

Panel III: Diagnostics – US and China Perspectives

**Moderator: Tang Hong, Institute of Biophysics
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. . . have a really tight schedule for this panel III because one of the speakers from the last panel will join us.

. . . now works in the Institute of Biophysics at the Chinese Academy of Sciences, the place George visited a couple days ago. So, I did my PhD in Rutgers University in New Jersey and then I went to MIT for my post-doctorate in Dr. Phil Sharp's lab. So, after that I returned to China to start my own life working on the immune response to the viral infections, that include the SARS virus and the HIV.

During this last period, my lab started to look at the serum biomarkers that might be specific to the SARS virus infection. So, we did the . . . analysis and tried to determine the biomarkers. Fortunately, we published that data in the clinical chemistry.

The point I want to address here as Moderator is since this is the dialogue or the discussion panel . . . China/American scientific field, particularly the genome research to fight against infectious disease, my understanding is that besides the cutting-edge science and tech that can be achieved by both sides as the heart . . . Professor Young and Eric, one thing we might have to pay attention to is the clinical practice in China that is way behind the norm in America.

For example, the specimens we got for the SARS serum – the only data you have is the name, the gender, and the PSAR results for the SARS positive or negative, but you don't have any information on the co-infections which is critical for the analysis. So, the standardized practice in clinics and hospitals are critical. I wish this workshop can form a consensus on how to promote the clinical practice in China that can catch up with the U.S.

The second point is although the science and tech can advance really fast and the laboratory techniques can be adapted to the application, however, we might still have to pay attention to the availability to most patients, for example, in China or other developing countries or poor countries. So, we not only need to achieve the faster, more sensitive and more specific diagnostic tools, for example by using the genomic approaches, but also we still have to make them cheaper and available to all the patients around the world.

So, our first speaker is Dr. Stephen Popper from Stanford University. He does his post-doctorate training with Dr. Patrick Brian and David Railman. His topic today is the DNA array analysis of the whole blood to try to determine the host response to infections.

Stephen Popper, Stanford University School of Medicine

Surveying Gene Expression Whole Blood: Host Response and Classification of Infection

First, I just want to thank the organizers and say how much I enjoyed my time here and the conversations I've had.

As was said, I'm at Stanford University working with Pat Brown and David Relman and the focus of my work and that of others in my group has been on looking at the immune response to infection through the prism or window provided by microarrays and gene expression profiling. What I thought I would do today is provide some background and mention some of the issues that we've been thinking about and trying to address in the course of trying to build up our capacity to both examine samples and specimens, and also more importantly, analyze data coming from clinical studies.

So, this panel has focused on diagnosis and as others have alluded to, it is a large and important practical problem. One crude estimate of the rate of successful microbiological diagnosis is presented here for various syndromes – sepsis, pneumonia, encephalitis, and acute diarrhea. So, clearly in many cases, the majority of infections or febrile illnesses actually have no concrete microbiological diagnosis with obvious implications for treatment for clinical management and for public health.

So, as I said, we're focused on using microarrays to look at immune responses, and the thought behind that is perhaps this presents a relatively unbiased approach to examine the host response. Clearly given the high density nature of most microarrays now, there is potential, at least, for high resolution and high specificity.

Now, in terms of why we are focused on the host response, the notion is, and was, that perhaps this presented us with a finite target set. In the context of diagnosis, you can imagine as again has been discussed, there seems to be an ever-growing set of pathogens which are of importance to us as humans.

The host response, therefore, represents some finite set that we know is always going to be present and doesn't, relative to the pathogen variance, vary very much.

Secondly, we hope that by looking at the host response, we are going to get some insight into immunity and also into immune mediated pathogenesis since much of the pathogenesis that occurs is not simply a function of the pathogen but its interaction with the host and the host response to that pathogen.

Finally, the studies that I'm going to talk about today focused on full blood sampling. The thought there is that whole blood is a relatively accessible tissue compartment. It is one that is commonly used in clinical settings. Because it is commonly used, it also is one that is familiar to people who are in clinical settings and it minimizes the need for processing.

So, I thought I would start by providing some vantage point – the vantage point from which we started these studies. This is simply a very crude sampling of the articles that are in Pub Med by year. What I did was I simply looked up gene expression profiling and . . .

(Tape 6)

. . . . have risen steadily. They have also risen for infectious diseases, but you can see how far it lags behind studies of cancer.

So, this is one of those cancer studies and one that I think is relevant for some of the issues we want to look at in infectious disease. This is a study of diffused large cell lymphoma and a gene expression which was published in 2000. I simply want to make a few points. They looked at a large set of diffused large cell lymphoma patients as well as samples from other patients with other cancers. They also looked at different cell lines and also some primary cells. What they illustrated was that even in clinical samples, using very simple statistical methods to organized data which we are all quite familiar with now, hierarchical clustering being one example, you can see coherent patterns of gene expression which represent sets of genes that have a common biological property associated with them.

The second point is that there is actually a real clinical and practical utility to this. So, this here represents survival curves for two sets of patients that are separated by the pattern of expression in the lymphoma samples. Here, in set B, is another segregation. This is done by a traditional scoring method for lymphoma. The point that the authors made was that even if they looked just within the low clinical risk patients defined by this traditional method, using microarrays and gene expression profiling, they were able to gather additional information which had practical consequences. So, even within this group, they were able to distinguish between those who were more or less likely to survive over a given number of years. So, the microarrays are adding value and information to what we are getting by traditional means.

So, the studies that we're working on in our laboratory involve looking for diagnostic markers in the context of febrile illness. There is a study we have conducted with collaborators looking at

febrile illness in Nepal. There is another one in progress in emergency rooms in the U.S. The third one is focused on Kawasaki disease.

We are also looking at prognostic markers. The additional issue here is that we are now interested in a time vector where in addition to saying can we distinguish things that are going on right now, we are asking can we actually also find things that predict what is going to happen tomorrow or some time in the future. This again, our pilot study is really focused on Kawasaki disease and we have looked for markers both for treatment response and aneurysm formation. If there is time, I will discuss this later.

Third, as I mentioned, we are hoping to get insights into immune responses. We hope to do this here as well, but our early study here is really focused on looking for genes whose expression is associated with the development of Denghi shock syndrome.

I'm going to focus in on the Nepal febrile illness study as a way of illustrating some of these issues. So, the first step that we decided we needed to simply determine the extent and the source of variation in infected individuals, and compare that to the variation that is present in the absence of infection. A second issue is simply how much of the variation is due to the presence of a specific etiologic agent. The third is, can we actually dissect these sources of variation even in a complex tissue like whole blood and in a complex setting represented by clinical illness.

We want to identify gene expression profiles that are characteristic of classes of infection. So, these might lead to the markers for diagnostic and clinical management that I mentioned earlier and again also provide some insight into molecular mechanisms that can characterize the immune response to an infection.

So, the tool we are using to conduct these studies is what we refer to as lymph a chip. It is a cDNA microarray. It has had about 37,000 or 38,000 spots on it, depending on which version, and that represents approximately 18,000 genes, perhaps more, of which 10,000 or 11,000 are not named.

I think everybody here is familiar with the basic technique, but just to quickly review, this is a two-channel microarray. So, we take our sample. We amplify it, if need be, and then label it with a fluorescent dye. At the same time, we have a universal reference sample which is hybridized in concert with each individual sample to this microarray and then scanned. And, the ratio of the two samples, the reference and the sample, provide us with essentially a pseudo-color on the array – either red where the sample is higher, green where the reference is higher, and yellow where they are roughly equivalent. These ratios are then log transformed and we then end up with relative measures where red means higher relative expression of a particular sample, green means lower, and black is somewhere in the middle.

As a first step, we tend to use hierarchical clustering to organize both the samples and the arrays just to look at the overall patterns. So, the first study we conducted was actually one we conducted in healthy donors. We simply wanted to identify the primary sources of variation in these sorts of samples, and use this as a baseline for future studies of disease. So, the samples we have used for that study and other ones are a pax-gene samples. Pax-gene is a commercial available product and it is essentially a vacutainer tube, something that is standardly used in clinical settings but it has a solution in it which is meant to disrupt all the proteins in the sample and stabilize the RNA at the time of draw. So, we are doing our best to look at RNA as its present in the individual.

For this particular study, we looked at 77 individuals (41 males and 36 females), and collected laboratory information as well as recording the time of day at which the sample was taken. This is just a brief overview of the samples, so each column here represents a sample, and each row represents a gene. You can see, as in the cancer study, we found coherent sets of gene expression that we could associate with particular biological properties. So, we found what we considered to be a reticula site cluster, another one which was clearly indicative of the number of lymphocytes that were in the samples, another neutrophil cluster. We also found a cluster of immunoglobulin genes which have very similar expression patterns. Interestingly, they were inversely correlated with the age of the donor.

We also found a set of genes which seemed to be associated with time of day at which the sample was taken, and another set of genes which, again, behaved in a fairly similar pattern and are known to be interferon-regulated. Interestingly, these genes were the ones that turned out to be most characteristic of an individual. So, in data sets where we looked at multiple samples from given individuals, we found that these were the ones that were best indicators of a particular individual.

The other point I want to make from this study is that simply we compared the overall variation in this data set to the variation in the cancer data set that I showed you earlier, as well as another data set. So, here is the diffused large cell lymphoma study, and this is essentially a measure of the variance in the expression of the genes in that data set. Here is one for chronic lymphocytic leukemia. Down here are the healthy donors. So, you can see there is much less variation here in the healthy than there is in these other lymphocytic data sets associated with diseased.

So, then we turn to the issue of febrile illness and maybe that is the one that is most appropriate for this particular panel. So, this represents a study that was conducted in Nepal. It involved active surveillance for consecutive cases of febrile illness in adult non-surgical patients. Essentially, they just had to have a fever, be at least 14 years of age, and provide informed consent. This was conducted in Katmandu in a large hospital with a lot of emergency room visits.

We collected pax-gene samples again so whole blood samples which were isolated no more than five days post collection. This was piggy-backed onto the study of the epidemiology of fever using more traditional techniques. So, they were already planning to use cultures as well as IGM serology. They used urine antigen tests in some cases, and they have done a little bit of off-site confirmatory PCR for some of these pathogens. What we did with these RNA samples from the whole blood was amplify them and then hybridize them to lymph a chip, in concert with this reference sample.

This is just simply a summary of the different microbiological diagnoses that covered the range of samples that we put on arrays. So, you can see there are a few e-colis, some leptaspiros, some

scrubbed-typhus, murine typhus, staff orius, strep pneumo, and finally over half of the samples were due to salmonella infections, and they were all bacterial infections.

