I think question No. 1, I agree with previous speakers. Again, it would appear that persistent infection in CIN 2/3 consensus endpoints, the accelerated approval I think we should do it. I think we should have as long as possible.

It would appear that the longer we go, the more evidence we'll have in terms of the efficacy in preventing cancer. I would just say I concur with previous speakers with those comments.

DR. DAUM: Thank you. I need to press you a little bit, though. The accelerated approval, where do you sit on that?

DR. FAGGETT: Yes. I think we should have accelerated approval.

DR. DAUM: The endpoint you pick would be? I just want to make sure I'm very clear on what you're saying.

DR. FAGGETT: Again, as previous speakers, that you would prevent the disease. I think the longer you go the more evidence you will have that you can prevent cancer so I would say it would be probable prevention of cancer would be an endpoint.

DR. DAUM: Which endpoint would you pick? Did I miss it? Did you say it? If you did, I apologize. Persistent infection?

DR. FAGGETT: Right. Persistent infection.

DR. DAUM: Okay.

DR. FAGGETT: I said persistent and CIN 2/3.

DR. DAUM: So you picked two different ones.

DR. FAGGETT: Yeah, those two.

DR. DAUM: I apologize. You want two different endpoints?

DR. FAGGETT: Right.

DR. DAUM: I think we know what you want now.

Ms. Fisher?

MS. FISHER: I thought of this in two separately so I'm making two separate statements.

DR. DAUM: Stop for one second. You said something that upset Dr. Mitthune.

DR. MITTHUNE: Just to clarify, Dr. Faggett, would you want both the virology and the CIN 2/3 for accelerated approval or would you want only the virology on regular approval? You want both? Thank you.

DR. DAUM: Thank you, Dr. Mitthune. Thank you, Dr. Faggett. Thank you, Dr. Katz.

Now, Ms. Fisher.

MS. FISHER: Well, first I'm going to speak about the endpoints. From the information that was

presented to us in both closed and open sessions of this meeting, it appears that if an HPV vaccine demonstrated prevention of persistent HPV infection with certain types such as HPV 16 and 18, it would suggest that it would be effective in preventing cervical cancer associated with those types.

However, much appears to be unknown about potential cofactors involved and why some women clear HPV infection and some do not and go on to develop cervical cancer. I think there needs to be more known about these potential cofactors because they may be important independent of HPV infection.

In prelicensure clinical trials demonstrating efficacy, the standard used should include follow-up of all participants to prove not only prevention of persistent HPV infection, but also prevention of CIN 2/3 as well as prevention of cervical cancer because CIN 2/3 is a more certain predictor that cancer will most likely occur and demonstration of prevention of cervical cancer is the only way the vaccine user could be reasonably confident that it is, indeed, a vaccine that could prevent cervical cancer.

The other statement is on the accelerated approval process. I think, needless to say, certainly

cervical cancer is a terrible disease for women, especially in developing countries and we need safe and effective ways to prevent it.

Because the majority of women clear HPV infection and a very small number go on to develop persistent infection, and an even smaller number go on to develop cervical cancer, I'm concerned about an accelerated approval process for licensure.

If the request for accelerated approval was for an HPV vaccine that would only be used by women known to be at very high risk for developing cervical cancer, then I might feel differently.

However, this discussion has been about an HPV vaccine that would target all healthy adolescent girls and adult women, perhaps even female and male children. That's entirely another matter. We need to have a better understanding of the biological mechanisms of long-term immunity of HPV infection.

We need more information about safety including the potential ability of this protein vaccine to induce autoimmunity in a subset of genetically susceptible individuals, as well as the potential negative impact on women with preexisting HPV infection.

Clearly it should not be an a priori

assumption that this vaccine has no long-term negative health consequences whatsoever. Long-term studies need to be done to measure for all morbidity and mortality outcomes.

I'm not talking about paying attention to car crashes and ski accidents that occur during the study but taking serious development of post-vaccination deterioration of health such as multiple sclerosis-like symptoms, arthralgia, arthritis, thyroid disease, etc., as well as exacerbation of preexisting autoimmune conditions during long-term follow-up.

If we don't ask for these kinds of studies prelicensure, an unknown number of young women who may indeed avoid infection with HPV and cervical cancer by using an HPV vaccine could be left with other vaccine induced chronic health problems because the vaccine was licensed too quickly without enough data. I do not think the accelerated approval process is appropriate for this vaccine.

DR. DAUM: Thank you, Dr. Fisher.

Dr. Palese.

DR. PALESE: This is obviously a very complex issue here. Human papilloma virus we don't have a good system, no good animal model, and

certainly no antivirals and no vaccines. On the other hand, cervical cancer appears to be almost 100 percent associated with infection by HPV.

Now, if we have a vaccine which basically prevents infection and we can't demonstrate virus, I'm sort of persuaded by persistent HPV as an endpoint and I would go along with Dr. Lowy's recommendation of a year. I guess he meant two assays. He didn't give a specific amount because of an interval but I think six months may be okay.

Clearly as a virologist I feel if there is no virus, then there is no disease so this is really for me very, very compelling that one would be able to prevent infection and replication of the virus that this must have some consequences and that's why I feel very comfortable with an endpoint which measures persistent HPV infections.

And having that rationale, I sort of also feel that an accelerated approach would be -- I would support that. Accelerated approval I would support, particularly if there is a provision for a long-term analysis in there and that the time would be large enough in terms of measuring other parameters. Again, I would be happy enough if it turns out that there is no virus replication that we would vote for an

accelerated approach.

In terms of the labeling I would also say if the vaccine prevents infection, then it is also most likely prevents cervical cancer so I would be quite happy with that kind of labeling.

DR. DAUM: Dr. Goldenthal, if I understood you, the accelerated approval scenario would be one that would be granted by the agency only if there were a confirmatory trial in progress or enrollment was completed. Is that correct?

DR. GOLDENTHAL: That's the way accelerated approval ordinarily works, yes.

DR. DAUM: So, Dr. Palese, let me come back to you for just one moment. You mentioned that you would have accelerated approval based on viral persistence, if I understood you.

DR. PALESE: Yes.

DR. DAUM: And if that's the case, then you would accept that caveat that the confirmatory trial be in progress but I didn't hear you say that.

DR. PALESE: Yes, with the same endpoint. I mean, I'm not -- maybe I didn't understand your question.

DR. DAUM: Okay. Agency people listen and if I'm not saying it right, please jump in. It seems

to me that you might ask for traditional approval with persistent viral infection as your endpoint.

DR. PALESE: No, that's not what I am --

DR. DAUM: Right. And then accelerated approval though would have to have an interim endpoint that approval would be granted for but a confirmatory trial in progress or underway as well for the agency --

DR. PALESE: What kind of endpoints? I mean, that's the question. For this confirmatory trial that's --

DR. DAUM: That's what we're asking you to comment on.

DR. PALESE: Okay. I will be happy with persistent -- if there's no virus, there's no disease so I will be happy with the confirmatory trial with the same endpoint of persistent HPV.

DR. DAUM: Does that fit with agency guidelines or are we okay with that?

DR. GOLDENTHAL: It almost sounded like he's more advocating traditional approval.

DR. DAUM: I think so, yeah. With persistent viral infection as the endpoint. I think that's what he's saying.

DR. PALESE: So what am I saying?

DR. DAUM: I'll be damned if I know.

DR. PALESE: I will keep going for an accelerated approval. If that requires a confirmatory trial going on, I would support that but with the assumption that the endpoint again would be persistent infection.

DR. DAUM: Okay. I understand what you're saying and it doesn't completely gel for me but that's okay. And the indication would be what?

DR. PALESE: That a vaccine, if it turns out that it really prevents infection, most likely prevents infection to a certain percentage and, therefore, is most likely to prevent also cervical cancer.

DR. DAUM: Very good. Thank you.

Dr. Myers.

DR. MYERS: The endpoint of interest is cervical cancer and I think the data on CIN 2 and 3 as a part of the natural history is sufficiently robust that it predicts a clinical benefit directly I think is clear and it probably serves as a surrogate for cervical cancer.

While I agree with a lot of the preceding comments, it's intuitive that prevention of infection, and specifically prevention of persistent infection

even without dislogic changes, it's intuitive that those could be endpoints. I don't think the data at this time are sufficiently robust. Like somebody said previously, at this time that is a qualifier.

I think some of the data we heard in closed session yesterday may imply that a year from now or so we may be able to say that persistent infection, in fact, in the absence of histology could be a marker but it's not at this point. I would suggest that infection are secondary endpoints and not the primary endpoint.

I would want data on the other high-risk HPVs as well looking for emergence of those. But also because I think for the next generation of vaccines that will be very important.

Going back to the secondary endpoint, the infection endpoints, I think, are also critical to collect that now as part of the study so that the next generation of vaccines we will, in fact, know whether we can utilize these as surrogate markers for the histology.

I mentioned this before a couple times. I would just like to say it again. I think it is important to understand that it will be important to examine the outcomes on an intent to humanize

perspective.

I think to look just at HPV 16 and 18, naive individuals, would be a mistake and that we need to understand what immunization of previously infected young women is as to whether that reduces the risk of persistent infection or has an adverse outcome because this vaccine will not be directed just at naive individuals. It will be targeted to specifically young women who are at high risk and, therefore, may already be infected.

As to accelerated approval, I'm unable to support that conceptually in that I think it would be very difficult to complete a study even if enrollment is completed. Once the vaccine is approved and is being marketed, I think it would be very difficult for the placebo arm to be maintained. Therefore, as I think the definitive endpoint is CIN 2/3, then I think it would be very difficult to support an accelerated approval.

With that said, I think if, in fact, early on in the process there were a significant difference between the groups for CIN 2 and 3 before the full duration of the study is completed, then I would consider accelerated approval at that point.

From the labeling perspective I thought

Steve Kohl and Dixie said it quite well and I would agree with that.

DR. DAUM: Thank you very much, Marty.

Dr. McInnes.

DR. McINNES: My certainty and uncertainty about papilloma viral infections and their relationship to cancer and the role of this vaccine waxed and waned. I think I'm left here with a fair amount of certainty that we are reasonably uncertain about lots of things here.

I'm moving forward on the assumptions that human papilloma virus infection is necessary for and does precede cervical cancer, although it's not sufficiently causal. I do understand that HPV infection with the oncogenic type is much more common than the resulting cancers.

Nevertheless, I'm also reasonably comfortable with the assumption that persistent infection is linked to risk of CIN 2, CIN 3, and invasive cancer.

