenic HPV types; persistent HPV infection by oncogenic HPV types; and regarding the persistent infection endpoint, we would also ask that you discuss the appropriate number of positive virologic results in the interval between such positive virologic results.

Again, another candidate endpoint is LSIL cytology, associated with oncogenic HPV types; CIN-1

associated with oncogenic HPV types; and CIN-2/3 associated with oncogenic HPV types; and finally cervical cancer.

For the second question, please discuss the use of accelerated approval regulations for licensure of HPV vaccines for the prevention of cervical cancer. Specifically, please discuss and identify possible surrogate endpoints to support accelerated approval.

In particular, please consider the following endpoints. Incident HPV infection by oncogenic HPV types; persistent HPV infection by oncogenic HPV types; LSIL cytology associated with oncogenic HPV types; and CIN-1 histology associated with oncogenic HPV types.

And also in the context of accelerated approval, please discuss and identify possible endpoints for the confirmatory trial. Thank you very much.

CHAIRMAN DAUM: Thank you very much, Karen. I would like to see if there is some comment or discussion from committee members specifically regarding clarification of what Dr. Goldenthal has said. We will start with Dr. Snider.

DR. SNIDER: I just would like to have

some clarification in the context based on conversations that we have had today, and I will try to be appropriately discreet about what information was presented in closed session or open session.

But my understanding is that we are being asked this question in the context of the deliberation by professional societies and guidelines making organizations which are considering the data available today.

It may or it appeared to be sometime in the near future coming out with some recommendations, and which may include recommendations for intervention in the context of persistent infection, and we don't at this time know exactly what those recommendations would be.

But that is the context in which we operate. We also are being asked to respond to these questions in the context of a large prospective study being analyzed, data from a large study be analyzed, which would be informative about optimal intervals to define persistent infections, and perhaps identify risk factors that indicate persistent infections might progress to cancer or to high grade lesions, CIN-2/3.

So we are being asked these questions in the context of there soon being some information that

will impinge on our answers, but we don't yet have that information. Is that correct?

DR. GOLDENTHAL: Yes. Well, I mean, you are absolutely right. It is difficult --

DR. SNIDER: Well, it is a moving target, but these are some very significant limitations I think that are going to be difficult for us to deal with, and let me just express my frustration in that regard.

DR. GOLDENTHAL: And the different therapeutic approaches may differ from country to country, which could be complex for a multi-country trial.

CHAIRMAN DAUM: Dr. Fleming, and then Dr. Kohl.

DR. FLEMING: Dr. Goldenthal, a couple of questions. First, I am assuming as you have laid out these two questions for us, one on endpoints, that might be appropriate surrogate endpoints to use for traditional or full approval and those for accelerated approval, I am assuming that we have flexibility in proposing alternatives, or combinations of these, or variations of these?

DR. GOLDENTHAL: Absolutely. These questions, they are both in a please discuss mode, and

I think you do have flexibility.

DR. FLEMING: Okay. Moving along in that direction, a couple of more questions. This is -- and following kind of Dixie's spirit, this is a very significant challenge, partly because it is always complex to talk about what are valid surrogates, and generally the type of information you need to even begin to truly address this with insight is not only natural history of it, but it is what are the relationships between various markers and clinical outcomes, and natural history.

But also what are those relationships, in the context of people who are receiving intervention of interest, and essentially we lack that type of insight.

We are also lacking as Dixie was pointing out some of the insight even in natural history. When I look at your list, you have listed as a major disadvantage for CIN-2/3 in particular logistical practicality, and that it is going to be a study that is going to be too large, and it is somewhat difficult to really assess that.

DR. GOLDENTHAL: Well, I was more thinking in terms of resource intensiveness per participant, compared to other preventive vaccines. The final size

-- you know, again we have trials in the 10 to 40,000 range, but this is a lot of follow-up on individuals in the trial, compared to many other trials.

DR. FLEMING: I understand. There are some very helpful sources of information in the literature. The Woodman article, for example, that you referred to, does give us a sense if you follow a cohort of uninfected individuals from time zero ahead, that we have something on the order of an accumulative risk of CIN-2/3 that might approach one percent by about 4 years follow-up.