So this was our first look at the data. It is an unsupervised approach to the data, letting correlation organize both the samples and the arrays. What we saw was that for the most part, the samples showed they weren't completely random and we saw some groupings. So, in this area here are the staph orius and strep pneumonia samples. But, it is also true that by and large these samples didn't cohere completely according to microbial species.

But, there appeared to be one almost exception to this and that is that six of the seven scrubbed-typhus samples were grouped together here. It appeared to be a function of the higher expression of these genes here. So, we went in and looked more closely at those genes. What we have done now is used genontology which is simply, again, something most people here are familiar with, but an attempt to annotate sets of genes according to molecular function biological process and cellular compartment. We found an overrepresentation of genes that were associated with the ribonucleo protein complex. They coated for structure components and were involved in RNA splicing, and also ones that were associated with the hydrogen ion transport, and these were ATP syntheses inside of chrom oxidase . . . So, these genes seem to be more highly expressed in samples from the patients with scrubbed-typhus infection. This suggested to us that there was some set of cells that was highly metabolically active.

To get a clue for what those genes were, we simply went back to this and looked at the set of genes which were most similar in expression to what we were referring to as the scrubbed-typhus cluster. When we looked in here, we found a set of genes which are strongly associated with positive CTLs and also NK. . .

That was our opportunity to ask what can we see using an unsupervised approach and just letting the data sort itself out.

The other thing we wanted to do is address the issue what would happen if we had a prior question. So, in this case, given the numbers, we asked can we actually tell who has the

salmonella infection in this case set? So, there are now a number of statistical approaches that worked for this. One which we used is called prediction analysis for microarrays and when we used that here, we found that it pulled out a set of genes which allowed us to predict in 63 out of 76 cases correctly whether a given sample came from a patient with salmonella infection or with some other infection. I've just grouped it here for visual purposes – the purple ones represent the salmonella infections, the light blue represent the other infections, and I added in the healthy donors for context. As it turned out, this set of genes consisted of and interferon-stimulated genes.

Given the two things I've just shown you, it is clear that some of the variation in the data set is due to the presence of different pathogens. That has been added in here to this graph I showed you before. Here are the two cancers. Here are the healthy donors. Here is the set of infections from Nepal.

When we focus in just on the typhoid patients, we see what appears to be less variation than here, which suggested again that some significant portion of the variation is due to the presence of different pathogens.

In addition to the issue of pathogens in variation, we also wanted to look at all the different parameters that we had information on. So, these actually are meant to be turned 90 degrees and what they represent are strengths of association for correlations between these continuous parameters – temperature, length of illness and age – and the expression of a given set of genes. So, if you can imagine these turned 90 degrees, the peaks out to the edges represent strong associations. What we saw was very strong associations for many of the parameters associated with the CBC – the complete blood counts of the different cell populations that were present. We also found some peaks that were associated with temperature, length of illness and age, as I mentioned.

But, the question then became how much of the variation is due to the presence of different pathogens, even when we control for these different parameters, and how can we address this

issue? So, the tact we took was using a linear regression model. That allowed us to adjust for multiple parameters simultaneously.

This is just a simple test case that I think is useful visually. We had 11 patients in whom we had matched samples of PVMCs and whole blood. So, really the only difference here is the presence of . . . in the whole blood samples and the absence of them in the PVMCs. What we did was simply adjust for neutrophil percentage in a linear model. What we found was it did a pretty good job of removing most of the differences between these two sets. There is a set of genes here which still seem to vary across this data set, and it turned out that these were the interferon-stimulated genes that I mentioned to you earlier that seemed to be very indicative of person-to-person differences.

So, we applied this to the overall Nepal data set and looked at for significance versus a variation. We looked at all the parameters I showed you before – gender, age, microbial diagnosis, white blood count, the different cell populations, temperature, . . . and length of illness. Out of all of those, the only ones that seemed to be significant sources of variation for the data set as a whole were microbial diagnosis and the large cell populations. So, we simply grouped these together, white blood count, lymphocytes and neutrophils, and adjusted for them in the model. So, this is what I . . . the curve for diagnosis. Over here is the same distribution of variance explained for each individual gene after adjusting for the cell populations. This turns out to still be significant.

So, we looked at what these genes were and in this data set, it turns out to be largely the scrubbed-typhus genes. However interestingly though it is hard to see in this figure down here, there were some other genes which popped out. There were some anti-microbial peptides, which seemed to be particularly highly expressed in the e-coli and strep-pneumo infections, and there is another set of genes here which are actually expressed at particularly low levels in the scrubbed-typhus infections.

This is another analysis we did where we actually removed the scrubbed-typhus and looked at those genes which still had significant variance from the data set after adjusting for cell populations.

So, in conclusion, what we found was the microbial species is an important source of variation in gene expression, and person-to-person differences do not dominate patterns of expression which is really encouraging because I think it means we can go to different populations and look at these issues. We also don't need to have multiple samples from each individual to act as their own control. This was true even after adjusting for differences in the cell composition. In this particular data set, it was driven by the presence of the scrubbed-typhus samples.

We can also identify pathogen-specific signatures represented by sets of genes. This includes the scrubbed-typhus using unsupervised approach and salmonella using supervised approach, and there is some potential utilizes as clinical markers.

Finally, the identity of these signatures I think is a particularly valuable aspect of this sort of analysis because it suggests biological pathways in critical aspects of the host immune response which can be investigated in other studies and by other means.

Finally, it seems that we can do this even though we are looking at a very complex tissue in a very dynamic process.

So, before acknowledging all of those that have been involved in this study, I simply wanted to mention a number of issues that have arisen in the course of conducting these studies. These were actually much more eloquently expressed by Dr. Ahlquist earlier today, so I'm just going to mention them and we can discuss them later if anybody is interested. But, one is the need for better, more diverse and more powerful statistical approaches. The real strength in these expression studies is in the patterns that we see and the groups of genes. The weakness is in the individual numbers. Everybody has too few samples and too many genes.

The second, which has also been alluded to, is the need for further biological insight. We think one important issue is to develop reference experiments which will aid our interpretation of these clinical studies. So, we have conducted experiments to look for canonical genes which would distinguish different cell subsets, and we have also done a simple study where we took PBMCs

and treated them with different kinds of interferon and have come up with patterns that distinguish Type I and Type II interferons that we can apply to some of these studies.

Of course, there is the need for further bioinformatics to meld all these different data sets together.

One very practical aspect is that in the time that I've been in the lab, we have found we have had a hundred-fold decrease in the sample size that we need to process for these samples, and we have also improved our throughput – the speed with which we can process large numbers of samples while minimizing technical variation. We hope this is actually going to get better in the next year. One of the reasons is that I wanted to mention a new platform that we're moving to, which are the mebo and hebo arrays. This acronym here refers to xenon evidence-based oligonucleotide arrays. These are based on a thorough examination of the good genome sequences that Dr. Eisenstadt and Dr. Yang and others have provided us with now and various data sets. The idea is that we have picked an oligo which represents constitutive exons for every well-defined gene, as well as other oligos which will allow us to look at alternate splicing.

Furthermore, there are many control spots on these arrays which will allow us to do a lot of modeling and model but sensitivity but specificity.

Finally, another issue which was alluded to is that we believe are going to contribute to the drive towards lower prices. In fact, these oligo sequences are freely available. You can go to the Alumina website and find them. You can also go to the website I have down here at the bottom of the page as well as websites at UCSF and some of these other institutes that were involved in the development of these arrays. It looks like the price of the oligos for these arrays is going to be under \$10.00 per array. There is still a lot of other things that cost way too much money.

So, having provided those last thoughts, I simply want to acknowledge the people that have been involved in this work – David Relman and Pat Brown at Stanford, as well as others in David Relman's lab. Our collaborators for the Nepal study were Barth Reller and Chris Woods at Duke, as well as others at the CDC in New Zealand and in Nepal; the Denghi study, which I

didn't mention, is being done in collaboration with the people in Vietnam; and finally the Kawasaki disease study is in collaboration with James Burns at UC San Diego.

Thank you.

Moderator – Thank you, Stephen, for a very nice talk. Due to the time limit, we are really encouraging every speaker to keep the time at 20 minutes. So, I will ring the bell at 18 minutes so when you hear the ring, you have two more minutes to go.

Our next speaker is Wenhan Wu from Xiamen University. He will talk to us on the diagnostic reagents development in his laboratories.

Wu Wenhan, Xiamen University

The diagnostic reagent development in National Institute of Diagnostics and Vaccine Development (NIDVD) in infectious diseases

Thank you, Mr. Chairman. I must first begin by thanking the organizers for inviting me to talk to you today.

I'm from this lab. My lab is called the National Institute of Diagnostics and Vaccines Development. We are the little brother just established under the auspices of the Science and Technology Ministry. So, we are given agreement, an impossible agreement I might add, that is to respond to newly emerged infectious disease and forecast of new infections emerging. Our task is to undertake to make sure the appropriate diagnostic and the vaccines are available for that. Of course, we cannot accomplish all that alone. This way, we are doing it in collaboration with various institutions.

What I thought I would do today is to highlight two projects that is going on in our laboratory. I would like to talk about bird flu and also my first love, Hepatitis B. The collaborating institutes are from Hong Kong, and parts of China, and so on.

Let me begin by talking about the bird flu. We started in late 2004. Our intention at that time was to try to build up a very large collection of H5 monoclonal antibodies. This is a summary of the panel of monoclonal and some of the monoclonal antibody we have developed. At that time, we had a total of 114. Now I'm told the number is up to 160.

Essentially, what we have done is to characterize these monoclonal antibody against 34 strains of H5 M1 influenza. These isolates were from various parts of the world, over a period of more than ten years. So, we selected these strains so that it may be representative of various strains at different parts, prevalent strains in various parts of the world over different periods of time.

As you see here, each monoclonal antibody exhibit a distinct reaction pattern against the 34 flu virus isolates here. These are independent isolates, so each one do have a unique antigenic specificity.

These strains actually when we follow genetics of these 34 strains, we can distinguish a total of several lineages. They are indicated by various colors. I'd like to draw your attention first to this lineage here – that was the ABC & H. If you were to look at this, these are representing strains which have evolved at different times. So, if you look at this, we can say this is my first lesson in my undergraduate study about antigenic drifts. What you are really looking at, at this lineage, you are looking at a progressive shut-off of the epitopes and that, for the first time I think, we are very, very happy about this.

Another thing is from this panel you will also realize there are several of these monoclonal antibody are reactive against all the 34 strains, indicating that these epitopes have been preserved throughout the 10-15 years or so we are looking at.