With the reality of having to accept a surrogate, I am comfortable with persistent infection as an endpoint, surrogate endpoint. The timing of that, I am somewhat concerned about the short interval that has been proposed.

Given the data that incident infections may clear within eight months, I am bothered by time frames that are less than that. I think I envision protracted trials rather than condensed trials.

I am somewhat persuaded that cytologic abnormalities are an endpoint for consideration in the trials because they certainly would give us a sense of the bad player HPV infections with more rapid progression to the CIN 2 and CIN 3.

I do not dismiss the role of cytological evaluation. Certainly it's a question of where it would be within the framework of endpoints, primary, secondary, tertiary endpoints.

The case definitions, I think I'm not totally resolved on which of the spectrum of clinical disease has a place and which doesn't. At this point I'm not persuaded that any of the spectrum doesn't have a potential place in articulation of an endpoint. I would leave open the possibility of a spectrum of clinical disease being incorporated with persistent viral infection into the endpoint.

Regarding the accelerated approval, I at this time am having a great deal of pragmatic difficulty understanding how sufficient safety data, how a considerable safety database will be brought to

bear for consideration for the accelerated approval.

Pragmatically when I lay out a time frame I don't at this point see much to be gained. I'm obviously open to being persuaded of something other than that. At this point I am advocating a traditional approval and I'm having difficulty understanding the role of accelerated approval for this vaccine.

DR. DAUM: Thank you very much. Quite clear.

Dr. Reeves.

DR. REEVES: Okay. To begin, I would be in favor of accelerated licensure because of the nature of the disease and the long time period and actually seeing the disease of interest. The disease of interest is prevention of cervical cancer.

For that reason I believe studies should be done in high-risk populations, the woman that actually get cervical cancer in the United States, and to the extent possible so that one can begin early on. They should involve populations that have cancer registry so that a long-term effect can be seen.

I think the only appropriate surrogate endpoint, and perhaps an endpoint in and of itself is CIN 2/3. In the United States in terms of body count

that is actually the primary cost, the primary morbidity. If treatments for that could be cut down significantly, it would be a significant public health advance so I think that is a very appropriate endpoint -- surrogate endpoint or endpoint.

I believe that the virology and immunology are also terribly important to studies and must be included as either co-surrogate endpoints or data that must be measured. I really don't have an opinion on what persistent infection is.

I think that viral studies must be very complete. The patients, or the subjects, should be followed by cytology as well. Every time that a cytologic sample is taken, a virologic sample taken also. Presumably high-grade SIL will go down but the patients with low-grade SIL, their virology is the important comparison along with the placebos.

Cervical immunology is terribly important to this and I believe should be included in any of the studies. I think the question of incident HPV infection is probably going to be an impossible one to address.

I think the term is used wrong. The first culture positive is not an incident disease. It's the first incident infection. It's the first infection in

a person that has not been infected with the agent previously.

I think it's going to be impossible to cover in the studies but it's been brought up a couple times. One of the primary epidemiologic risk factors is age at first intercourse. That group of women in the high-risk group is going to be part of this but only a small part of it.

As far as the package insert, I believe it's premature to be discussing that.

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

Dr. Goldberg.

DR. GOLDBERG: Thank you. I think that the accelerated approval --

DR. DAUM: Sorry. We may have a procedural problem.

DR. MITTHUNE: I would just like to ask for a clarification, Dr. Reeves. You said that you thought that CIN 2/3 would be your basis for accelerated approval?

DR. REEVES: That's correct.

DR. MITTHUNE: What would be your confirmatory study endpoint?

DR. REEVES: My confirmatory study? I think CIN 2/3 in and of itself would be sufficient. I think

that's an important enough public health problem that if that could be dramatically reduced, I would be quite happy. I think cervical cancer is going to take decades which is, in fact, the final end product.

DR. MITTHUNE: Right. So are you actually advocating a traditional approval based on CIN 2/3 as your endpoint?

DR. REEVES: That's correct, with obviously evaluation of the virologic and immunologic data that is collected along with it.

DR. MITTHUNE: Thank you. One further clarification. Dr. McInnes, you said that you did not support and you advocated a traditional approval. It wasn't clear to me what endpoint would support that traditional approval.

DR. McINNES: I would use vaccine type DNA persistence so viral persistence. I talked about some spectrum of clinical presentation ranging from vaccine type DNA positive, cytological abnormalities to some clinical endpoint. I'm not opposed to the CIN 2/3. I'm just considering that it may need to be broader.

DR. MITTHUNE: Thank you.

DR. DAUM: Thank you.

Before we go to Dr. Goldberg, Dr. Goldenthal, I think there's a little bit of confusion

still about accelerated approval. I would like you to just say the sentences you said before so that the remaining committee members can have it straight. What does accelerated approval mean?

DR. GOLDENTHAL: Okay. Accelerated approval means that you would have, I guess, a drug development plan in place where a product would be initially approved, receive the accelerated approval based on a surrogate and, at the same time, there would be a confirmatory efficacy trial that was also well controlled and well under way at the time of license application submission.

This would mean, again, a committee and FDA would have to review the interim data. That interim data could be used for the accelerated approval. Then we would be very interested in the timing, of course, of the confirmatory trial and when it would be completed in comparison to when the license application was submitted. You can have -- you know, we've heard various scenarios.

One thing that was mentioned was, I guess, sort of an early look at CIN 2/3 and then those people might be followed for another year. You might get more follow-up data on other participants in the trial. That was one example of the sort of

accelerated approval. In that case, it was the same endpoint.

Usually you think of a different -- when I've seen it used in other context, it's been usually two endpoints, one for accelerated approval and one for the confirmatory trial endpoint which is something different. Perhaps we can work in the CIN 2/3 for both.

DR. GEBER: I just wanted to add that maybe a way of thinking of it that the accelerated approval is in a way a preliminary approval and if that were granted, the sponsor would have to then meet their endpoint in the confirmatory trial to keep their approval. If one decided that a persistent infection was an endpoint for a confirmatory trial, or if one were not satisfied for an endpoint for a traditional approval, then that would be a preliminary approval.

DR. DAUM: Question about this?

DR. FELIX: Procedure.

DR. DAUM: Please go ahead.

DR. FELIX: If in the confirmatory trial does it have to be a failure to achieve significance or would it -- I'm sorry. Would it have to achieve significance or failure to achieve significance? Would that belie the preliminary approval gain at

accelerated?

DR. GOLDENTHAL: Certainly we can withdraw approval if the confirmatory trial is a failure, so to speak. In other words, if they don't find a significant result, than we can withdraw the approval. We do have that authority.

DR. DAUM: Dixie and Steve, we need to hear from the other half of the table.

DR. SNIDER: I think they need to understand the question.

DR. DAUM: Right to this point. Go ahead but we're running behind. Go ahead, Dixie, and then Steve.

DR. SNIDER: My question is that, if I understand correctly then, under accelerated approval the product would be licensed and available and, therefore, the individuals who participated in the confirmatory trial would have to be informed about the availability of the vaccine and presumably the IRBs would require that is included in the consent form and the IRBs would have to approve such a trial.

DR. GOLDENTHAL: Right. That actually speaks to my major concern which is continuing the trial following approval. I don't believe that there is a major issue in continuing a trial during the FDA

review of the BLA.

As I have said, I think that accelerated approval might buy you a year but it's still got to be -- that's a very good question. Would an IRB concur with, you know, an ongoing placebo-controlled trial following approval. In the U.S. that's pretty unlikely.

DR. HILDESHEIM: If I could provide some factual information.

DR. DAUM: Tell us who you are and your affiliation.

DR. HILDESHEIM: Allan Hildesheim with the National Cancer Institute. If we did any interim analysis to submit to the FDA, we would have to present it to our IRB data and safety monitoring board.

We've discussed this and it's clear that any trial that had early CIN 2/3 as an accelerated outcome with confirmatory long-term CIN 2/3 would not happen because we would vaccinate our placebo group at the instant that we saw any evidence of protection against CIN 2/3.

DR. DAUM: Other the other hand --

DR. HILDESHEIM: Possibly even persistent infection.

DR. DAUM: On the other hand, if the first basis for interim approval were some viral marker like viral persistence and there was an indication for viral persistence and the trial were ongoing to look at CIN 2/3 or cervical cancer, that trial wouldn't necessarily have to be aborted because the vaccine would be available for prevention of viral infection.

IRB would certainly have to address that and it's hard to know how it would come out. It's not as clear as the example you gave where it's very clear an IRB shouldn't go along with it if they were willing to even.

DR. HILDESHEIM: You are correct, it's more murky. However, my sense from the discussions I've had with IRB and DSMB members for our trial is that if we showed something was protected against persistent infection for a reasonable amount of time, that we may not have to abort the trial but we would be required ethnically to inform all of the women.

If they wanted, they could withdraw from the trial. In effect, any follow-up data after that would be highly biased by who stayed and decided not to stay in the trial.

DR. DAUM: Perhaps. I think that's very helpful. I think we understand what accelerated

approval means and I'm really anxious to hear from this side of the table so let's go on.

DR. GOLDBERG: I'm not sure I know anymore but I believe there can be a well-designed trial for vaccine efficacy based on the CIN 2/3 or worse including any cases of cervical cancer that would be included in that endpoint.

With that said, I do believe you have to monitor for persistent HPV and the length of the interval has to be studied. I guess from everything we've heard so far, probably a year is the interval.

What I would suggest as an interim analysis for CIN 2/3 efficacy requiring that persistent -- that the endpoint is supported at that interim analysis if there was a recommendation to stop the trial early with the persistent HPV also showing efficacy.

I believe that we should be studying this in high-risk populations as well. I think there should be some stratification and HPV positive at entry should be retained as a stratum file so that you will have some information on possibly higher risk women.

The length of the study is an issue. It could be a much longer study than was anticipated, but I don't believe that even if we did a vaccine efficacy trial with persistence as the endpoint that we would

ever be able to complete a confirmatory trial.

I think we have to make the effort to expand both enrollment initially and lengthening the follow-up but having a carefully planned interim analysis or interim analyses based on the best available planning mechanism that you can put in place.

I also think that there needs to be a mechanism in place for the long-term follow-up for the occurrence of untoward events as well as cancer. You also during the trial should be monitoring for other types of CIN 2/3 and/or cervical cancer associated with other types of HPV than just 16 and 18 to be able to access the impact of this on that.

DR. GOLDENTHAL: Could you just clarify are you advocating CIN 2/3 for traditional approval?