You have also given some specific indications of lifetime risks, where for a thousand people lifetime risk of death due to cervical cancer might be three in a thousand, and maybe nine in a thousand would have a diagnosis of cervical cancer.

And you have also told us that the actual annual incidences of CIN-2/3 might be 20-fold annually higher than diagnosis of cancer. So clearly one is left with a sense that over time there is a very high cumulative risk of CIN-2/3.

One of the things that I am struggling with though is to get a sense of if that risk based on the Woodman data over 3 years to 4 years might approach 7/10s of a percent to one percent, is there

a sense as to whether that risk is essentially linear?

Will that increase at that rate? Let's say you are looking at a cohort of 16 to 23 year old women at randomization, and if their risk is .25 percent a year for the first several years, is that likely to, if anything, go up a bit as those women approach their mid-20s and late-20s? Does anybody have a sense about that?

CHAIRMAN DAUM: Who would like to take on that question?

DR. GOLDENTHAL: It is interesting, because I would have predicted that it would -- that the increase would not be linear. That it would go up a lot. In other words, in year four of a trial might be more than year two of a trial.

And I think you will get some of that in a trial, but I have been a little bit surprised, at least in two of the longitudinal studies, how quickly HPV -- how quickly CIN-2/3 developed following HPV infection.

I think, for example, in the Woodman study that they mentioned that the median time from the first detection of HPV to diagnosis of CIN-2/3 was 26 months. So I do think that your -- let's say your second, third and fourth year will probably be

enriched compared to your first year, in terms of diagnoses.

But I don't know -- you know, assuming that the HPV infection rate or whatever stays high -- and it certainly did seem to stay high in some of these cohorts. But that is a difficult question to answer. I think that there would be definitely value added from going from four years to three years, for example, in such a trial. Perhaps some of the sponsors can or others can address this better.

CHAIRMAN DAUM: If people have information about this very issue, we will entertain that. Dr. Felix, do you?

DR. FELIX: Well, there is natural occurrence of CIN-2/3 that is in an older age group. So, every subsequent year one would predict the frequency of that diagnosis to increase not -- and most of the studies that that has been shown in are not HPV -- do not examine virologic status.

The Woodman study perhaps, as well as the Koutsky study, showing the very brief interval, is because of the prevalent HPV in those two studies. I mean, a significant or a very large majority, and in Koutsky, all of them already had HPV positivity at entry.

And again your point taken, you don't know how long that has been and what time zero was in those women, and we don't have a naive cohort analyzed so far. So I think there is very good existing natural history data that would predict an increase in incidents as the population matures in age.

MR. JONES: My name is Bruce James, and I have a personal communication from Eduardo Franco, who is one of the co-principal investigators for a cohort study, a natural history study, in Sao Paulo, Brazil, in which women 18 to 60, were enrolled over a 3 year period, and then followed initially in the first year of observation three times, and then annually thereafter.

And we asked him to -- well, the median age of this group was 33, with about one-quarter of the enrollees under the age of 25. And we asked Eduardo recently to look at his data and tell us in women who were initially HPV negative, and had no SIL, what the pattern of HSIL was.

And what he saw was if you looked in all age groups, the accumulation of lesions really stopped after about 3 years; and if you looked at women who were under 25, it stopped after 2 years of observations.

So there are small numbers involved and this is a group of about 1,100 women that were followed, but it would suggest that there is not clearly a linear increase.

CHAIRMAN DAUM: Thank you very kindly. Dr. Schiffman.

DR. SCHIFFMAN: In Costa Rica, in our Portland cohorts, we see a much greater than expected percentage of what we initially thought was incident high grade, and it turned out to be missed-prevalent high grade.

I caution you when you are looking at any study of the old cohorts or whatever to think carefully about how did they rule out small high grade lesions, and what did they use, and how many techniques did they use.

And as you were saying before, how many were colposcopic, and was there any kind of an attempt to look at or for false negatives, because the more that you look -- we have dropped progression rates from 17 percent to 6 percent by more careful review of initially what was thought to be totally negative initial entry criteria.

In Costa Rica, in Portland, what we see is this burst in HPV infected women who are normal,

cytologically and totally normal, and we see this burst of apparent incident detection that we think is false-negative detection. In other words, it was already there.