We further characterized these monoclonal antibody by neutralizing tests. Basically each one – we have a small panel now of the so-called neutralizing antibodies. Interestingly, there are certain neutralizing epitopes that have been preserved throughout the ages as well too.

This is exciting in the sense that once you have this, I think the first thing that comes to mind is maybe this would be the antibody that would be very useful for selection of the vaccine strain, and also to look at the efficacy of the vaccine strain. After all, we are really looking at the neutralizing activity of these strains.

So, using some of these monoclonal antibodies that are common for all the 34 strains, we have undertaken a multi-center trial. Those centers are in different places – Hong Kong, China and so on. But, the bottom line here is that these trials are carried out with direct detection of the viruses from the The sensitivity of detection of which we have about 70%, the sensitivity varies from places to places. But overall, we do achieve a 70% sensitivity. More importantly is that we have also non H5 M1 . . . swap where we achieved a 99% specificity. So, actually we are still continuing on with this type of evaluation to ascertain this one.

When you look at these, we have produced two types of tests now – one is a rapid test which is designed for use in the field; the other is a elizer test designed for use in the lab. They all use all four direct detection of H5-specific direct detection tests. You can see that the H5 base reach a sensitivity down to .02 HA units. There is another test which detects the end – the group A specific direct . . . and this sensitivity is around the level of HA.

I guess in summary, essentially, we have now a very useful collection of H5 specific monoclonal antibody and this can be used for direct detection in different types of test formats. Its usefulness would be in differential diagnosis of fluid, and I think it would be most useful in the sense in monitoring outbreak among chickens.

I think lastly the conservation of neutralizing epitope probably give us the stimulation to go ahead and try to develop the target-specific intervention.

So, that is all I wanted to talk about here.

We are now onto . . . tension – Hepatitis E. We have been working on this for some time, so we are relatively advanced. We also have the benefit of an industrial partner to help us. Right now, we have three Hepatitis E specific tests which is already registered with the State Drug Administration. Then we also have a vaccine which has just completed Phase II clinical trials, and we are planning on Phase III trials soon.

Why do I want to talk about hepatitis? The conventional wisdom has it that the world has two equal parts – the endemic and the non-endemic. So, we in Asia and Africa are the endemic part. The rest of you in the developed world is the non-endemic part. But, I think this is oversimplification. I think this is an overly complacent view. Because today, if we were to look at these virus and we just isolated the virus, carried out genetic, we find the various population is highly diverse. Basically, we can divide these viruses into four groups: Group 1, Group 2. Now, Group 1 and Group 2 were the ones that causes all the large, extended waterborne outbreak – never been isolated in animals. Group 3 and Group 4 are basically we now feel these are the natural hosts which are animals – swine and other animals as well too.

So, the Group 3 is widely distributed. You name everywhere. So, according to our present view now, I think it is clear and it is better for us to conceive the world into two parts again, an endemic and a pre-endemic because a devised population continue to evolve, I think there would be certainly at the time when we could be emergency of certain virulent strain that would come out in the future.

So, let me now just briefly describe to you our developments to far, and the making of the diagnostic kit and the vaccine. The virus has been very kind to us because it only has one cerotype. So, although you have four different genotypes, there is only one cerotype. So, you have only one virus to worry about when you talk about vaccine or diagnostic.

The second thing is that it is even more obliging because it has only one single structural protein. So, it is the single protein we need to worry about.

But, the virus does have its secrets because in that single protein, the basic unit is a dimer or two dimer. Then in the two dimer, its dimer has three separate domains. The part called the end terminal is responsible for packaging the viral genome. The monomer here is a shell. These monomers engage in monomer interaction to form partially enclosed shell in the virus here. But, an outstanding feature about this is the virus has this protrusion. This protrusion, by electron-microscopy, if you were to analyze it closely, then it is formed by three of these dimeric units into a protrusion here. So, these are the domain.

There is two main discoveries that give us the breakthrough we needed. One is that we have cloned and expressed a P2 – a peptide expressed in bacteria which models the protrusion. Then later on, we also derived another one from here that also includes part of these shell domain. This then will form an aggregate. So, we finish up – this one, the whole dimer here is a perfect because it models the protrusion. It is a perfect antigen. But then what is with the P239, it form aggregate, we would finish up with a particulate vaccine.

So, as you see, the two – the PE2 and also the 239, the vaccine, they naturally occur as dimers as in the protrusion in the presence of .1% SDS. So, it is a mildly denaturing condition. Now, all these antigen activity identified by convalescence serum rests on this dimeric structure and not on this monomeric structure.

Then we have race 2 neutralizing antibody against HEV, neutralizing four primates. Then you would see this is where the neutralizing activity rests as well too.

So, we assessed the assay in a recent monkey model. Here we were lucky at that time we were developing two monkey models for our vaccine studies. So, we have 86 monkeys to use to try to establish an infection for evaluation of this one. So, in this model, you would see viral excretion . . . precedes disease. Then typically with immunopathology, the disease begins with antibody responses. Antibody responses to PE2 . . . every animal respond to it. Then the IGG persists for . . . prevalence persists for over one year. The IGM typically drops after about three months. Then

you have the other, if you were to prepare other type of peptides, their response is delayed and thus the response rate is low. That is in the monkey model.

We also have really looked at it as to develop the IGG as an epidemiological marker. In a very large study, you would see specific sero prevalence increases at about 1% per year until 60 or older. This actually shows us that the sero prevalence is a reflection of cumulative lifetime exposure to the virus. The second thing is that you would see the negative sero conversion rate of IGG is very low indeed – 1.4%.

We could also make use of this test, the IGM, to evaluate IGM against enteric hepatitis. One can find that our test will achieve a sensitivity of 97% and 100% specificity in this. I don't have time.

If you were look at evaluating the vaccine now, to look at the immunogeneity, you can see the hepatitis, the 239, the ID50 is less than .25 micrograms, and whereas if we don't have the s-extension, then it is about 250 times higher.

Efficacy of a monkey – I don't have time. But, let me show you some of our clinical trial phase II data. This is our clinical trial phase II data. The monkey we have here now is 10 micrograms three times, 20 micrograms two times, 10 micrograms three times, and so forth. In other words, the actual . . . antibody level of all these vaccinated group were essentially the same. But, I show you the control group here is very interesting because these are nine individuals who become infected during the time of our study. So, with our vaccinated group level achieved by our vaccinated group is definitely higher than that achieved by natural infection. We know that we don't get hepatitis E due to re-infection – usually it is a primary infection. So, we can safely assume that natural infection with hepatitis E is sufficient to afford protection and our vaccine is very likely to afford protection.

So, I'd like to spend some time with you, but to carry out three different kinds of epidemiological studies, but let me run through. We do three types – one is in a pig farming district in eastern China. You can see this is a pig farmer and this place is cross-crossed by

canals. So, the sewage from the pig farm goes straight into the canal into the water. So, the answer we want to see is how does pig farming impact on a community downstream and upstream, and how does it impact on the thing.

The other setting is in a rural community where virtually everybody keeps pigs in or near their home. So, we want to see how does pig population level infection relate to human infection.

Finally, in the district that we are talking about, in the pig farming district, the phylogenetics suggest that the concurrent isolates from the pig population and that from a human population is indistinguishable. In other words, there is unrestricted transmission between human and swine.

Second, but the . . .

Moderator – Sorry, but I really have to interrupt you. One more courtesy minute . . .

Wenhan – So, in any case I don't have time to finish. Let me just conclude now. We find the swine now is really the dominant reservoir now. The infection is from swine to human. And then through pig trafficking, that virus is spread to different parts of the world. But, human-to-human is very inefficient – still not quite efficient. So, we still have a window of opportunity.

So, in conclusion, because we have a diagnostic rating, because we have the vaccine ready, we have an opportunity now to institute control measures before this . . . eventuates. It is a very exciting option here. We can first actually monitor – we must now routinely diagnose a cure for hepatitis. We also must now routinely look after the outbreaks in pigs. We have to keep surveillance at risk populations, animal and man.

Finally, if we have the stomach for it, we can actually carry out vaccination of human and also the swine population.

Thank you very much for bearing with me.

Moderator – I apologize for the rude interruption. Our next speaker is Patrick Murray from NIH. He is now the Chief of the Laboratory of Clinical Microbiology in the Department of Laboratory Medicine.

Patrick R. Murray, National Institutes of Health

The Impact of Genomics on the Diagnosis of Infectious Diseases

Thank you. I'm wondering how many extra minutes can I negotiate up-front. Two courtesy minutes – all right. It is about 3:15.

First of all, I'd like to thank our host. This is really a privilege to be here at this conference. What I'd like to do today is to try to put into perspective some of the technologies that we've heard about and how it is currently being used in a rather sophisticated clinical laboratory. I think at the end, the conclusions will be that we have a long way to go, but I think we see the path we are going to follow.

The diagnosis of infectious diseases, the challenge both to the clinicians and to the microbiologists, new infections such as West Nile virus encephalitis, meta pneumo virus respiratory disease, SARS, and aviation influenza are all now well-known as we have heard today. Additionally, older pathogens have acquired new virulence factors that cause severe diseases such as community-acquired pneumonia caused by staphorius and severe colitis caused by clostridium difficile.

Likewise, the number of newly-recognized microbes responsible for infectious diseases has expanded dramatically in the last few years, in large part due to increased sophistication of detection and identification procedures.

Historically, tools for the diagnosis of infectious diseases included microscopy, culture, biochemical testing, antigen detection, and serology. The relative value of each tool was defined both by the technique and by the organism. For example, microscopy is a useful procedure for the detection and identification of organisms like the fungi and parasites, but it is a relatively insensitive technique for direct detection and is very labor-intensive for the technologist performing the test.

Culture and biochemical testing has been useful for bacteriology, in particular, the culture is a rather slow procedure, requiring days for many organisms, months for some organisms, and biochemical testing is a rather non-specific and unreliable procedure, particularly for fastidious organisms.

The use of antigen detection and antibody detection is commonly used for virology among other diseases, but again, the quality of the testing is depending on the reagents that are available and so these tests can be both insensitive and non-specific. So, it is within this technologic framework that newly introduced molecular tools are applied.

The application of molecular diagnostic tools in the clinical microbiology laboratory should be viewed as the evolving technology that is focused in four general areas: the detection of microbes; the identification of microbes; the ability to determine their anti-microbial susceptibilities, and to use the technologies to perform epidemiologic investigations. We have heard a little bit of information about most of these procedures so far.

What I'm going to focus on in my presentation will be in the first two – the ability to use molecular diagnostics to detect organisms, and also to identify organisms.