DR. GOLDBERG: Traditional approval.

DR. GOLDENTHAL: Okay.

DR. GOLDBERG: I don't believe accelerated approval is really possible here. But I do believe that if the trial were well designed and the results compelling somewhere during that trial, there could be an early stopping but there still needs to be follow-up for safety.

DR. DAUM: Thank you, Dr. Goldberg.

Dr. Fleming.

DR. FLEMING: Thank you. Clearly the prevention of cervical cancer is a critically important public health problem and that leads to an obvious need for effective, responsible, and timely evaluation of various vaccines here for targeting the risk or for targeting the reduction and the risk of cervical cancer.

However, the use of surrogates has always raised complex issues. There is clearly a tradeoff when we're using surrogates between the timeliness and the reliability of conclusions. In this specific setting what we have in hand is strong evidence that HPV infection is a necessary factor.

But there is significant uncertainty from the information we have at this point regarding whether reduction in various virologic, cytologic, and histologic markers and what duration of effects on those markers translates into being reasonably likely to predict benefit which is the condition put forward before us by the FDA.

To me this leads us to the wisdom that Steve Kohl had pointed out yesterday, "When in doubt, be cautious." I think there is a lot of doubt in this setting. I think there is additional reasons to be cautious. Dr. Fisher pointed out how broadly this

vaccine is going to be used. To my way of thinking, that does mean this is a setting where we have to be cautious.

I'm also concerned that the trials that lead to the initial approvals have a particular burden to be well designed. It's going to be extremely difficult in the future. You won't be looking at future vaccines addressed through placebo-controlled trials. Inferior trials will be even that much more problematic.

All of this leads me to being very cautious. My sense is from what we've heard the marker that has the strongest evidence for reliability, even though it, too, is not fully reliable, is CIN 2/3 and, in particular, CIN 3.

My sense here, as I think through the two stages, Bob, in this accelerated approval leading to full approval, ultimately the full approval from my perspective needs to have considerable evidence that we're influencing CIN 2/3 in two ways here. One is relative to the targeted types of HPV 16 and 18.

I think we need to be ruling out 33 or 50 percent reductions. We need to have sufficient evidence that we have something on the order of an 80 percent reduction that we can rule out 33 to 50

percent reductions. Relative to untargeted types, I would argue that we need to have evidence that over all we're seeing a reduction in CIN 2/3.

Ultimately the data that we would have in hand in the final approval, I think, has to address all of the dimensions of strength of evidence, breath of effect, and durability of effect.

Now, working backwards could we do an accelerated approval? I think this is very controversial. I think there is a potential here for doing an accelerated approval.

Just to give you a sense of what I'm thinking, the type of trial that I think can address what I would think we would need for an accelerated and for full approval is one that might only involve -- I say only in contrast to what would be in some settings even bigger trials, 10,000 to 15,000 participants in a two-arm trial where they would take a year of accrual and about three years of follow-up to get what I'm getting at here for the accelerated approval target and an additional two to three years of follow-up for the full approval, essentially what could be the accelerated approval.

If we had significant evidence of a reduction in targeted HPV 16 to 18 type CIN 2/3, that

would take on the order of 20 to 25 specific cases. The problem with that is what we may be seeing with that is simply the ability of the vaccine to reduce this risk of progression to CIN 2/3 in the rapid progressors which may not represent a more global effect.

For that reason I would strongly support that there would be a duel endpoint in the accelerated approval that would be based on persistent infection, specifically persistent HPV 16/18 infection.

I'm not comfortable at this moment, though, defining over what interval. I think there is a lot that is unknown, although the good news is in the course of finalizing the design and implementing these trials, there will be some additional time to tap into natural history data.

What I would specifically focus on here is getting additional data from prospective cohorts that allows us to follow up incident cases that are going to allow us to understand more clearly what will be the time of persistent infection as well as potentially viral loads. This could be multi-variate.

What is the duration of persistent infection and level of viral burden that translates into fairly reliable evidence that when these markers are

achieved, there is going to be a high level of risk of progression of CIN 2/3.

The reason I want this as part of the accelerated approval evidence is that if an accelerated approval is going to be based on 25 cases relating to CIN 2/3 which is all it takes to rule out quality when you have an 80 percent reduction, I want to have additional evidence to give me a sense that when I'm going to get more durable evidence later on about more global effects on CIN 2/3, that this is going to be achieved and that is where I think the added data on persistent infection as a co-marker for accelerated approval is complimentary in its insight.

What I haven't mentioned is there's a third dimension I would ask for to be explored in the accelerated approval. In addition to persistent infection where you are going to define much better than we can today exactly what that is after you follow these incident cohorts, I would like to see the CIN 2/3, HPV 16/18.

I would also like to see consideration of need for reduction in invasive therapies because that is, in fact, part of the tangible benefit that is going to be achieved here.

Where I would define invasive therapy,

certainly what I'm thinking are of, for example, what typically would be clinical care when you have a CIN 2/3 infection, diagnostic decisional procedures, electro-loop excisions, for example.

Interventions which by their very nature have such clinical importance that it exceeds the cost inconvenience and toxicities and side effects of the very intervention, i.e., the vaccine you're going to be delivering to prevent these. They have to be significant events.

Now, with this as an accelerated approval, what we don't have, just to repeat what I said earlier, in my view is adequate insight about strength of evidence, breathe of effect, and duration of effect.

Ultimately this trial would need to continue and I'm guessing to approximately a six-year median follow-up point to be able to have sufficient evidence to rule out a 33 to 50 percent reduction, that we have even a better effect than that.

If we have an 80 percent true reduction, it's going to take 40 to 60 events to do this. If we have at least an 80 percent reduction on targeted HPV 16 to 18 based on Karen's projections, that's going to translate into about a 50 percent global reduction.

At the same time this trial is going to be powered to rule out no reduction. My standard for untargeted CIN 2/3 is to at least conclude that you are achieving this roughly 50 percent reduction ruling out no reduction.

But for targeted 16/18 I want to see an 80 percent reduction ruling out 33 to 50 percent combined with the additional evidence that invasive interventions are also being reduced.

Final comment. Is this doable? My sense is this is a strategy of about a 10,000 to 15,000 person trial. We're going to be about four years into this trial when we would have this information that I outlined for the accelerated approval. We're still about three years away from having the final data.

There will be certainly a lag time of approximately a year from the time the data are essentially realized and when they would be analyzed, presented, and reviewed for regulatory approval which essentially would mean if at that point the vaccine was now available for potential access to the control arm, there could be some cross-ins.

This gets to, if I don't call it a flaw, a risk of accelerated approval and it's been acknowledged here. If you have an accelerated

approval, does this truly compromise your ability to answer the question of interest. If it does, then I don't think the accelerated approval is acceptable because I think we need to get the answers here for the full approval.

On the other hand if the judgment is this is late enough in the process that any crossing in or lack of adherence to the control intervention would only in a minor way dilute this assessment, then I would consider it to be acceptable.

I'll just note here I acknowledge my NCI colleagues as they point out the ethical dilemmas. I've had this ethical dilemma for a long time as we've implemented accelerated approval in HIV settings and oncology settings.

Is it ethical to say I have enough evidence to bring forth to regulatory authorities approval of a new intervention and, yet, I'm still going to enter or follow people in a controlled trial to be able to get at what we recognize to be the ultimate answer that we know we have to get. That's a dilemma that I think all of us have to face.

But if in our judgement it is ethical, and we can adequately maintain adherence, then I think it is appropriate to consider this accelerated approval

and this strategy so long as we're insured that this six to seven-year answer is going to be achievable.

DR. DAUM: Thank you, Dr. Fleming.

Dr. Sheets.

DR. SHEETS: Can I say I agree with everything you said?

DR. FLEMING: You sure can. The committee would be grateful and so would everyone else.

DR. SHEETS: I find the constraint in the argument of Dr. Fleming to be the ethical consideration of early termination or accelerated approval. Although the trial that he outlines and others have outlined ahead of him is probably doable, I think you would get to the point where clinically speaking it would be very difficult to continue on with the placebo arm.

I believe that the endpoint is CIN 2/3, although I would caution that we should continue to collect data on cytology, as was pointed out previously, because we are assuming here that colposcopy as the gold standard has no downsides and that's not true by any means.

As much as we would like to say that we are experts at this and we are perfect, we're not. The sensitivity and specificity of colposcopy leaves

something to be desired. I think we need to look at the endpoint of a high-risk cytology and histology together.

People who have colposcopicly negative yet persistent high-grade abnormalities on PAP smears will in excisional data have high-grade histology. We just missed the lesion so we have to keep that in consideration as we go forward with the trial that you might outline.

I would advocate also to approach, as has been said before, high-risk women and try to endeavor to try to keep these trials demographically representative of what the future may be very soon in America and certainly include high-risk groups within that not only in terms of ethnic groups but socioeconomic groups that are at greater risk for the development of high-grade precancer and invasive disease with or without screen being present in those communities.

I do think that safety data, as has been pointed out before, is very important. Although we are using recompetent material here that represents what is probably exposed to a woman in general transvaginally, we are giving it now systemically in certain a larger dose than it has ever been inoculated

into someone before or vaccinated to someone before. I do think it's important to continue on with rigorous safety controls in regards to these trials.

I'm not a clinical trialist and I won't tell you how to do that, but I think it's important to bring that data forward because in America although cervical cancer continues to be persistent, we are not going to impact on that cervical invasive rate, as has been pointed out, for 10 to 20, maybe longer years. In the interim the safety data is very important for these women who have been given this vaccination.

That's all I have to say. I would vote for only accelerated approval if it does not impact on the placebo group for the CIN 2/3 outcome. In regards to labeling, I think it might be premature but it would be for the prevention of persistence of high-risk infection and also CIN 2/3.

DR. DAUM: What was your interim endpoint?

DR. SHEETS: I would use CIN 2/3 as the interim endpoint with persistence of viral high-risk oncogenic type of analysis like Dr. Fleming has pointed out with the final endpoint being CIN 2/3.

DR. DAUM: Thank you very much.

Dr. Unger.

DR. UNGER: I think that this is a

nontraditional vaccine and probably for that reason I think it's very important that we are cautious. For that reason I feel that this is our chance to really understand what's happening with this virus in a natural history setting so the studies have to be able to help us understand what we're preventing.

I, therefore, feel that we need a CIN 2/3 histology as an endpoint. I agree that the study has to be designed to help us understand what that CIN 2/3 endpoint means in terms of viral persistence of all the types and immune response.