And then a trough, which appears to be some kind of latency that is fairly short, and then we see a pickup where CIN-2 is fairly steady, and it is ticking along as people get HPV, and then pretty rapidly get CIN-2, even young women who are virginal and then get their incident infection.

CIN-3 with some delay, picking up a little bit later, and the predictive value of HPV DNA test in those women by 7 years or so has dropped off entirely. So it is a complex phenomenon, and I do not think particularly that the Woodman article captured all those subtleties.

CHAIRMAN DAUM: Thank you very much.

DR. GOLDENTHAL: Although they certainly did have a frequent follow-up. That has been the best that I have been able to find in terms of a longitudinal cohort.

CHAIRMAN DAUM: Thank you very much. Dr. Kohl, you have been patient.

DR. KOHL: I have been patient. My head right now is in the Dixie mindset of contextual

issues, and a couple of things come to mind. If we advise studies that involve higher grade findings, CIN-2/3, for instance, then those studies essentially will have to have also virological data associated with that I would think, because it would be CIN-2/3 with high grade virus.

And then the context is that we obviously have lots of other evolving information coming; guidelines and natural history studies. And my question is do we need to make some advice for all time, or can it be a temporary advice, in which case there might be a more rigorous criteria, which as more information evolve, we could then fall back to a virological surrogate if that proves to be feasible and useful as we get more long term natural history done.

DR. GOLDENTHAL: Well, as you know -- I mean, we take advice from committees, but then things can potentially evolve, and what is used to prove -- well, a good example of that over a longer period of time is what may be used for one approval may not be used -- you know, may not be for the next approval, in terms of endpoints, and so on, and so forth.

So we certainly do consider evolution, and it is possible that we may bring this question back to

the VRBPAC at some point in the not too distant future. But I think for the purposes of the meeting today, I think you have to advise us on the available information.

I mean, I could argue that we maybe should have had this meeting six months ago, but that is the problem with this area. Every time I wanted to have a meeting, I thought, oh, good, some new data came out, and it sort of narrowed things down.

And I understand this point a little better, but then there are these other three points that we don't know about. I mean, I think you have got to give us advice for now, and if you want to make comments on advice for the future, you are welcome to do that, too.

CHAIRMAN DAUM: Karen, I would like to ask a question actually about the implications of accelerated approval for patients in the actual use of the vaccine.

And I am wondering if you could accept an assumption that, for example, some early endpoint is chosen for the initial trial, or the initial checkpoint. I may not be using quite the right terminology here.

But let's say a vaccine is shown to

prevent HPV infection, and let's say that on the accelerated approval track that a confirmatory trial is set up, and the agency begins processing data and preparing for licensure, and allowing use based on the HPV infection prevention dataset.

The trial is then going on to say, prevent CIN-2/3, and just to make something up for the sake of the question. And so at what point would the accelerated approval actually allow use of the vaccine publicly, and would the trial, the CIN-2/3 prevention trial, still be going on?

And there are two obvious questions there. Would placebo people still be enrollable at that point; and then secondly -- well, would the vaccine be out there in use and people actually able to use it earlier with this accelerated approval?

And would people still be able to be able to be enrolled into the placebo arm of a confirmatory trial?

DR. GOLDENTHAL: Well, here is my thought on the issue. I think that potentially -- and we have not done this before in the agency, at least for preventive vaccine. But my thought in this setting is that accelerated approval might buy you a year in terms of coming out on the market earlier.

I mean, this is what I would envision. A sponsor would have a CIN, and let's say for example a CIN-2/3 efficacy trial ongoing. And then for that particular -- and that study would be very well enrolled in my opinion even at the time a BLA would be submitted with the virology endpoint.

And then that BLA would be under review by the agency and it would go through its review cycle, and so on and so forth. And at some point -- and let's just take an example. Let's say that all issues were resolved a year after approval, or a year after BLA submission.

I would think that that confirmatory, at least in the U.S., I would think that that confirmatory CIN-2/3 trial would basically have to be finished at about the time of approval, because I think it would be untenable to have that trial ongoing.

So again by my math, in the U.S. it would buy you about a year, and some may argue that that year might be valuable. It might even buy you more than a year, because again you would have that CIN-2/3 -- well, because at the point that you get the result of that CIN-2/3 trial, the sponsor has got to QC the data, and they have got to QC the trial, and they have