The detection of microbes in clinical specimens is based on fundamental questions – does the patient have an infection, and if so, with which organism? The answers to these questions can be broad, such as the patient has an infection with a gram-positive bacterium, or the answer can be narrow such as the patient has an infection with a specific organism like micro bacterium

tuberculosis. The value of the diagnostic test is dependent on the question that is asked, and the tools that are used to answer these questions.

The most common molecular approaches used in the clinical laboratory for the direct detection of organisms in specimens is hybridization of a nucleic acid probe with a specific target sequence present in the organism. Two variations of this approach have been developed – the use of non-amplified probe-based tests, and the use of amplified nucleic acid tests.

For the non-amplified tests, the clinical indications and the needs are fairly specific. There has to be a clinical need for the test. There has to be a simple procedure for preparing the sample. A large number of organisms must be present, or at least a large amount of the target nucleic acids. The test has to be cost-effective, and it has to be accurate.

There is only a few companies, at least in the United States, that have developed tests that meet these criteria. Three examples of these are Genpro, Beckton Dickinson Diagnostic Test, and the Advantix Fish Test, or fluorescent in situ hybridization.

The more common test that we see used in the clinical laboratory are the amplified tests. There are three approaches to amplification. We can use amplification of the target signal. We can use amplification of the target itself. Or, we can use amplification of the probe. Again, I've listed on this slide examples of companies that have developed tests that fit within each of these categories. So, we have two commercial companies developing tests for amplification of the signal. We have 4-5 tests that amplify the target itself, which is the most popular approach. We have three companies that have amplified the probe itself.

This is a summary of the commercial tests that are currently available using nucleic acid amplification tests. Two of the companies again have used signal amplification and four companies have used target amplification. We have amplification tests for six viruses and four bacteria. It is interesting when you look at the spectrum of tests that are available here, four of the viral tests are developed for screening donated blood samples in the clinical laboratories. So, we have test for HIV, hepatitis B and C virus, and West Nile virus. The cytomegalovirus test is

developed primarily for immuno compromised patients, particularly those with stem cell transplants, and human papyloma virus tests developed for screening women for cervical cancer.

For the bacteria, two of the bacteria are the most common causes of bacterial sexually-transmitted diseases, . . . gonorrhea, and chlamydia tracomitus, micro bacterium tuberculosis is an organism of clinical interest both in the United States as well as worldwide, and micro bacterium . . . is particularly an organism of interest in HIV-infected patients in the United States.

The commercial companies have also developed amplification tests for research applications. To say that for research applications needs to be defined a little bit. Although they are developed for research applications, if a clinical laboratory can validate the utility of the test, it can be used for clinical diagnostic purposes. So, both quantitative assays as well as qualitative assays have been developed commercially. The quantitative assays that we have are the provo-virus B19, Epstein Barr virus, and hepatitis A virus. The qualitative assays have been developed for bacteria, for fungi, as well as for viruses. So, we see a spectrum of nucleic acid amplification tests that have been developed by commercial companies.

To extend that, research laboratories have also developed nucleic acid amplification tests and this is an example of tests that we currently use in my clinical laboratory at the NIH. So, for viruses we have a large spectrum of viruses; fungi – we have a test for pneumosistis. For bacteria, we have a spectrum of tests as well as for parasites.

Now, at least the clinicians will look at this list and say there are some unusual patterns that are here. For example, we have the microphlaria and the parasites. Typically, you wouldn't see many microphlaria infections in the United States. However, we have a very large research program studying microphlaria disease and patients fly from all over the world into the NIH as part of their diagnostic work-up. So, the purpose of my laboratory is to develop tests both for the clinical diagnosis of patients that are our clinical center on research protocols, as well as to develop test that will support the research protocols of investigators at the NIH. So, we have a

spectrum of tests. I would say the same approach applies to many of the laboratories that are seen throughout the country.

One of the things I never like to do is have a prepared talk because I start varying from the text and then I lose my place.

The second type of question that can be considered as more complex, and that is of all the organisms that are responsible for a particular disease, which one is important for a specific patient? Now, to appreciate the complexity of this test, we need to consider some specific diseases. Cerebral spinal fluid is normally sterile, so any organism present in a well collected specimen from a patient with meningitis should be considered clinically significant. Additionally, large numbers of organisms are generally present in the specimen, so test sensitivity should not represent a diagnostic problem.

We heard a discussion earlier today as you try to pick which types of diseases you would pick in order to develop a molecular-based test, and for aseptic meningitis was one of the examples that was given, and it is for these reasons. Any organism that you would find is going to be important, and generally they are in large numbers.

The primary issue with meningitis then is that there is a large number of pathogens that need to be considered. On this slide, what I've done is listed all of the organisms that I could think of that could be associated with meningitis. So, if you are trying to develop a test to look for the most common diseases, you only need a test that can recognize three or four pathogens. If you're trying to develop a test that recognizes all pathogens, that is a stand-alone test that would replace all other tests used in the laboratory, then you have a much more difficult challenge to face.

The use of nucleic acid-based tests for the diagnosis of pneumonia offers a different level of complexity. As with meningitis, there is numerous bacteria, fungi and viruses that are responsible for pneumonia. Additionally, the lower respiratory tract is frequently contaminated with the upper respiratory tract secretions. Where molecular assays are most useful is for the

detection of fastidious or slow-growing organisms whose detection is always associated with disease. Some examples of those organisms are listed in this slide.

The last example of a disease-oriented molecular diagnostic testing is processing blood from septic patients. Septic patients generally have fewer than one organism per milliliter of blood, so any molecular assay is likely to require specimen processing steps where the number of organisms must be concentrated from a large volume of blood. Blood specimens may also be contaminated with non-viable organisms from the skin surface so this could complicate the interpretation of the assay result. Finally, numerous organisms are associated with sepsis, so it is likely that the first molecular assays for the diagnosis of sepsis will complement the existing systems and not replace them.

With these limitations in mind, it should be stated that many commercial companies are working in this molecular diagnostic arena and are developing tests that can be used for the diagnosis of sepsis. This is because the blood cultures are the highest volume specimen processed in the clinical microbiology laboratory, are clinically significant, and cultures traditionally must be incubated for up to one week.

The second application of molecular diagnostic tools that I would like to discuss is identification of isolated organisms. As with the approach to detection of organisms in clinical specimens, both non-amplified probe-based tests and nucleic acid amplified tests have been developed. The U.S. company, Genprobe, recognized the need for rapid identification of significant microbes with non-amplified probe tests. They developed a large number of probe tests for the identification of bacteria, micro-bacteria, and fungi. These tests are highly sensitive and specific, require minimal specimen preparation, and can be performed rapidly. The limitation of the test is that the identity of the organism must be suspected. That is, the probes are used to answer a specific question such as, is this organism micro bacterium tuberculosis, or is this fungus just a plasmic capsulotum.

The use of non-amplified molecular probes is useful if a specific question is probed. A different approach is generally used for the more general question such as what is the identify of this

organism? Indeed, this is the question that is most frequently asked by the clinical microbiologist. Nucleic acid sequencing is currently the most useful procedure for answering this question. Although a variety of target sequences have been used, . . .

(Tape 7)

. . . transcriber spaces in fungi.

The advantages of the 16s ribosomal gene is a target we've already heard about. It has both conserved and variable regions. Universal primers have been identified for all bacterio isolates, and there is a large database that we can draw from in order to analyze the data that we generate. For this reason, commercial kits are available for doing these types of tests.

The problems are that the database have many errors – that is, the identification of the organism associated with the sequence information is inaccurate. In addition, some well-known species have identical or very similar 16s ribosomal RNA genes, and so for these organisms, the test is not discriminatory enough, and some organisms can have multiple different copies of the 16s ribosomal RNA gene which presents some problems in the analysis of the data.

One approach to resolve these types of issues is to look at other target genes, and this is frequently done for some organisms in order to more accurately identify them.

One of the targets we've used in the last year is the sec-A gene and the reason for this is the sec-A gene is an essential gene that is present in all bacteria, but is not present in eukariotic cells. The sec-A gene recognizes and binds pre-secretory proteins. Many of these proteins are virulence factors for the organisms and so that is one of the reasons why this is a highly conserved gene. The gene also has both conserved and variable regions which allow us to identify the bacteria.

Finally for fungi, we see sequencing has also become commonplace and there is a number of targets that have been used, both large sub-units of the ribosomal RNA gene, the 28-s as well as

the 18-s, and the internal transcribe spacers, the ITS region, has been used. The reason for this is that each of these targets has universal primers that have been identified and there is a very large database that has been generated particularly with the environmental fungi for these sequences.

What I would like to do is give two examples in my laboratory where sequencing has been useful. The first is a slow-growing gram-negative rod that was isolated from the lymph nodes of one of our patients with chronic granulomitus disease. The organism was relatively inert by biochemical testing. In fact, it really performed very poorly in virtually all of the biochemical tests that we performed. So, we proceeded with gene sequencing in order to identify the organism. What we found was that it was a novel organism that had not been described before. It is a member of the family of pseudo-bacteria aceii which is primarily an environmental group of organisms. But, this organism was distinctly different from the other pseudo-bacteria aceiis. This is the analysis of our 16-s ribosomal RNA gene. The red arrow indicates where our organism is, and it is quite distinct from all the other members of that family. The same was found when we analyzed one of the other housekeeping genes, the rec-A gene.

This organism was particularly interesting and the question was raised earlier about when do you do sequencing. This organism was completely sequenced. It actually was completely sequenced before the biochemical identification was made. We found there was a number of unique virulence markers that we believe play a particular role in this group of patients – patients with chronic granulomitus disease. Maybe later I can talk about that in some of our discussions.

The second diagnostic problem was a 48-year-old man with an undefined immunodeficiency that was seen at the NIH. He developed an infection of his . . . that spread to his cheeks, sinus, pallets and skin. Obviously, it was in a very far advanced stage when he was seen at the NIH. The patient required extensive surgical debridement and from many different specimens we isolated a mold. The mold grew relatively rapidly. It took about a week to grow, but it took us nine weeks for the mold to form characteristic structures so that we could identify it based on its morphologic properties as the . . . for mold. The molecular approaches we used were 28s ribosomal RNA gene failed to identify this organism and in large part because this sequence was not in the database. That underlines the importance of the database that you're using. In

contrast, the ITS sequence did have the organism in the database and it was identified as a chryso-spore . . . cola. Now, I say this because we were able to identify the organism within a couple of days after we isolated it. The other techniques took much longer – nine weeks for the more traditional techniques.