The trial does need to be conducted in appropriate populations. I really don't see a public health imperative to do an accelerated approval. The package as far as what the recommendation would be, I think that we only -- we have to stick with what we know and what we've shown.

If we end up approving it based on prevention of persistent infection, that's how it's labeled and we can say what we think that means. Until we show something else, I think it's ill-advised to label it as showing something we haven't demonstrated.

DR. DAUM: Thank you very much.

Dr. Wilkinson.

DR. WILKINSON: First I would like to congratulate all involved in this commendable meeting on prevention of cervical neoplasia, a very important issue in women's health. I encourage accelerated approval considering the evidence at hand and the importance of the issue that we're dealing with.

I would favor an accelerated approval study format with the CIN 1, CIN 2, or CIN 3 as the accelerated interim and confirmatory points considering that CIN lesion is the usual source of the HPV infection and that CIN 3 lesions rarely regress. I would also make emphasis that cytology and HPV testing need to be applied in this process and safety net issues need to be addressed. Thank you.

DR. DAUM: We thank you, sir.

Dr. Felix.

DR. FELIX: I will make first a comment. I think one of the things that we saw presented were power calculations. Eventually it won't make a difference because if their studies are inappropriately powered they will not reach statistical significance.

But I will say that the numbers that I've heard in the power calculations look to me to be in error because of the studies examining or the studies

that were used to determine that were lacking in taking into consideration incident disease.

I think the preponderance of data on persistent HPV is robust enough to use it as a primary endpoint. I think that a negative predictive value of nonpersistent HPV is very robust for a prediction of CIN 2/3.

I would favor using that as an endpoint or a co-endpoint. I would favor only accelerated approval once. If for primary endpoint for accelerated approval, I would chose persistent HPV at a minimum of one-year interval.

But I would favor only granting accelerated approval if FDA is aware that there is a complete maturation of the data for the confirmatory process already delivered, and that was very nicely stated would reduce the interval by one year.

The secondary endpoint for the confirmatory trial I would consider CIN 2/3 with a co-endpoint of cytology because, again, we don't know what a vaccine could potentially do to the reduction or change of predicted ability of cytology once a patient has a reduced inoculum. I hope that that data would come out.

As far as the labeling, for the preliminary

approval I would label it, if effective, a reduction of HPV, a persistent infection by HPV that has been associated with development of precursor cervical cancer lesions and, if confirmed by the secondary endpoint, a prevention of cervical cancer precursor lesions.

DR. DAUM: Thank you very kindly.

Dr. Freeman.

DR. FREEMAN: I'd like to start with complimenting the NIH, NCI, and the industry and many others not here who have contributed so much to this important problem of HPV.

After giving this careful consideration, I feel that the traditional approach with CIN 2/3 as an endpoint is the safest in this particular situation having been involved and actually chaired the data monitoring committee.

I understand the complexity of how data monitoring committees can evaluate data and decide to make decisions for early termination. It's not a trivial matter and I would emphasize the data monitoring committee needs to be an independent committee totally and the principal investigator should not be involved in these decisions.

The reason that I favor this as an endpoint

is this is an important decision here that will affect many millions of lives. We don't know the outcome.

There have been randomized trials, and I can think of one in particular recently in lung cancer where a product was given to patients with lung cancer to try to prevent secondary lung cancer where, in fact, it turned out that the treatment was worse and more patients were dying in the treatment.

It was a subset of patients who continued to smoke as it turned out. It was very, very good preliminary lab data and clinical data to support this clinical trial. But that is one of the reasons I think that we need to be especially cautious with a study that can impact so much on patients' safety and their outcome.

However, I would say that the virologic, immunologic studies are very important and other studies. For example, we talk about mucosal immunity and factors in the vagina that could impact on infection.

For example, women in certain countries are prone to use vaginal medications more frequently than in others. Particularly if you're doing a study in different locations, these factors could possibly impact on the infection. We need to study these as

well as part of the design of the clinical trials.

I would use the CIN 2/3 as an endpoint that both men and women will understand who will be receiving the vaccine. Eventually men possibly in other trials. Also that the physicians that have to take care of these patients will understand the significance of the trial and the endpoints.

Also I am concerned that if you give an accelerated approval, the information that's on the label may very well influence what happens to the definitive trial.

Obviously informed consents would have to be changed based on that your control arms may be affected, particularly if patients have not been fully entered and followed for enough time. The definitive answers here, or the most proximal answers to the real question which is whether the vaccine actually prevents cancer may never come to us. I think that is particularly important.

DR. DAUM: Thank you very kindly.

DR. GREENE: Thank you. A few comments. One is the idea that even a perfectly effective and perfectly administered vaccine could possibly eliminate all cervical cancer is somewhat naive. I would say it's comparable to expecting the elimination

of cigarette smoking to eliminate all lung cancer. Nonetheless, it would be desirable if no one ever smoked cigarettes. I think there is a certain analogy there.

Next is a question that came up earlier in the discussion. What is the number we need to treat with a vaccine in order to eliminate one case of cancer? The number needed to treat, of course, is one over the absolute risk reduction.

In this case we don't know what the absolute risk reduction is so that we can't a priori calculate what the number needed to treat is. It may be that this kind of a trial might help to give us some notion as to what the number needed to treat is.

But it should be fair to people interested in vaccines that the number needed to treat could be very large to avoid one very serious outcome. Certainly that's true for hepatitis B.

It would be true for the number needed to treat with varicella vaccines to avoid one death from varicella pneumonia so that a very high number needed to treat I think would still be an acceptable indication for vaccine.

Next is that to me the most important point that we don't understand yet is the durability of a

vaccine affect and this has very important implications in terms of who and when you would suggest to receive the vaccine. Who should the recipients of the vaccine be?

In theory, if the immunity was life long, then the appropriate recipients would be children at birth because that way you could be 100 percent confident that all persons would be protected at the time of first intercourse.

However, if the vaccine effect wans after 10 years, you would wind up with no net reduction in the incidence of infection so that we definitely need to know what the duration of the effect is in order to know who to treat with the vaccine.

The question of identifying "high-risk population" is extremely difficult. Women who are celibate life long basically do not get cervical cancer. Everyone else is "at higher risk."

Among those people you can identify people who are even at further greater risk on the basis of age at first intercourse and total life time number of partners. Women, for example, who are commercial sex workers have ultimately the highest risk next maybe to persons who are immunosuppressed, HIV, transplant recipients, etc.

It would be difficult for me to imagine asking parents to bring in their girls for immunization if they expected their daughters to have a very young age at first intercourse or to have a very large number of sexual partners life time. I would think that would pose some difficulties. As the father of a 16-year-old daughter, I'm particularly sensitive about this issue.

I'm not sure who we should label, how the vaccine would be labeled in terms of who the vaccinees should be and who should the targeted population be.

Finally, with respect to the definition of persistent infection, it's quite clear from the data that is already available in the literature, Woodman's paper and Lancet, Hoe and Burk's paper in the New England Journal of Medicine that two cultures separated by less than 12 months really describe only incident infection and not persistent infection.

Not cultures but PCR assays so that you would need -- it would seem to me that you would need to have two positive assays at least a year apart to define a persistent infection.

Finally, I am persuaded by the discussions that I've heard from the past two days as well as Dr. Goldenthal's assessment that the difference between

accelerated approval and final approval might be 12 months.

I am persuaded from that, as well as the difficulties that have been acknowledged with attempting to complete a definitive trial in the wake of an accelerated approval, that the accelerated approval mechanism is not appropriate for this vaccine. My recommendation would be that the standard approval mechanism be used and that the endpoint be CIN 2/3.

DR. DAUM: And I thank you for your very cogent comments.

Dr. Pagliusi, please.

DR. PAGLIUSI: I'd like to thank the FDA for the opportunity to participate in this meeting first.

Secondly, I would like to express our respect to all the scientific community who worked on the development of these vaccines and brought this field so far.

Now, at this stage we believe that cervical cancer is not a visible endpoint and that an intermediate endpoint should be considered. We would favor CIN 2 and 3 as the most appropriate endpoint.

However, if trials should take longer than expected, infection at endpoint would be a surrogate

endpoint to consider only if a sustained protection is proven because from the public health point of view, a vaccine that should be boosted every year or every second year would be a challenge for coverage and compliance and may not be useful at all.

In this sense the laboratory wishes to accelerate the vaccine development and consistent with this line we would welcome the accelerated approval but provided that robust data is created to support long-term duration of protection and that the persistent infection is well correlated with CIN 2 and 3. We would favor the one-year interval between PCR positive points.

DR. DAUM: Thank you very much.

As a penultament speaker, Dr. Decker.

DR. DECKER: This vaccine is an exciting prospect. This has been a fascinating discussion. Prevention of ICC cervical carcinoma is a major public health goal so the stakes are high, as are the penalties for missed steps.

Based on our presentations and discussions, I conclude that first there is not presently the necessary proof regarding the effect on cervical carcinoma of vaccine induced changes in virologic or cytologic endpoints. As much as we think they're

correlated, there is clearly some uncertainty as to what the effect on ICC would be are changes in those prior measures.

Secondly, a study endpoint of cervical carcinoma itself is infeasible for multiple reasons.

Third, as CIN 1 is not a clearly defined homogenous group, it's use as a study endpoint would be problematic.

Fourth, it appears that the study using CIN 2/3 as the primary endpoint could be conducted within time frames and expenses that are commensurate with other modern vaccine efficacy trials.

Finally, my own observation, I believe that licensure of a vaccine is likely to lead to widespread use irrespective of license indication. This has implications regarding the need for high confidence regarding the outcome of confirmatory studies to reduce the risk that a widely used vaccine would later be shown to be poorly effective.

At the same time, concern regarding the ability to conduct these confirmatory studies, and moreover the need for complete safety data in both males and females before any licensure because it seems to me that any rational use of such vaccine would target both males and females such as we do with

rubella vaccine.

Accordingly, I would recommend that the primary endpoint should be efficacy against CIN 2/3. The study should incorporate secondary or observational objectives regarding virological and cytological outcomes so that we can improve our understanding of the relationships between these outcomes and CIN 2/3, and perhaps allow for simpler studies subsequently.

Similarly with Dr. Katz, I would encourage the inclusion of nested substudies to explore related epidemiologic and clinical questions. The study design should not be predicated on a plan for accelerated approval but a design such as I have just described would permit reconsideration of accelerated approval should findings during the course of the study warrant that reconsideration.