So, let me summarize and say that the benefits of the genome-based testing are well recognized in clinical laboratories today. Probes are commonly used, both non-amplified and amplified probes. The nucleic acid amplification test for viruses such as HIV, hepatitis B and C, and West Nile virus are used by our blood banks. Nucleic acid sequencing is used in many of the large laboratories for the identification of bacteria and fungi, and I believe this will be a technology that will be adopted more and more commonly as the costs of the technology decreases.

The continued expansion of the genomic-based testing is going to require integration of the sample preparation, amplification, and detection into low or moderately priced instrumentation utilizing CL-disposable assay systems. That is the ideal system, much like what you saw with immunoassay development over the last 20 years.

The development of comprehensive, accurate sequence databases is also important.

Finally, it should be recognized that today's molecular diagnostic successes should not be viewed as an end point, but rather the foundation for the next generation of diagnostic tests. Although we should define technical limitations for today's test methods, such as their sensitivity and their cost, this analysis should be our motivation to continue to refine the technology and not perceived as insurmountable roadblocks.

Thank you.

Moderator – Thank you, Patrick, for the very comprehensive summary of the monoclonal diagnosis and a particular thank you for keeping the time. Our next speaker is Gao Fu. He is also my colleague and now serves as Director of the Institute of Microbiology, Chinese Academy of Sciences. He is going to update us with the bird flu and streptococcus in China.

Gao Fu, Institute of Microbiology, Chinese Academy of Sciences

***Recent Outbreaks of Aviation Influenza in Migratory Birds and
Streptococcus Infection in China***

Thank you. Good afternoon everybody, my friends old and new. I'm pleased to be here to share some of our thoughts and also . . . a talk that is sort of a descriptive science.

As you know, . . . in China last year we have . . . avian influenza, especially with some infections in the migratory birds, and also some streptococcus . . . sero type II in human populations. We have 30 . . . that has evolved. So, I'm here to represent some of my colleagues from China CDC, from China Agricultural University and Academy of Military Medical Sciences and Chinese Academy of Sciences – lots of places. With their efforts, we tried to control these two types of our outbreak, and also we were able to write-off those outbreaks into the . . . papers and report it. By the end of this talk, I'm going to tell you that those two outbreaks, we wrote the paper, is published. One is published; the other one is published soon.

As a very serious question everybody would ask – what about the role of the migratory birds might play in avian influenza virus. What is the role the migratory birds and avian influenza would play for the public health so we don't a pandemic in the near future. So, that is the sum question and everybody is asking. Suddenly something happened last year.

Let us remind you – I think everybody who works here on the flu field will use a slide to show you and those four pandemics recorded in history with H1 and H2 and H3 for the last almost 100 years.

For H5 A1 influenza virus, the most important question we are addressing as everybody in the whole world – all the scientists are addressing – why we would have H5 A1 suddenly enter into a new species. Inter-species transmission is the key and also the key point of the scientists of the whole world working together to try to get the answer. So, inter-species transmission is the very important point for this outbreak.

I want to give you some outline for so far, what we found for the H and N subtypes for the – not just avian – for influenza which would infect human beings. So, so far we know H1 A1, H2 N2, H3 N2, and H5 A1, H9 N2, and H7 N7. So, nothing else yet for the human beings for the outbreak.

As you for the genetic basics for the avian influenza virus variation, and first one is antigenic shift. So, that means it is a combination of genes. As all you know, because they have eight genetic segments for their genes, and also antigen drift -- that you would see for all the viruses and bacteria because the gene mutations.

Before I can tell you something about the . . . outbreak, we need this slide to outline the importance of this outbreak. As you know, so the roles of migratory birds before early May, 2005, everybody think that migratory birds would play a very important role as a reservoir or just a host without any symptoms. So, the natural cycle of the . . . virus would go like this. You have a water bird, a coast bird, all these migratory birds, that is the cycle. Just before the May 4th – the exact date – May 4, 2005. Everyone think this is the cycle. Just some of the virus would dump out to infect poultry or pig or humans. So, that is the cycle you would see either textbook – so everywhere.

Also, I want to point out just before May 4, 2005, there were some sporadic cases of the migratory birds or water birds infection with H5 A1 or other influenza virus. But, you never see population infection. So, that is the key – also the importance of the outbreak in the . . . So, that also tells you that H5 A1 avian influenza virus overcame another species.

Of course, as I said from the very beginning, everybody is working on trying to find out what is the key point for the inter-species transmission. Of course, I should tell you we don't have the answer yet in the whole world. Recently, . . . published a paper in nature claiming something about the importance of the receptors . . . lower respiratory tract and also the upper respiratory tract because they have different distributions of the receptors. So, that is just one explanation. You still cannot explain everything. If you have an interest, you should read that publication in nature. Also another group that published a paper . . . same thing. They claimed because you have some receptor for the H5 A1 in a lower part of our respiratory tract.

Anyway, I'm just trying to give you a description of this outbreak . . new species. . . is a very, very important tourist site. As you know, every year the international cycling sports and competition yearly happens there. So, last year because this outbreak, they stopped. So, it is very important for sightseeing and also important for the natural reserve for not just China but for human beings to reserve those important endangered birds. So, suddenly and I will use this picture that I want to show you. This time, a major victim of this outbreak is . . . goose. Why is that? Though the lake is very broad, because that is a salty lake, yearly the birds would stay in some shallow water-side like this. So, the area is very, very limited – a limited areas for those birds because they need not salty water – and they stay there for breeding. So, this is why they will stay together in this area. So, this is why we believe from ecology this is the reason why the . . . goose – this is why they are infected from the very beginning. As you can see how extensive the population in the area. This is the shallow water area near the lake. Yearly they would hatch their eggs in the area quite near to this shallow water.

What was . . outbreak? As you see, mainly from the very beginning three major species were One of them is a bare-headed goose, very similar to the Canadian goose, and also some sea goose, and the typical symptoms and pathology you would see – . . . pancreas. The picture I show you . . . and also very serious neurological symptoms. So, we don't know why because I used to work on the . . . disease. I know all the virus, if any virus or bacteria, if they infect ducks – they have neurological signs. So, you would see this bare-headed goose – this is very typical neurological signs of these birds.

Of course, when we isolated this virus, the outbreak occurred on the 4th of May and we sent someone there and my colleague . . . from China Agricultural University and from The Institute of Zoology. So, they went and collected the samples. We isolated the virus immediately. Then we tried to reproduce the disease.

So, . . . 100% fatality . . . So, it is a typical, highly pathogenic H5 A1. I also want to tell you what we put into the ducks. The ducks . . . around 30-50% of the fatality. Also, we know – I want to show you later the migratory birds fly away and we know at . . . University in Japan. They isolated one virus from Mongolia. That was very similar to this virus. So, you can see the virus was carried to Mongolia. Also, the . . . 100% fatality for mice; 100% for chicken. But, 30-40% to ducks and also goose.

So, when we did the sequencing analysis, we found . . and all those what we call the molecular markers for the virulence or is three major mutations were there for this . . . to HA1 and HA2. Also, . . . in gene. And also one mutation discovered from the we found all these isolates we have from the chicken . . . mutation. However, you have to remember when they published this paper, they claim this is the mutation must be related to the infection of the H5 A1 to the mammals – the mice. But, we found this mutation in the isolates from bare-headed goose.

We also drew a picture to show the migratory birds – their fly route. So, you can see this is the fly route of the migratory birds through the . . . lake. So, this is why you would see the virus from Romanian, . . . from Europe and also from Nigeria and Africa. The virus was very similar to the virus we isolated from . . .lake. So, that is the conclusion.

So, you can see the virus carried by all these flying birds, maybe through this EuroAsia fly-away, . . . then Europe. So, this is the flyway for this bare-headed goose. So, this was downloaded from FAO . . . You can see, this is somewhere . . . lake. The . . . lake bird would fly in a fly-route, but they always have the overlapping areas. So, they must get some area to stay together. So, that means they have a mixing ecological niche for those migratory birds to stay together. So, we speculated this is why the isolates from Mongolia and . . . lake are so similar to the virus

isolated from Africa, Romanian and Greece and Turkey. So, maybe this is the reason. That is what we found for this migratory birds effects of H5 A1 avian flu.

I just want to give you some brief introductions about what is . . . in July and August of last year in China. It will be surprised if I'm telling you . . streptococcus . . . because everybody knows streptococcus . . . is a sporadic disease for the human beings. So, in total, the whole world, we have roughly about 200 cases in total, but they are always sporadic and also many societies and You never get a population disease. This time we got 200 . . . with . . . 38 that has evolved. That is a very severe case. Also you know, although a lot of experts evolved from , it is still very difficult to control the disease. So, there must be something special. It is not just the factor of the hospital or the conditions or the environment either hospital or rural area. It must be something special.

So, we collect all this information. I worked as a . . . to get all this information together and then we figure out it was streptococcus toxic shock syndrome. So, for this definition, we defined this disease through the clinical criteria. So, you have an emergent high fever, hypertension, acute respiratory deficiency syndrome, and and shock . . . So, in a lot of the cases you would see the patient die within hours. So, we know that patient contacted with an infectious agent because of a skin cut. All these people have a history of the skin cut. So, either the . . . involved in farming or involved in the slaughtering of these pigs. So, it is very obvious. But, it is so obvious but it is very difficult for the clinicians to control the disease. Some of the places they died within hours. Most of the patients have these symptoms, especially those who died immediately. So, that is what we found.

I also want to give you some background about this toxic shock syndrome. As you know, toxic shock syndrome is notorious already, but it was always disease caused by streptococcus orius, and also the super antigens. That is the overreaction of your immune systems. Super antigens form streptococcus orius . . . Also, the already know is a . . . protein and another protein that has evolved. That was the last five years' work. So, super antigens were known for the toxic shock syndrome caused by either staphylococcus orius or streptococcus But, I also want to tell you we tried very hard – so far, we haven't found out which of the components from

the streptococcus . . . would be the responsible super antigen to cause this STSS. We are working very hard to find out and also Dr. and Dr. this morning mentioned we did the sequencing for three strains of the streptococcus So, we are ready to write up. We found something new, but we are not ready to tell you, but we are ready to write a paper.

I also want to tell you – respectively, we went back to see . . . we also found in the 1990 25 cases of human beings with 14 When you go back to all these clinical records, we found it is also typical toxic shock syndrome. So, we put all this together into one paper. We did some laboratory work and some of the artificial infections with some STFPs and the disease can be reproduced and also we did some sections – this will all be in the paper published soon. typical streptococcus-type Also, what we did was immunochemistry staining of the . . . so this is a staining with a normal and this staining with the . . . peak sera to human few hundreds of pigs were also infected and died during this infection.