Finally, the license indication should be based on the outcomes proven in the study which should also explain the basis for a belief that these outcomes are relevant to the prevention of cervical carcinoma.

DR. DAUM: Thank you. To bring this discussion to a close before I forget to say it, I would like to really commend all members of the

committee and members of the sponsors who presented data to us and, of course, our FDA colleagues.

I think this has been a very wonderful discussion. I think the committee has transcended their usual degree of excellence by having a very lively debate and consideration of all points of view.

Having said that, I will very briefly give you mine. I think that one of the things we haven't said a lot about is that if we have a potential preventive strategy to stop a disease like cancer that we ought to do everything in our power to ensure -- I think everyone in this room would agree with this -- to ensure that it be developed to get the definitive answer of does it or doesn't it prevent this horrible disease as quickly as we possibly can.

Having said that, I favor an accelerated approval strategy but only if things can be put into place during that. First of all, what persuades me to be in favor of it is Dr. Goldenthal's notion that we might be able to get a confidently effective vaccine to the public a year earlier. I am sort of shooting for that.

It would have to be done predicated on something in place to definitively answer the question about an important endpoint. I agree with everybody

else that CIN 2/3 is a reasonable surrogate for the definitive endpoint.

I think viral persistence were it to be shown could be the interim endpoint. I don't know what the exact definition is. We've heard many different attempts at it. For lack of a better one, I think I would accept the one-year cut off as a definition of viral persistence.

I would not favor interim approval if it compromised gathering of appropriate safety data, or if in the views of the people responsible for the study design compromise the integrity of the study to get to the definitive endpoint.

In that circumstance I would rather let the year pass because I would not want uncertainty or erosion of public confidence once it was decided this vaccine were good enough for general public use.

I, like others, have commented, particularly on this side of the table, am very excited about what I've heard here in terms of a prospect of getting a preventive measure like this out. I would like to encourage everybody who is working on this problem to move things forward as fast as they possibly can.

I think that brings this discussion to a conclusion. Before anybody moves, there are two items

of importance to deal with. One is we have a minor and very quick presentation to make. It will take about 10 or 15 seconds to walk the presentation over. Another two or three to gather it.

This is your moment in the sun here, Ms. Cherry. Nancy, on behalf of the agency, the committee, and I think really everybody else in this room if I could just extrapolate for a moment, there is only one Nancy Cherry, folks, in this universe and she cannot be replaced and will be missed sorely. Thank you so much.

MS. CHERRY: Thanks to all of you. I was going to wait until the end of the closed session today to say goodbye to my committee because it's been such an honor and a privilege to work with them. Those are hackneyed words, I know, but I really, really mean them.

I've taken this job probably more seriously than I should have and more personally than I should have and I've never gotten over my feeling that a kid would have of being totally awed by the group I'm working with. Not just your reputations, not just the importance of what you're doing, but also how good the people are. How good all of you are.

It took a lot of thinking to decide when to

announce that I was ready to retire and you all are certainly making it difficult today.

DR. DAUM: Maybe you'll reconsider.

MS. CHERRY: Thank you all.

DR. DAUM: Now, before everyone starts milling around, I would like to briefly take a line of demarcation here from Dr. Goldberg over and just ask the troops whether you would like to break for lunch or whether you would like to continue working through. Break for lunch? Work through? Okay. We will then take a 10-minute pause, let the room clear, potty break, etc., and we'll reassemble with the review of the lab.

(Whereupon, at 12:24 p.m. off the record until 12:43 p.m.)

DR. DAUM: Okay. Welcome back everyone, committee members, FDA folks. This is an open session on the briefing on activities in the Laboratory of Bacterial Toxins. We are going to try and complete this in a succinct but thorough style and begin by calling on Dr. Walker.

Did I see him? There he is. Thank you, Dr. Walker. Welcome. He will talk to us about the organizational structure and overview of research and regulatory responsibilities in the Division of

Bacterial Parasitic and Allergenic Products.

Dr. Walker.

DR. WALKER: Thank you. Good afternoon. Hopefully everybody can hear me now.

In a few minutes you're going to hear presentations of the research of Dr. Vann and Dr. Schmitt. For this reason I've been asked to give a little introduction to their presentations by giving you an overview of the Division of Bacterial, Parasitic, and Allergenic Products.

I'll do that in two ways. First, I'll talk about the functions of the division and then I'll talk a little bit about the organization of the division to meet these functions.

Very briefly, our mission of functions is to assure safe and effective products for control of bacterial, parasitic, and allergenic agents affecting human health. This involves a number of activities by the people in the division. One of those activities is research. The other is review.

Something I might mention about putting these two together, it's sometimes hard for these people to manage their schedules because things coming in for review are not always something that somebody can plan for so this creates a scheduling problem. As

you'll see when you hear the presentations this afternoon, these people do get the work done anyway.

In addition to the review, there's post-licensure surveillance with the things that it involves like inspections, log-release testing, and review of label and promotional activities. Then we continue to consult with outside organizations like WHO and others that are dealing with problems that are pertinent to the FDA.

I just want to show this to illustrate the involvement of the FDA research and reviewers in the lifetime of a product. As you can see here, the important take-home message from this slide is not all the individual components under each section of the development of a product, but the fact that there's activities that go on under each section of the development of the product.

Like here in very early stages meeting with sponsors, providing some guidance, review of original submission and subsequent amendments, technical advice for product and assay development, review of product manufacturing data, determination of product specs, and, of course, continued discussion with sponsors.

I don't want to belabor this since we're moving along with regards to time but, as you can see,

after license activities there is present the product to the advisory committee, continue to have dialogue with the sponsors, and continue to evaluate the products and review the procedures that are being used in manufacturing and so forth.

The point, like I said, that comes out of this is once a product is licensed, the story is not over. The job continues. Post-licensure we still have to review biological deviation reports from industry, participate in inspection of licensed products, view post-approval commitments, so forth. It's a long-term ongoing process.

To make the challenge even greater in dealing with all these products, there's a tremendous variety of products that research and reviewers have to deal with. You can see by looking at this figure about new and improved products that might be possible in the next 10 years.

There are respiratory pathogens dealing all the way from life-threatening diseases to those that cause ear infections, sexually transmitted pathogens, diarrhea-causing pathogens like campylobacter and so forth, and other mucosally trafficking pathogens like salmonella, heliobacter, and so forth.

There's quite a variety of pathogens, most

of these mucosal pathogens that are shown on this slide. Also we need to think about pathogens that are not necessarily mucosal like those that are like materia, lyme disease that are encountered by penetrating inoculation.

And then something that is, of course, is very relevant these days, special pathogens, biological terrorism type agents like franciscella santhrasious, clostridium botulinum, franciscella tularendous, and arensia peskas.

In addition to these pathogens, we also have products, the allergenic antigens dealing with latex antigens, cockroach, and various plant antigens, and skin test antigens. I'm just trying to give you the picture that there is a variety of types of products that our people have to be able to deal with over the next couple of years.

To meet these challenges the Division of Bacterial, Parasitic, and Allergenic Products is divided into eight laboratories. There's the immediate Office of the Director with myself. I have an excellent deputy director Carolyn Deal.

Carolyn and I are supported by people who are regulatory and administrative staffs. We work together to help all these people in the various

laboratories accomplish the jobs that they have to do.

We have eight laboratories. The Laboratory of Respiratory and Special Pathogens, Laboratory of Bacterial Toxins, which you'll be hearing from today, Laboratory of Mycobacterial Diseases and Cellular Immunology, Laboratory of Methods Development and Quality Control, Laboratory of Immunobiochemistry which is allergenic products, Laboratory of Biophysics, Laboratory of Sexually Transmitted Diseases, and Laboratory of Bacterial Polysaccharides.

If you look at these laboratory names, you'll see that they are identified by the types of pathogens and types of approaches they use. I think it is also important to realize that the talents and the resources that are present in these different laboratories we brought together on certain focus areas.

These are some of the focus areas that we're currently dealing with in our division. One of those is standardization of assay methods for bacterial, parasitic, and allergenic products. Also a large group is focusing on pertussis and other toximediated diseases. You'll hear a little bit of that in just a few minutes. Mycobacterial and other intercellular parasites.

It was mentioned this morning how important mucosal immunization is and how much we need to know about that. We've got work going studying mucosal pathogenesis and immunization, products to combat bioterrorism. We also have a very active group dealing with allogenic products.

What I'm trying to show in this slide on the screen now is that you can take those laboratories and those focus areas and sort of think of it in terms of a matrix. You can just see how we pull our resources together to accomplish things.

All of the laboratory names are shown across the top. I've abbreviated some of them just to make it easier to read. The various focus programs are shown going vertically.

If you look at assay standardization, that's something that involves everybody, all the laboratories to some degree or another. Pertussis and toxinmediated diseases involves work from the Laboratory of Methods Development and Quality Control, as well as biophysics, toxins, and the respiratory and special pathogens groups.

Microbacteria is really one laboratory that's dealing with that. Mucosal pathogenesis immunization, one laboratory dealing with that. We

now have six laboratories out of the eight that are dealing with some aspect of bioterrorism agents and two of the laboratories, Biophysics and Laboratory of Immunobiochemistry, they are dealing with allergenic products.

I'm going to go very quickly through this. I have identified these laboratories and this will just give you a little bit of flavor of the type of research that is going on. The Laboratory of Biophysics they use various instrumentation such as NMR to characterize biopolymers.

Examples are given here. And macromolecular assemblies so they bring a lot of technology that really opens up new doors for some of us in the other sections. They also have computer or simulation methods for collector myogen analysis.

Laboratory of Bacterial Toxins, we're not going to say anything about that because you're going to be hearing about their program in just a few minutes. Laboratory of Respiratory and Special Pathogens conducts structure and fluction studies of various toxins and regulation of virulence factors, B. pertussis and B. anthraces.

Laboratory of Bacterial Polysaccharides is another rather large laboratory in our division. They

characterize immune responses to polysaccharide and conjugate vaccines and work toward standardization of methods for relevant clinical applications and develop physical and chemical methods for improved evaluation of license and experimental vaccines. There's quite a lot of work there to do with the polysaccharide and the conjugate vaccines.

Mycobacterial Diseases and Cellular Immunology are evaluating immune responses to intercellular bacteria, mycobacteria and tuberculosis. They are assessing vaccine strategies particularly for tuberculosis.

Enterics is looking at pathogenic mechanisms such as invasion mechanisms of campobacteria and shigella, hormonal controls of conococcual pathogens, mucosal immunity that will help us understand not only these but other pathogens infecting mucosal services.

The Laboratory of Methods Development and Quality Control, as the name suggest, is one set up to develop, standardize, and evaluate quality control methods for bacterial vaccines and develop and evaluate and apply the serological methods to measure immune responses in vaccine trials.

Also an aspect of the overall lab accreditation project the FDA is doing now, people in

this laboratory help coordinate the quality assurance activities within our division, provide leadership and initiative to accredit to the CBER Quality Control Testing Laboratories.

The final laboratory I want to mention is Laboratory of Immunobiochemistry where they are looking at not only the allergen structure inflections but the immune responses caused by these allergens, as well as ways to modulate these immune responses.

As you can see, we have a variety of things going on. We have a lot of talented people. In just a couple of minutes you'll hear from two of them and have a better appreciation for what they are doing. Thank you.

DR. DAUM: Thank you very much, Dr. Walker. Are there any comments or questions, concerns? Thank you again. I appreciate your time.

I would like to next introduce Dr. Willie Vann in one of two hats that he'll be wearing in the next little while. This hat is as the Director of the Laboratory of Bacterial Toxins, one of the laboratories that Dr. Walker mentioned.

When Dr. Vann has concluded his remarks as director of the laboratory, he will then transform into Dr. Vann in charge of his program and tell us a

little bit about that. We'll actually have a hiatus in between for committee comment if there is.

Dr. Vann, as director of the laboratory tell us what's going on.

DR. VANN: The Laboratory of Bacterial Toxins is organized into three sections, Neurotoxin Section, Glycobiology Section, and Corynebacteria Section with three PIs. I'm currently the acting PI for the Neurotoxin Section. We have recently since our review completed the hiring of a new PI, Dr. James Keller for the Neurotoxin Section.

This section currently has two post-doctoral fellows and a biologist. They work primarily on clostridial neurotoxins botulinum and tetanus. The Clycobiology Section has currently a post-doctoral fellow and a biologist.

This post-doctoral fellow is working on an anti-bioterrorism project with anthrax. The Corynebacteria Section currently has a post-doctoral fellow and a microbiologist, newly hired microbiologist technician. This post-doctoral fellow is also working on an anti-bioterrorism anthrax project.

The laboratory has product responsibilities for bacterial toxoid vaccines against botulism,

diphtheria, and tetanus, vaccines containing toxoids as components of polysaccharide conjugate vaccines, and botulinum toxins, active toxins, as therapeutics for diseases involving muscle contractions.

Our review responsibilities include review of biological license applications, biological license application supplements, and investigation of drug applications relating to these above products.

In addition, we have responsibilities for review of lot release protocols for botulinum toxin, annual and prelicense inspections of manufacturing establishments. We participate in efforts to monitor and improve vaccine safety and potency. We evaluate manufacturing deviations reported to CBER. In addition, we provide expertise to the Office of Therapeutics on glycoprotein therapeutics.

The FDA has an anti-bioterrorism initiative. The Laboratory of Bacterial Toxins has incorporated into its existing program research projects on bacillus anthraces which are on polysaccharide biosynthesis and iron metabolism.

The laboratory has organized to meet its existing and future obligations. Existing toxoid vaccines require a maintenance of expertise in C. diptheriae and neurotoxins. The therapeutic use of

plastritial neurotoxins requires a clear understanding of how these active toxins and not toxoids work.

Glycoconjugate and recominant vaccines require expertise on molecularbiology and carbohydrates. CBER has an expanded obligation in glycoprotein therapeutics. Thus, this requires a leveraging of expertise across the center of which we are a part. We have integrated into our existing programs an anti-bioterrorism effort.

The general research objectives of the Laboratory of Bacterial Toxins want to determine the function and structural basis for the potency of vaccines and neurotoxins. Questions we're answering is, (1) what determines the specificity of the interaction of a neurotoxin with the nerve cells, (2) can we replace expensive and low-precision in vivo assays with in vitro assays that are based on biochemical measurements that are functions of toxins?

A large part of our effort is to define new targets for the control of viral bacteria. To this end we are asking two questions. (1) What are the systems for iron metabolism and C. diptheriae, an essential component of virulence, (2) and how do bacteria make their protective coats? Later you'll hear Dr. Smith talk about his efforts in this area.

That concludes my summary of the organizational laboratories.

Yes, sir?

DR. DAUM: Dr. Faggett, please.

DR. FAGGETT: I have just one question. I've had a lot of action with botox injections and all that. Do you folks look at efficacy of botulinum toxin? Is that part of your responsibility, too, or is it just the basic science of it?

DR. VANN: We do review license applications. We have the product lab for reviewing license applications for botulinum toxin indications if that answers your question. We also review IND submissions for various indications using botulinum toxins.

DR. FAGGETT: So you would have information on efficacy of toxin?

DR. VANN: Exactly. We are part of the committee that -- we are part of the committee that actually assesses the efficacy of the toxin for various indications.

DR. FAGGETT: Of course, in D.C. we've had a little experience with anthrax recently and I was wondering are you also involved in development of the anthrax vaccine or what is the relationship?

DR. VANN: The objective of our research on anthrax is to actually develop targets for development of vaccines against anthrax. For example, if there were a key protein that we found on the surface of anthrax or that was produced by anthrax involved in iron metabolism, that could be developed by someone as a potential vaccine.

Or if there was something we found in our research that was essentially for the survival of an organism or the virulence of the organism, that would provide information for someone to develop an antagonist against the organism. This is lying foundation research for the development of new things to combat anthrax.

DR. FAGGETT: Thank you very much.

DR. DAUM: Dr. Kohl, then Ms. Fisher.

DR. KOHL: Several questions. In the material we got there is a little table on the funding of the Laboratory of Bacterial Toxins.

DR. VANN: You're referring to the book?

DR. KOHL: I'm referring to the book from 1997 to 2001. Is that funding -- that does not include personnel, I presume?

DR. VANN: The funding that I have listed there does not include personnel.

DR. KOHL: Okay. So that is basically research funding.

DR. VANN: That is research funding, yes, which includes materials, services, and everything else that is not personnel.

DR. KOHL: Okay. If you had to estimate what the total budget is of the division, could you give me an estimate for that counting personnel?

DR. VANN: I didn't understand your question.

DR. KOHL: How much of the personnel work?

DR. VANN: I don't have an answer to that.

DR. KOHL: What percentage of your time is regulatory? It looks like a lot but I would like to get a feel for that. Who is primary involved in the regulatory activities?

DR. VANN: Okay. That depends upon the person. Dr. Schmitt and I, I would say, could spend up to 50 percent of our time doing regulatory work. Sometimes it's a little bit more. Sometimes a little bit less. If you look at the organization chart, that varies with some of the other people in there.

For example, the post-doctoral fellows, for example, IRTA fellows who are post-doctoral fellows don't spend any of their time on regulatory work. It

depends on the personnel.

DR. DAUM: Thank you. Ms. Fisher.

MS. FISHER: It looks like you have a very important function, particularly now that anthrax has been added. Your organizational chart looked very small though to me. Do you have enough people in your organization to fulfill all of the duties that you are supposed to fulfill?

DR. VANN: Thank you.

MS. FISHER: I mean, could you use more help is what I'm saying?

DR. DAUM: What was that green stuff you guys were exchanging?

DR. VANN: We do our best and we are always trying to get additional resources to actually help. Some things are beyond our control but we do the best we can.

MS. FISHER: Well, I understand that. I guess as a consumer I'm very concerned that you have adequate resources and staff to fulfill the function that you have.

DR. DAUM: Dr. Deal, do you want to respond to that?

DR. DEAL: Yeah. My name is Carolyn Deal.

One thing I think might be helpful to the committee is to clarify that in addition to Dr. Vann's lab other laboratories within the division also have a regulatory responsibility for the anthrax vaccine.

In fact, his lab is not the primary one for that vaccine's review work. That may be helpful in your consideration of some of the workload of the regulatory responsibility.

DR. VANN: Right. There's an entire division that deals with the clinical aspects that are related to these things. What we are responsible for primarily is product and things that are related to product but that is still a lot of work.

DR. DAUM: Dr. Kim, please.

DR. KIM: You briefly indicated that CBER has expanded to include glycoconjugate therapeutics. Can you give me just a couple of examples of what they are?

DR. VANN: Yeah. One of the ones that is very -- probably one of the ones that earns the most money for biotech companies is monoclonal antibodies. One of the first was TPA, erythropoietin just to name a few examples. There are many others that I don't know anything about.

The reason I actually am somewhat involved

in that is that I started out and my major expertise is in carbohydrates.

DR. DAUM: Okay. I think we should move on to the next presentation which will be by Dr. Willie Vann where he will describe his own research activities and his own research program.

Dr. Vann.

DR. VANN: For the sake of clarity of presentation, I will discuss two of the research projects in the research program. The other projects are actually listed in the book.

We are investigating the biosynthesis of capsule of polysaccharides in our model system. We're studying the biosynthesis of polysialic acid. Work has been done largely by a post-doctoral fellow who is no longer with us and a technician.

E. coli and niceria meningitides which are encapsulated with polysialic acid are generally associated with invasive disease such as meningitis and urinary tract infections. These are the structures of the common known polysialic acid capsule of polysaccharides. We are using in our system the E. coli K-92 which is alternating 28/29 polymer.

As an added bonus to studying polysialic acid, we also are studying polysialic acid metabolism.

Polysialic acid plays several roles in microbial pathogenesis. The same system general pathway that is used to synthesize polysialic acid is also important for synthesizing other virulence factors for pathogens like polysaccharide, capsule of polysaccharide, and the eukariotic cell receptors for toxins and adhesives.

The genes that encode polysialic acid synthesis are arranged in three regions. The central region, Region 2, is specific for the polysaccharide. We have concentrated our efforts on the understanding the function of the genes that are in this region.

Our approach has been to purify and characterize enzymes encoded by the gene cluster and in the process assign various genes to functions within the pathway.

Our recent efforts have concentrated on the polysialytransferase, the enzyme that actually perlimerizes the substraight CMP sialic acid into a polymer and exports it through the cell surface. This enzyme is a membrane bound enzyme and is characteristic of glycosialytransferase and other pathogen bacteria.

What we would like to know under the question of the mechanism of this enzyme is, one, how

the reaction is initiated, how the chain is elongated, what components within that complex are responsible for initiation and elongation, and how is this chain fidelity of the repeat unit maintained?