My talk for those two pieces of the outbreak in China in 2005, so H5 A1 influenza virus for the description of this outbreak published in Science last August and the streptococcus . . . causing the STSS will be published in . . . magazine in the second week of April.

Thank you very much for your attention.

Moderator – Thank you Gao for bringing us some really exciting data. Our last speaker, Professor Yuan Zhenghong, who just flew from Shanghai to this meeting, will give us the update information on hepatitis B virus.

Yuan Zhenghong, Medical College of Fu-Dan University

*New insights into the interactions between hepatitis viruses with IFN using cDNA microarray:
implications in identifying new tools for combating infectious diseases*

Thanks, chairman. I'm very sorry for my delay. Five days ago, a patient was confirmed to be infected by avian flu so a lot of measures have been implemented in Shanghai.

I don't want to talk about these very serious disease. I prefer to keep to hepatitis and . . . simple and easy.

Before I want to talk about hepatitis B virus and interferon, I just want to give you a few minutes introduction of my lab. This is the . . . my lab is located and we have three floors and Our lab have been mainly working on the . . . viral infections for the last 20 years and especially the B virus and the C virus. We try to understand why in China the patients become chronic very easily. We found . . . balance . . . In the end, we try to develop new strategies, especially it develops . . . vaccines for the patients.

About five years ago, our lab also expanded to the . . . genomics and cell microbiology. For example, . . . infections and in collaboration with the patients and doctors and the hospitals, we try to . . . in the patients with different outcomes and different response to treatment of the mutations of the surface, structure and non

(transcription not continued – too disjointed)

Moderator – Thank you, Zhenghong, for the nice talk. I guess it is still in panel III. Since we will have a banquet around 6:30, so we will have to finish the panel discussion as well as the poster session before 6:20. So, I asked the organizers to have the authority to squeeze 15 minutes from the poster session as a panel discussion here. After that, we will have a short

poster session and finish around 6:20. So, you guys will have 10 minutes to prepare for the banquet.

Question and Answer Panel Discussion

Moderator – This panel discussion will be open to all the speakers today, not only this Panel III.

Question – I just have a question for Dr. Stephen Popper about the whole . . . blood. It is very easy to get the . . . microarray and I think that is a doctor's hope in the clinic. . . . interferon treatment . . . So, I just want to know, can you tell the difference of the gene expression . . . in the whole blood caused not only by the . . ., but by the symptoms, or my question that can you find some of the symptoms and patterns from the gene expression Also, for the patients, I'm sure if you have gene expression . . . in the patients from the onset to the . . . what difference.

Popper – The short answer is yes. We have found, and I tried to show some of this, that we found patterns of gene expression associated with both the pathogen and all the clinical variables for which information has been collected in the patients. The issue is that very often what we're getting is a statistical association. So, some of these are essentially confounders. So, what we are finding is that in whole blood we are able to get a first look at this and tease it apart. Then if we really want to look further at whether a particular parameter, whether it is the pathogen or the clinical symptom is, in some sense, causally related, that ultimately what one might want to do at that point is go back to additional patients and obtain samples or purify samples that would allow one to more closely address that.

Question – I actually have two questions for Professor Yang Huanming about the human and the SARS. The first one is about the SARS. Tell me, how did the sequencing of the SARS help stop the epidemic?

Huanming – Genome study first has a contributive to the identification of the pathogen. That is very important. That would be the first step towards the victory of SARS virus. After that, after the first generation of the . . . actually was just an inactivated virus. Later, of course, we also identified many episodes which strongly react with the serum from the patients. Then the third . . .diagnostics with RTPCR without a sequence as the basis, no PCR detection would be done.

Comment – I agree with you the detection was facilitated by the sequencing, but didn't the epidemic just stop by itself?

Huanming – After the 17th of April, really took a strong or decisive measures – I think the general method to block the transfer of the virus by major way to help stop SARS spread.

Question – Okay. The second question is your contributions to the HAP MAP was considerable. However, I was wondering why is it that Caucasian Europeans were trios which allowed for haplotype dissemination and the African populations were trios. But, Beijing was not. There were no offspring. Can you explain that?

Huanming – That is not a ready-known issue at all. That is just a balance of scientific issues. Three types of materials can be used for this project. First, agriculturally. That is the best way to get of one generation. . . . The . . . efficiency would be rather low. Then the other, or the third one is just a genetic unrelated individuals. Fortunately, the consortium just developed the software to identify haplotypes with the material or for genetically unrelated examples. Then the middle one would be the best – just to balance the efficiency and the information for haplotypes that is the smallest family or pedigree trial. The parents and a single Then just because the family wouldn't stay together in . . . and then also because the Europeans already . . . in taking **(remainder not transcribed)**

Comment – The algorithms for imputing haplotypes are ineffective when rare alleles are involved particularly. So, you have sacrificed in the Chinese sample the precision of haplotype definition by using those algorithms which they have not done in the Caucasians and the Africans. I wonder who made that decision?

Huanming – The decision was made by the consortium. I myself, as a general member, take equal responsibility. The control, . . . Asians actually get more information by using genetically unrelated individuals because they . . . for these individuals obviously is higher. Then for . . . of this time, we just . . . to identify two things: First, . . . inheritance; the second, the determination or confirmation of haplotypes. Here now that hypothesis concerning efficiency and confirmation haplotype have been confirmed. Next project – if our similar goals were totally . . . genetically unrelated individuals, . . . really good . . . , **(not transcribed)**

Question – I have a question for Dr. Gao. You describe the streptococcus . . . type 2 infection has been potentially associated with toxic shock syndrome. It is very rare in the United States, but I actually saw a case of streptococcus . . . type 2 infection from a Phillipino man who came back to the U.S. – he had eaten raw pork. He presented – and this is the only case I’ve seen – with meningitis as well as a sepsis-like syndrome, did have some features that were consistent with toxic shock syndrome, but not a rash. My question is, is there something different about the particular isolates that you generated from this particular outbreak, or do you think in general that because of the sporadic nature of streptococcus . . . in general, that it already had the potential or that all isolates have the potential of causing a toxic shock syndrome.

Fu – I think that is a very good question. . . . STSS caused by streptococcus . . . , that is a question we are asking ourselves. I think what we found from the whole genome sequencing, Dr. . . . and also Dr. . . . is involved. We found something new. As you know, there is one . . . sequencing from Sanger Center. It is published in their web page. They didn’t publish the paper yet. So, we found something new, but we are not ready to see whether or not those new . . . or those mutations or sequences would be responsible for the special cases in China, especially for the STSS. So, we try to see any analogue or homologue how you would find in staphylococcus or you would find streptococcus But, as far as I know, there are several groups in China that are working on it. So, we haven’t found anything really special which would be responsible for this STSS. I think that is a very good question. I hope within a year or two, with a group effort in China, we should be able to identify something. Thank you.

Question – I have a question for . . . You mentioned, and also I know that you guys set up a branch in Tibet for the high altitude specimens to discover the gene diversity. Is that money-driven or science-driven, or otherwise my imagination is the biggest gene pool is in the ocean. So, why don't you guys go into the ocean and do this similar research?

Huanming – From my own point of view are concerned, . . . tourism . . .to transportation since the first of July. They estimated a number of tourists would be one million to Tibet a year. Now, according to the . . . statistics, 37% of the tourists would help the response to high altitude at the different level **(not transcribed)**

Huanming – I have been criticized by our American colleagues -- we are here to encourage the young people, both staff and students, to ask questions. We have to create that atmosphere that is our responsibility.

Question – My name is . . . and I came from Institute for Viral Disease Control and Prevention. I have a question for . . . My question is H5 A1 avian influenza have circulated in the . . . for several years, maybe from the 1996 and it is difficult to . . . because the H5 A1 . . . bird circulating in other animals. So, it is nearly impossible to keep this virus from these animals. So, . . . circulate in these animals, one day it may be a mutation. So far, it still didn't get the ability from the person-to-person transmission, but maybe one day it happen. What is your opinion?

Answer – Thank you very much. Maybe it is a very tough question. As for my opinion, as I said, what I said from my talk is a descriptive side. I just described what we found. As for my opinion, I think it is very difficult to say. Since we do not have any evidence yet, the H5 A1 would transmit into human beings. As I also said in my talk, it is possible because of the receptor . . . , there are a lot of factors would have some effect for this inter-species transmission. I'm the one who is very positive for this pandemic. So, I do not think – as you know, in the world, if Robert Webster was here, he would say pandemic is very soon. If Peter . . . he would say no, it is a long time to go. So, in this case, I would stand – that is a personal view – I would stand along with Peter because you think whole evolution – since I took up my job as Director

General of Microbiology Institute, I found my . . . are fascinating. So, through evolution, they tried to find their own niche to survive. So, what was clear in the lake . . . outbreak, I remind you of that circle. So, the migratory birds, they were infected with the H5 A1. The virus itself was striving to the dead end because theoretically the circle would give the virus a positive selection. It will stay there forever – at least for some time, and then they jump out for human beings or whichever species. Now, they just killed their reservoir. So, that means they kill their storage – whatever you would call it. So, . . . I'm right. So, you are asking my own opinion – that is my opinion. That is just one explanation for the evolutionary point of view. But, because everybody . . . if Robert were here, he would explain it in a different way. That is the first point to support my view as to why I'm positive. The second view would say, you can't imagine though the globalization and traveling is extensive, but think about techniques and we can approach to control the disease, especially infectious disease. We are far ahead for this combating than we were in the 1918. . . . we were not ready for the molecular biology. We were not ready for the vector . . . vaccines. We were not ready for the reverse . . we are ready now to change all these different kinds of antigenic shift, . . . so easy for us to make a new vaccine.

The second point is that the technique we can approach at the moment . . . That is my opinion. Thank you.

Question – I'm . . from the . . . Laboratory . . I have a question for Dr. Eisenstadt from TIGR. From your talk, you mentioned that there are no more than 10% of all the sequenced bacteria that have had over two isolate sequences. As I know TIGR have been sequence many strains from the same species. But, you also mentioned when the sequence genomes increase, the unique genes you got decreased, and the key . . . for several unique novel genes got from genome. So, my question is, do you think two sequence genome unique genes is valuable, or does the TIGR center ever try other techniques except genome sequencing to find novel genes. In my opinion, the genes for bacterial species may have a So, we all try to get other genes in this pool. But, I think to increase the sequence strains to finish the . . . seems too expensive. Do you think a cheaper way?

Deleted: Question – I have a question for Dr. Ahlquist who came from the University of Wisconsin. In your presentation you mentioned you did some study on relationship between the Epstein-Barr virus the . . . carcinoma. Did you find some gene relationship with?¶

¶ **Ahlquist** – Yes, and actually that was going to be the subject of some of my remarks tomorrow. But, inasmuch as time is short in these sessions, I can preview that by saying that yes, we found some very strong correlations between the up and down regulation of some cell genes and variations from tumor to tumor in levels of EBV. So, EBV (Epstein-Barr virus) is associated with all of the tumor cells, but in varying copy numbers, providing some of these correlations. As I believe is briefly indicated in the Abstract, these include suppression of some antigen presentation functions, as well as some anti-apoptotic effects and a few other functions that are involved in tumor progression and the proliferative state of the cell.¶

¶ **Question** – What were the genes that were regulated off by MPC?¶

¶ **Ahlquist** – The single strongest effect is down regulation of all of the HLA 1 oligos and several other associated oligos – HFES and some others. ¶

Eisenstadt – I completely agree. Yes, we do use other approaches and prominent among them is comparative genome hybridization analysis using microarrays. That is a major activity that is a part of the pathogen functional genomics resource center. We are increasingly turning to those kinds of technologies because it is very expensive to sequence one strain after the other. And, it doesn't make sense going into a project to first do the sequencing. You could imagine doing a localized sequence analysis if other technologies reveal that there is variation or novelty that is appearing using, say, hybridization approaches. So, I think a combination of the two is in order and is much more affordable.

Question – My name is from Molecular Virology Research Center in the Institute of Microbiology. I have a question for from Xiamen University. My question is what is the definition of a HA unit.

Answer – I hardly get my hands dirty these days, so I have to recall my earlier teaching. HA is a traditional – it is a hemagglutination unit. So, what you do is take a virus and serially dilute it. Then you add the appropriate kind of red blood cell to it. Hemagglutinating is identified by its activity to agglutinate the red blood cell. So, then you dilute it – you do a limiting dilution to the point where it no longer is . . . agglutination. So, the basic unit of hemagglutinate is one because if you dilute it further, you will not get a regular agglutination. So, this is the conventional and the traditional way of identifying the virus. So, what we are doing then when we have this direct test, then we compare this. Can we detect relative to HA as to what kind of sensitivity? So, it is a relative measure rather than a definite unit. Is that okay?

Moderator – We are running out of time. Before we stop, I'd like to suggest George to give us a conclusion remark for today's discussion.

George – It comes as a surprise to be up in front of you and before dinner. Everyone knows you never stand in front of an audience and its food.

I would note two things: One is I'd like to find myself agreeing with Henry again. I know this is shocking, but I think it is extremely important that those sitting behind the front table become

engaged in this conversation. I'm very appreciative of the young ladies here and the gentlemen here for asking questions. I think that this relates to the second observation. It is clear that the Chinese community has made tremendous contributions to the discussion of the entire landscape that is being treated here today. But, it is also clear that we have a long way to go, and I think the Americans in this audience would agree very quickly that much needs to be done.

So, when the conversation a little while ago diverted toward money and resources, I would like to interject that it isn't just the dollar. It is the human resources that are clearly needed in this conversation. So, I would like very much to encourage the next generation behind us here in this seating to be heavily engaged in the conversation because I think they will carry much of this conversation forward long after this meeting is over.

So, those are two observations. I want to not take my full two minutes because I don't want to keep you from your dinner or the poster session which I know has been cut short. But, with your permission, I will leave it at that for a discussion at dinner. Perhaps I will be disagreed with, but hopefully not too vigorously.

Thank you.

Thursday, March 30, 2006

Panel IV: Vaccine Development

Moderator: Toni Marechaux, U.S. National Academy of Sciences

If everyone could sit down, we will be ready to get started. This morning we will follow on our presentations yesterday and start talking about development of vaccines and how we will cure some of these problems that we talked about in our meetings yesterday.

My name is Toni Marechaux. I'm with the National Academy of Sciences in Washington, DC, and I have learned very much yesterday and I look forward to some exciting talks today as well.

In our session this morning, we have four speakers and we will begin with Dr. Li Dexin, from China CDC, who will talk to us about vaccines and viral diseases in China.

Li Dexin, China CDC

Vaccines against Viral Diseases in China

Good morning ladies and gentlemen. I will talk about viral disease and some new vaccines being developed in China.

(Not transcribed)

Moderator – Thank you very much for staying on time. We will wait and take questions for all the speakers, except for one speaker, at the end of our session. Our next talk is – and I didn't

even have to use my newly learned Chinese hand signals. The next talk is from one of our American colleagues, but born in China, Dr. Xiaoyan Zhan, who is over here all ready to go. She is from St. Jude Research Hospital in Memphis, Tennessee.

Xiaoyan Zhan, St. Jude Children's Research Hospital

***Development of Sendai Virus-based Vaccines to Prevent
Pediatric Respiratory Infectious Diseases***

Good morning everyone. First of all, I'd like to thank the workshop organizer to give me this opportunity to present our year's work on the vaccine development.

Today, in the next 20 minutes, I'm going to tell you how we use Sendai virus which is a mice virus to prevent pediatric respiratory virus invasion.

The so-called pediatric virus we focus on one year's human Para influenza virus type 1, we call it HPIV1 and type 3, HPIV3, and also the human respiratory virus.

As you can see, in the same genus as the HPIV1, there is another murrine HPIV1, murrine Para influenza virus 1, and commonly called Sendai virus. So, genetically this Sendai virus is close related to this human HPIV1.

So, our vaccine has a three aspect. One is you . . . Sendai virus . . . to prevent HPIV1. Second is used . . . Sendai virus, which is used as a vehicle to carry the RSV foreign gene and then prevent RSV. The third one is using the . . . Sendai virus carry HPIV3 gene to prevent HPIV3.

So, I will start from first part, use the . . . type Sendai virus as a . . . attenuate vaccine for the human HPIV1.

As you may know, HPIV1 mainly causes disease in the younger infant from 6 months old to three years. In the U.S. annually, they cause about 600,000 cases per year. In this 600,000, about 5%, which means about 30,000 per year, children have to be hospitalized. Vaccine study start from about 40 decades ago, but even people try many different kind of way and until today we still don't have good, effective vaccine for this virus.

Generic vaccine approach teaches us an antigenically related virus strain from an animal host could be used as the immuno. . . to reduce and protect against a human virus. So, the purpose of the study shows the highly notable immunoassay similarity between the Sendai virus and HPIV1. As you can see here, the HN . . . shared about 72% immunoassay identity. So, under the . . . protein . . shared about 68%. So, the most significant . . . we found a sequence . . . So, this is a perfect animal virus we can use as the vaccine to test on the human to prevent human disease. This . . . and people have known for a long time, but a lot of time today you cannot find ideally the animal virus used on the human, but in that case and we found a perfect match.

So, since we have this idea, we test this idea on a small animal model and we got big success. Then we moved to bigger animal models. In that case, we used the Africa green monkey. On the first green, six Africa green monkey gave the Sendai virus . . . and under the group and . . . saline as a control, about 126 days later and vaccine group B boost. Another group gave . . . What I want to say is Sendai virus can . . . very . . . gave a very high titer viral . . . So, control group here just gave . . . flu. About a month later, we . . . Africa green monkey with HPIV1.

These results is . . . data. As you can see, the . . . was collected from day zero to day 140, just before the . . . Y-axis means how high the amount of the antibody and this . . . is one to 300 dilution and a lot of . . . was coated by Sendai virus. As you can see, start from day one to day ten, an antibody amount increased dramatically and a high amount of . . . this colored line indicates the vaccine group. This blue line indicates the control group. So, you can see from day one to day ten, the antibody amounts increased dramatically. This amount will last for the whole time of the experiment. This data tell us the vaccine group has the high amount of the antibody

and then we tested if this antibody has the capability of the neutralization and we also get a similar result.

Then the animals were tested for the susceptibility . . . with HPIV1. The top six monkeys was Sendai virus immunized group. The bottom one is the control. Nasal swabs were taken and indicated that from day zero to day eight, and the example was to try to see if they can recover any virus.

So, as you can see the vaccine group from day zero to day eight you never see any virus recover from the nasal swabs, but in contrast, the control group start from day two and all the animals see recover the virus. So, protection against HPIV1 was achieved in all Sendai virus immunized animals.

Based on this result, and other facts like Sendai virus do not cause respiratory symptoms in tested primates, and the Sendai virus has never been demonstrated to cause disease in humans, and we started to test the safety and the immunogenicity of the Sendai virus in humans. Young infants, especially the one with HPIV . . . , is the name target of the vaccine. But, we don't want to start from this infant and for the safety concern we like to start from the healthy adult. Then we move to the healthy children. Then we move to the HPIV1 serum positive younger infants, and then move to the final target which is HPIV1 serum negative infants.

A couple years ago we started the phase 1 test on the healthy adult. The paper already been published and to make a long story short and simply tell you the subject is not healthy adults, average age about 29 years old, and they being immunized in . . with the Sendai virus, three different dose. The results tells us in . . Sendai virus was uniformly well-tolerated.

Currently, we were doing the phase 2 study. So, summarized for this Sendai virus as a vaccine for the HPIV1 and we found the Sendai virus is an effective vaccine for HPIV1 in the non-human primates model. Sendai virus is well tolerated in human trials to date. We are also doing the phase 2 and we try to push to the phase 3.

Next, I'm going to tell you how we make . . . Sendai virus as a vaccine to prevent RSV infection. RSV is the leading cause of the viral respiratory illness in children and high-risk adults. It was estimated more than 120,000 infants hospitalized every year in U.S. Worldwide, it is estimated to cause approximately 900,000 deaths per year. But, so far, the vaccine study also started 40 years ago, but so far we don't have effective vaccine.

Sendai virus behaves so well on the HPIV tests, so we were thinking maybe we could use Sendai virus as a carrier to carry the foreign gene, for example the RSV G gene or RSV F gene and through express . . we can prevent RSV infection. So, we . . . Sendai virus like this. This is a whole genome of the Sendai virus. Between the F and HN gene and we put the RSV G or RSV F here. So, the whole Sendai virus particle now because the new particle and this particle carries the foreign gene and can express a foreign gene. But, we also test this . . . Sendai virus, the foreign gene will not express on the surface of the virus, so make this virus host to . . . means still will infect mice instead of the human.

Then we tested if this . . . Sendai virus can give us some information about the prevention. So, this is a strategy of the vaccine variation. The animal model we used here is a cotton rats. We pre-breeding and immunize on day zero, and about four weeks later, we breeding them again to just get enough serum to do the test. A week later we . . . then with either . . . Sendai virus, carry RSV F gene or carry G gene or carry half F or half G. Also, we have some Sendai virus . . . as a control. Then we charged them on week five and we charged with RSV.