We can summarize our findings thus far on the elongation reaction here. The K92 polysialytransferase itself cannot initiate synthesis, thus it requires other components. It will elongate or use all of the polysialic acids that we know as acceptors. It has a preference for 208 link acceptors. It seems also to have a preference for a hydrophobic aglycon.

We can use our current model to explain data elongation or list it here in the next two slides. The first model proposes that there are three sites, an acceptor site which binds the preferred alpha 2-8 acceptor and two catalytic sites, one that forms a 2-8 linkage and one that forms a 2-9 linkage.

Once the 2-8 and 2-9 linkages are formed, the newly formed 2-8 linkage moves to the preferred site and then the reaction starts over again. The alternative mechanism proposes that there is a 2-8 binding site and a single catalytic site. Once the 2-9 linkages form, the enzyme undergoes a comfirmational change to allow it to bind the newly formed 2-9

linkage and then a 2-8 is formed and then you start over again.

We have approached the initiation reaction in using two different types of methods. The question is how many things are involved in initiating the reaction and how does that occur.

One of these is using complementation. We know that we can separate the initiation reaction from the elongation reaction by simply cloning out the polysialytransferase gene. In the complementation experiment what we've done is genetically add back various components from the gene cluster and ask the question can we restore the ability to initiate synthesis.

Another approach that we've taken is to try to estimate the molecular weight of the complex that is actually doing the reaction. We've done this using a method that is particularly suited for crude systems, namely radiation target analysis. The size of the active complex is inversely proportional to the amount of radiation.

Our current model for the initiation reaction is listed in this -- given in this slide. First of all, what we've learned is that the initiation reaction and the elongation reaction

probably uses the same size complex that consisting of a dimer of the polysialytransferase.

The complex actually transfers to some membrane bound glycolipid acceptor. The groin chain stays attached to the membrane acceptor. Now, what we believe is that the acceptor is probably some glycolipid. The next phase of our research involves understanding what this glycolipid acceptor is.

Our efforts to do that will be to chemically synthesis sialic acid analogs that get incorporated into the membrane but terminate, and then selectively using some of the newer chemistry to tag this acceptor and then extract it and characterize it structurally.

The other project that we are studying is the binding of tetanus toxoid C fragment to ganglioside. This has largely been done by post-doctoral fellow Heather Loach, along with a graduate student in the Laboratory of Biophysics.

Clostridial neurotoxins are very lethal and they are produced by C botulinum and C. tetani. They are the active agents in tetanus and botulism and they chemically inactivate toxins or serve quite well as vaccines.

We have mentioned before botulinum toxins are a potential bioterrorism agent. However,

botulinum toxin is also a therapeutic for diseases involving severe muscle contractions.

These toxins are organized structurally into three domains, catalytic domain, central translocation domain, and a receptor domain. The receptor domain is the part that actually binds to the ganglioside on the surface.

This domain is organized to be two domains itself. One of those is elected jelly roll domain, and the second is what we call a beta tree foil.

The binding of tetanus or botulinum toxin to ganglioside is an essential first step in pathogenesis. It binds to the nerve cell, gets internalized, and then eventually goes and cleaves a snare protein that prevents neurotransmitter release.

Most of the protective antibody to tetanus and botulinum toxin is against the binding domain. For that reason scientists are currently developing recombinant vaccines against the binding domain of botulinum and tetanus toxin.

Our approach to determining the binding site is to use available biochemical and crystallographic data and then to make use of molecular model and to predict likely binding sites. We then test these likely binding sites by site directed mutagenesis and

then look at the binding properties of the resulting mutants, and then in a reiterative process refine our model to hone in on the binding site.

We use two types of molecular modeling experiments to actually do this. The first is homology modeling. In this type of experiment what we would do is superimpose the three dimensional structure of proteins that bind carbohydrates on the C fragment of tetanus toxin and look for motifs.

Once we found and narrowed in on an area, we then use our molecular docking which is based on the crystal structure of the oligosaccharide and then dock or look for energy at various locations of that oligosaccharide on the three dimensional structure of the C fragment.

Using that type of methodology we came up with two regions for mutagenesis. Both of these regions are located on the beta tree foil section of the toxin. We mutated all the residues here and what we found is that one of these is actually essentially for binding. Thus, we've concentrated on Region 1 as being the potential binding site to ganglioside.

We later went back and did further modeling using this docking methodology and identified two other residues here, histamine 1271 and aspartic acid

1222. We mutated those, did binding studies. In these traces which illustrate the extent of binding, both of these indeed are involved in the binding of the ganglioside to the C fragment.

Thus, we've refined out model here and thus far we have defined the binding site on to tetanus C fragment as including these residues, the histamine and the aspartic acid I mentioned before, the tryptofame 1289. During the process of this research these residues were identified in literature.

In the future what we would like to do is refine our model using experiments based on the entire ganglioside since most of these experiments were done with a fragment of the ganglioside. We would like to determine the effective side chain characteristics on the kinetics and thermodynamics of binding. It determined the effect of these mutants on binding to nerve cells.

DR. DAUM: Thank you very much, Dr. Vann. Do we have committee questions or comments?

Dr. Griffin, please.

DR. GRIFFIN: This is probably totally obvious to anybody who is a bacteriologist, which is not me. The polysaccharide glycosialytransferase that you are studying, I assume that they are involved with

developing the capsules of these E. coli and are important for virulence of these particular organisms and are potentially targets or something like that?

DR. VANN: That is exactly right. These glycosialytransferase are the enzymes that actually make the polymer. They are part of the machinery that make the polymer and export it and put it on the cell surface.

More specifically, if glycosialytransferase is negative mutants of bacteria, encapsulated bacteria are a capsular.

DR. GRIFFIN: And are then less virulent?

DR. VANN: And are then either not virulent or less virulent. In strains were capsule is essential, they are not viral.

DR. DAUM: Pneumococcus for example?

DR. VANN: Pneumococcus, for example, has been shown with E. coli K1.

DR. DAUM: Dr. Kim.

DR. KIM: I guess one question, I don't know, you may have that on your slide, do you cross-complement between K1 and K12 glycosialytransferase?

DR. VANN: We've never tried that.

DR. KIM: K12?

DR. VANN: No. What we can do is we can

cross-complement it between K5 which is a totally different polymer and certain regions of that genome.

DR. KIM: My question is if you have a K1 mutants what happens if you complement with the K12 glycosialytransferase?

DR. VANN: With K12?

DR. KIM: K92. I'm sorry.

DR. VANN: K92. Oh, okay. That's a different story. K92 and K1 polycosialytransferase are interchangeable. In fact, that's the way we do the experiments. We do the experiments using mutants of K1 since most of the mutants have been made with K1. What we do is we take K92 glycosialytransferase gene and put it into K1 to study the system.

DR. DAUM: Dr. Palese, please.

DR. PALESE: Which laboratories are sort of competing in your field?

DR. VANN: Which laboratories out in the rest of the world?

DR. PALESE: In the rest of the world, yes.

DR. VANN: There are a number of them. One, Dr. Vemmer at the University of Illinois in Urbana. Dr. Silver who is also a collaborator but he is also somewhat competition. Dr. Troy who is --

DR. PALESE: Where is Silver located?

DR. VANN: University of Rochester. There is Dr. -- in the synthesis of polysialic acid, Dr. Stephens was part of the advisory committee. Then in Germany Dr. Frosch works on niceria. There are a number. And there are a few that occasionally you see another paper pop up with something on the system.

There's Cheuw Wong who is a synthetic chemist who actually did some experiments directly on solid transfers which was directly competition. Then there's a group in Taiwan. I don't know whether they are still working on it. Does that answer your question?

DR. PALESE: Yes.

DR. VANN: Okay.

DR. DAUM: All right. If there's no further input -- there is further input. Dr. Snider.

DR. SNIDER: I wondered if you would just briefly comment since there is a bit about the anthrax in your section what you're doing and what the objective is and how that fits in to the rest of things that are going on.

DR. VANN: You have to understand that these anthrax projects are actually new projects. When we wrote this, these projects were just getting started. Briefly I can tell you what I'm doing and I can sort

of hint to what Dr. Schmitt is doing but he can answer that question himself.

The fellow in my lab is looking at a group of genes that were discovered on a virulence plasma for bacillus anthraces which seemed to include how uranic acid synthesis and how uranic acid has actually been associated with other pathogenic bacteria such as Group A scrapococcus.

The question is why is it there, what's it doing. We are just in the beginning of characterizing those genes to see whether they're functional, what those gene products do, and then later ask questions like what does it have to do with hurdles.

DR. SNIDER: Thank you.

DR. DAUM: Okay. If there are no further questions, Dr. Vann, we thank you for both of your presentations. We will now hear from Dr. Michael Schmitt who is the Director of the Corynebacterium Laboratory and the overall structure of the laboratory of bacterial toxins.

Dr. Schmitt, welcome. We need you to probably adjust the microphone down. Talk right into it.

DR. SCHMITT: How is that?

DR. DAUM: That's fabulous. Thank you.

DR. SCHMITT: So what I would like to present today is a very brief overview of my research program in the Laboratory of Bacterial Toxins. The focus of my research is the characterization of iron transport systems in the corynebacterium and bacterium diphtheria which is the causative agent of diphtheria.

While the incidents of diphtheria has declined dramatically in recent decades in the United States and in other developed countries primarily due to the widespread use of the vaccine, a number of recent studies have indicated that greater than 50 percent of the adult population lacks adequate immunity to diphtheria and is potentially susceptible to disease. This is primarily due to waning immunity and failure to receive booster doses of the vaccine as adults.

Now, the vaccine is in the activated form of the diphtheria toxin known as toxoid and it is recommended for adults every 10 years. Additionally, since the vaccine is primarily directed against the toxin, it fails to eradicate the carrier state of the organism. Fully vaccinated and healthy individuals can potentially be carriers of highly virulent organisms and potentially introduce these into susceptible populations.

Another alarming factor regarding diphtheria was the recent epidemic in the newly independent states of the former Soviet Union which occurred in the mid to late 1990s.

This is the largest outbreak of diphtheria to have occurred anywhere in the world in the last 40 years and I think it illustrates the important point of how quickly a disease like diphtheria can reemerge when we fail to keep an adequate vaccination of the population and also when there is a partial breakdown in the medical infrastructure which had occurred at this time.

So the organism I study is corynebacterium diphtheriae. It is a gram positive aerobic nonsporulating bacteria. It is related to the microbacterium in streptomyces, a group of organisms. And it is the causative agent of diphtheria with the primary virulence being diphtheria toxin which has been extensively studied at the biochemical level. We actually know quite a great deal about its structure and function.

We also know a great deal about how the toxin is regulated which has been an interest of mine over the years and, in fact, has been known for over 60 years that the diphtheria toxin is regulated by the

iron concentration in the growth media.

In fact, the human host is generally believed to be very limited for iron with regards to invading bacterial pathogens and, in fact, this low iron environment if the host is generally thought to be a signal to activate certain virulence factors such as diphtheria toxin.

The tox gene, which is the structural gene for diphtheria toxin, is regulated at the transcriptional level by DtxR, the diphtheria toxin repressor protein, and iron when iron functions as an essential co-repressor in this system.

When the organism is grown in a high iron environment, iron will bind the DtxR causing it to undergo a conformational shift which allows it to bind to a region that overlaps the promoter for the tox gene, thus inhibiting transcription and blocking production of diphtheria toxin.

In a low iron environment, which is the environment thought to exist where the bacteria colonizes in the upper respiratory tract of humans, iron is not available to bind to the DtxR and, therefore, DtxR cannot block transcription and transcription of toxin proceeds and production of diphtheria toxin occurs.

So my primary research objectives are to identify and characterize new virulence determinants and C. diphtheriae whose expression is predicted to be coordinately regulated with that of diphtheria toxin. That is regulated by iron and presumably DtxR.

My primary emphasis has been looking at heme-iron transport systems in C. diphytheriae. Heme-iron transport systems or heme-iron utilization systems have been well characterized in gram negative bacterial pathogens where they have been shown to be important virulence factors in many cases.

They have also been shown to be iron regulated in a manner very similar to how the tox gene is regulated in C. diphtheria. Some of my initial studies when I arrived at the FDA was to demonstrate that C. diphtheria could, indeed, use a variety of host compounds such as heme and hemoglobin and transferrin as essential iron sources.

However, the mechanism for how it used iron from heme and hemoglobin was not known and this was one of the projects that I initiated. I set out a strategy to try to characterize this system.

So the strategy I followed was to initially isolate mutants in corynebacterium that were unable to use heme and hemoglobin as iron sources. And to

complement these mutants with a plasma library carrying C. diphtheria DNA, and that ultimately characterized the genes and the products on these complimenting clones. And also to look at the molecular mechanism of how some of these genes might be regulated. Are they regulated by iron like the tox gene.

So I isolated a number of mutants in corynebacterium that were unable to use heme or hemoglobin as iron sources and then proceeded to compliment these mutants with a plasma clones carrying C. diphtheria DNA and identified two distinct groups of clones, one represented by placid PCD293 which carried a gene that I term HmuO, and another group of clones represented by PCD842 which carried a small operon of three genes which I call HmuTU and V.

The product of the HmuO gene encoded heme oxygenase which have been well characterized in ucariotic systems but this was the first report of the heme oxygenase in bacteria. What heme oxygenase do is they degrade heme shown here, but the subsequent release of iron in a heme breakdown product.

What we think HmuO is doing in the heme iron utilization system is that it will act on the heme once it is traversed the cytoplasmic membrane breaking

down the heme and releasing the iron into the cytosol making it available for the cell.

The other clone that I identified that could complement some of these heme transport mutants encoded three genes that appear to be organized in a single operon termed HmuTU and V. These showed a high degree of homology to heme transport systems that have been identified in gram negative bacterial pathogens. We think it has a similar role. Actually, we went on to demonstrate that it had a similar role in C. diphtheria.

What I'm showing here is a model of what we think is going on in C. diphtheria and possibly other gram positive bacteria with regards to heme transport and the utilization of heme as a iron source.

What we believe is at the HmuT protein, which we showed was a lipo protein is anchored to the side of plasmic membrane by means of a lipid moiety so it's basically tethered to the cell and the remaining portion of the protein which is exposed on the extracellular surface is available to bind to heme or hemoglobin.

We then believe it delivers heme to a permease complex located in the side of plasmic membrane which is composed of HmuU and HmuV proteins.

This facilitates the transport of heme into the cytosol where then the HmuO protein, the heme oxygenase, can then act on the heme breaking down the molecule and releasing the iron.

As I said at the outset, my interests were not only to identify the components and proteins involved in the transport utilization system, but also to understand how some of them are regulated. Are they coordinately expressed with the toxin.

In the process of sequencing the HmuO gene, I identified overlapping the promoter region for the HmuO gene. Just upstream of the actual coding region was a sequence that showed a high degree of homology to the consensus DtxR binding site which could indicate that the HmuO may well be regulated by DtxR and possibly iron.

Subsequent studies, DNA footprinting and various promoter fusion studies went on to show that HmuO was indeed regulated by iron in DtxR in a manner very similar to how the tox gene was regulated. However, the regulatory system for HmuO proved to be more complex than what was found at the tox promoter.

So in addition to regulation by DtxR and iron which was very similar to how the tox gene was regulated. We found an additional layer of regulation

in that in order to see any appreciable expression of the HmuO gene, he heme source was required, either heme or hemoglobin.

Not only was there repression by DtxR but the promoter was also activated in the presence of a heme source. This we found to be very interesting and unusual since heme activated genes had not been previously identified in bacteria.

Additional studies to try to identify what were the factors involved in this heme activation went on to show that this heme activation was mediated by a two component signal transduction system in which one of the components of the system was involved in sensing heme at the cell surface and then transmitting this signal to a second protein located in the cytosol which then activated transcription of HmuO.

What I have shown here is pretty much a summary slide of the heme transport and heme regulatory network that we think goes on in C. diphtheria. The two component system I just mentioned is composed of a sensor kinase protein, which I have termed ChrS, which has at its end terminus a number of transmembrane regions and some loop regions that extend to the extracellular environment which we believe are involved in the binding or heme.

Upon binding heme we believe a signal is transmitted to the C. terminal portion of the protein which contains a hystine kinase which becomes phosphorylated on the binding of heme and then can transmit this phosphate group to the activator component ChrA which upon being phosphorylated will undergo a conformational change allowing it to bind upstream HmuO promoter and activating transcription.

Now, in high iron environments this promoter can still be repressed by DtxR. Optimal expression of HmuO would occur in the presence of the heme source, either heme or hemoglobin in a low-iron environment where DtxR is no longer acting as a repressor on this promoter.

Optimal levels of HmuO would then be predicted to be made under these conditions and then it would be able to act on any heme being transported to this heme transport system.

We now believe there is an alternate or second heme transport system in C. diphtheria since site directed mutations in the HmuT protein do not abolish the ability of C. diphtheria to transport mutalized hemes and iron source.

Regardless of which transport system is bringing in heme, HmuO would act on this heme coming

into the cell breaking it down, releasing the iron, and making the iron available to the cell in order for it to grow in the low-iron environment of the respiratory track.

Some of my future aims are to identify this alternate heme transport system in C. diphtheria and to develop improved mutagenesis methods for C. diphtheria. There are very few molecular tools in this organism and the development of improved mutagenesis methods for the chromosome would greatly enhance our genetic analysis of this organism.

I also intend on pursuing structure-function cellular with the ChrS protein. This is the sensor kinase for the two component system to understand the mechanism by which it senses heme in the extracellular environment and how it transmits this signal to the activator component.

I would like to acknowledge some of the people who helped me in this work. Post-doc Sue Drazek, Craig Hammack, and Carrie Brickner who worked with me on this diphtheria project. Collaborators on this project include Angela Wilks at the University of Maryland and Shelly Payne, University of Texas, John Fulkerson who is currently a post-doc in my lab looking at iron transport systems in bacillus

anthraces. Thank you.

DR. DAUM: Thank you, Dr. Schmitt. Downloading a lot of information very quickly.

Committee questions? Comments?

DR. KIM: I guess one question I have what is the current status of sequencing of genome of diphtheria?

DR. SCHMITT: That is actually an interesting question. It was in progress at the Sanger Institute but actually logging on to the website last night I discovered that they actually just completed the genome of diphtheria.

DR. DAUM: Who did that?

DR. SCHMITT: Sanger Institute.

DR. DAUM: Is that public domain kind of information?

DR. SCHMITT: Yes, it is. It's available.

DR. PALESE: Anthrax. How far is anthrax?

DR. SCHMITT: Very, very close. They are still filling gaps so it's not entirely complete yet.

DR. PALESE: Who does that?

DR. SCHMITT: That's Tiger.

DR. DAUM: Dr. Kohl.

DR. KOHL: I'll ask you a lead question that Dr. Vann already got. What would make your life more

productive in your lab? What do you need that you don't have?

DR. SCHMITT: I'm in the process now of hiring a new post-doc for my lab. I think certainly once I get that person on board --

COURT REPORTER: Can you hear him?

DR. SCHMITT: I'm in the process now of hiring a new post-doc for my lab. That certainly will make life easier once that person is on board. Other than that --

DR. KOHL: Do you find within the constraints of the FDA that you can collaborate with people who you would like to collaborate with?

DR. SCHMITT: Right. Absolutely. I looked at a number of collaborators here. Certainly some of the important people in the field that I developed collaborations with that have been very productive.

DR. DAUM: Thank you very much.

Ms. Fisher, did you have a comment?

MS. FISHER: I don't know if it's specifically for you but any of the bioterrorism money -- the money to fight bioterrorism that Congress is appropriating, is any of that going to the FDA?

DR. SCHMITT: I believe so. I'm probably not the most appropriate person to comment on that.

DR. DAUM: Let's call on Dr. Goldman to answer that. I suspect we've already heard the answer.

DR. GOLDMAN: Yes, indeed, Dr. Fisher. In fact, the FDA has received $104 million to support bioterrorism. They got it only about a week ago.

DR. DAUM: Thank you, Dr. Goldman. Thank you, Ms. Fisher. I think at this point, thank you, Dr. Schmitt. That brings to a conclusion our open session. We thank very much the speakers and participants in it. I think we'll take a five-minute break to let the room clear and then we'll go into closed session and try and finish up.

(Whereupon, at 1:40 p.m. the open session was adjourned.)

CERTIFICATE

This is to certify that the foregoing transcript

in the matter of: VACCINES AND RELATED BIOLOGICAL PRODUCTS ADVISORY COMMITTEE

Before: FOOD AND DRUG ADMINISTRATION
CENTER FOR BIOLOGICS EVALUATION
AND RESEARCH

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8120 WISCONSIN AVENUE
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