The experimental assay we used is . . . and then is in . . . assay and . . . assay and the . . . I'm going to detail the way . . I show you the data.

First, we test the . . . and they show very nicely. Then we did the . . . assay. So, this figure shows the . . . data.

Each vaccine group includes the five cotton rats and we got serum, mixed the serum from these five cotton rats, and to the serum dilution like 1 to 64, 1 to 256 and 1 to more than 1,000. So, this serum dilution and this is a control group. . . . This is one group got the RSV F and another

group got G and this group got half F and half G. This is the percentage of the . . . reduction. So, as you can see here, compared with the control, either you gave F or you gave F + G and can efficiently reduce the virus to infected host itself. The G and a little bit lower than just F or F + G, but still significantly compare with the control.

So, it means you gave the cotton rats with this . . . Sendai virus . . . and secret a big amount of the antibody and this antibody can function very well.

Then we also tested T-cell response and this is the result of For somebody here who maybe don't know . . . , I just simply tell you that day one you . . . with anti-interferon . . . and on the second day you added the cell. The cell is from immunized cotton rat. We collected . . . and also we . . . from both immunized group and non-immunized group, and do the single cell suspension Then we added the peptide. So, if you immunize RSV . . . with the Sendai virus RSV F and you stimulate the cell with F peptide pool, and if you immunized the cotton rats with RSV G and you stimulate with the G peptide pool, the peptide pool is a peptide synthesized . . . the whole gene. They are the . . . This results show here.

We tested all the pool but finally we focused on the pool for the F gene and we . . . and the pool 5 and this is no peptide control. As you can see, the control group is significantly different from the immunized group, and immunized group has a very good T-cell response as well as if you give the G, you will see very good T-cell response. So, it means you give the cotton rat with a Sendai virus, either carry F or they carry G, and they can stimulate both T-cell response and B-cell response. So, this is all the in vitro data.

Then we tested for the suspicion of the virus The cotton rat . . . group from number 1 to the . . . cotton rat, and this cotton rat got the controlled Sendai virus. Another group got a vaccine that carried F. Three days after . . . , you collect homogenize . . . and culture the suspicion and try to recover the virus from the As you can see here, the control group you see the high amount of the virus recovered from the lung, in contrast the vaccine group, there is no virus recovered from the lung. The same case happened if you just give the F or you give the G or half F and half G. So, this data tells us this vaccine can

(Tape 9)

... for RSV vaccine. Then G, F and G + F combination ... cotton rats generate RSV specific antibody and the T-cell responses. F, G and F/G combination vaccines protect cotton rats from RSV

I also have some data but I don't have the time to show you here. I just tell you the conclusion is G, F and G + F combination vaccine also capable to protect the cotton rats from the ... RSV ... because on the vaccine study, I think one of the very important issues is that you have to not only ... or protect the homologous virus ... and most importantly, you had to see the cross-protection. It means you gave virus A and you had to cross-protect virus B. So, we see very well cross-protection. We tested ... a bunch of clinical isolated virus.

Also, we found ... Sendai virus, ... antibody response and we have data that shows after a year after immunization, you still see very high titer of the antibody. So, this will be a benefit for the pediatric vaccine.

Another vaccine ... Sendai virus at this time, we carry the human Para influenza virus type 3 gene and F or F and G, and then prevent the HPIV3.

I just tell you the conclusion is ... Sendai virus expressing F and HN of the HPIV3 ... HPIV3 ... antibody response. Also, this response can protect the cotton rats from HPIV3

So, I was kind of excited by this vaccine strategies because I just feel like this is a cocktail – the top tier is a recipe of the pediatric respiratory virus vaccine. Why I say it is like a cocktail is you use ... Sendai virus to prevent HPIV1 infection. So, it is just like you added the first one on the top. Then you added the second ... Sendai virus and express RSV gene to prevent RSV infection. Then, you added the last layer, a Sendai virus carry HPIV3 to prevent HPIV3 infection. So, for an infant if you give the drop of this cocktail, just one drop, and they will be prevented from this RSV virus.

I stop here. I finally I want to acknowledge Karen Slobaw and Julia . . . who are the head of the vaccine project and we have been in close collaboration with Dr. and their lab makes all the . . . Sendai virus . . RSV gene. And also Dr. . . . lab make all the . . . Sendai virus express HPIV3 gene and also we have the close collaboration with from the . . . Children's Hospital.

I'd also like to say this is just one of the vaccine projects in our lab and we have another main vaccine project in the lab trying to develop a vaccine to prevent HIV vaccine project. We are already working on this project for more than 15 years, and we now have a strategy or cocktail . . . and we have a more than 15 sequence information the DNA mixing and we have around 20-30 different sequences on the vaccine. We also have around 4-5 protein vaccine in the protein vaccine. So, we have the DNA . . . and the . . .virus and the protein boost strategy. So, I didn't talk about this, but I bring the poster in the back if you are interested.

Finally, I just want to show this – I already like to show off this – this is

Thank you.

Moderator – We have our third talk this morning is on studies of HIV, preventative vaccines in China, and our speaker is Dr. Wang Youchun from National Institute for the Control of Pharmaceutical and Bio Products. Unfortunately, Dr. Wang will have to leave directly after his talk, so we hope to have time for a few questions before he leaves.

Wang Youchun, National Institute for Control of Pharmaceutical and Bio Products

Studies on HIV Preventive Vaccines in China

Good morning. First, I think the chairman and organizers who give this chance to join this meeting. I'm from the laboratory of the National Control Authority. I am merely responsible for the quality control and . . . of HIV-related products such as . . . assays and the vaccine. So, today I just give you a brief introduction about the studies of HIV preventive vaccines in China.

So, HIV is a very serious program so I . . . CDC report up to the end of September, 2005 . . . HIV infections in China have reached about 135,000 which include about 31,000 . . . cases and about 7,000 death cases. So far, all the HIV infected cases have been identifying all of the 31 provinces in China.

So, the highest accumulated . . HIV infections come from the Hunan Province, province, province to identify the HIV infection. Most of the HIV infected cases are identified in the southwest and northwest parts of China, come for the drug use in the population. Most of the HIV infected cases are identified in the southeast China and the major city of China comes from the commercial sex population.

The males compromise the major part of the whole HIV population in China of all the accumulated reported cases. The males make up about 65%. So, it has continued to be the major target population of the HIV infection. Most of the infected cases are founded in the age group of 20-29, which comprise about 50% of all those infected. The age group of 30-39 and that of the 40-49 comprise about 30 and 10%, respectively.

So, the transmission . . . analyze by Chinese CDC are called into their report about 44% was transmitted by intravenous drug users, and about 36% was transmitted by heterosexual and about 8% was transmitted by the form of plasma donors, and about 7% was transmitted by homosexual

and 2% was transmitted by blood transfusion or by using blood products, and about 1% was transmitted by mother to child.

So, the . . . have done a lot of work on the . . . determining the subtype of the HIV in China. In his group, he collect about 1,500 . . . from the different provinces in China . . . determined the subtype of the HIV. Through this result, we can find about 40% belong to the type B – we call this type B because this subtype is similar to the . . . from the . . . So, about 30% belong to the . . . and 15% belong to the A . . . So, in China, the type B . . . and the AE . . . comprise more than 90%. So, in China we also find another minor subtype including the A, B, C, D, and F. So, the minor subtype comprise about 10%.

So, the distribution of subtypes in different groups was also analyzed so through this table you can find the amount of donor populations. Most subtypes and most of the HIV belong to the subtype B, which comprise more than 90%. Other minor subtype . . . type B, . . . and AE . . . comprise about 10% or less.

So, in the drug user populations, most of the subtypes belong to the BC . . ., which reach about 60% and the . . . type B can reach about 30%. The B, . . . comprise less than 10%.

In the sex populations, you can find almost all the different subtypes so the highest subtype is the AE . . . can reach about 40% and the . . . BC . . . and third . . . This is the normal B, not the type B.

So, so far Chinese government have taken so many different intervention measures, but I think the development of the HIV vaccine is also very important to control the spread of the HIV family. So, I called into the . . . several different HIV vaccines should be . . . The whole based on the traditional technology that has not yet gone to the clinical trial just because of the safety concerns.

Another vaccines such as the peptide vaccine, protein vaccine, . . . vaccine and the . . . vaccines have been developed in China or worldwide. So, some vaccines show very good promise.

The main concern for the development of HIV vaccine in China include several kinds of HIV vaccines should be developed in China, including the protein vaccine, vaccines and others. Several subtypes such as subtype B and BC . . . in China, so the vaccines should include the genomic regions of those genotypes to ensure the vaccine . . . protection of those subtypes.

. So, in order to induce the antibody . . .

(remainder not transcribed)

Moderator - . . . and Dr. Monto is from the University of Michigan.

Arnold Monto, University of Michigan

Influenza Pandemics: Their Origin and Control

Thank you very much. It is my pleasure to be here to talk about a very important topic which has attracted the attention of the people, the press, and the policymakers all over the world. I would like to put this into context by looking at not only pandemic influenza, but what we call seasonal influenza, inter-pandemic influenza because as the song goes, you can't have one without the other. You really have to prepare for the inter-pandemic influenzas which probably kill about 100,000 Chinese every year. We estimate that 36,000 Americans die each year because of seasonal influenza, 200,000 get hospitalized, and these are preventable events. So, we need to look at one while we are looking at the other because seasonal influenza or inter-pandemic influenza is relatively predictable in terms of its occurrence, whereas pandemic influenza is utterly unpredictable in terms of where it comes from, what virus will be involved, and the impact.

So, with inter-pandemic influenza, we have predictable risk groups, those who are more likely to get complications and die. We get vaccine protection in younger individuals with a single dose. We have new interventions. We have developed new anti-virals which have now come into their own paradoxically because of the occurrence of avian influenza and we have good markers to be able to tell us that our vaccines are protective laboratory markers. Pandemic influenza we don't know about risk groups. We'll talk about that.

We will all be new, naïve hosts for these viruses, so we will probably all need at least two inoculations of virus. We may also need other substances adjuvants to get good response. Standard efficacy studies aren't possible because we don't have sufficient events taking place. So, we have to look for surrogates of protection and they don't really exist, at least for the avian strains, to help us in saying who is protected and who is not protected. So, there is a lot of difficulty in working with pandemic influenza which is why we're not there yet. We are not prepared.

Now, just to review seasonal influenza, and these curves actually come from the pandemic which will resemble seasonal influenza. I just want to make a few points, and I'll do them pretty rapidly because for many people, this is review. Most of the morbidity of influenza occurs in younger individuals. Most of the mortality occurs in older individuals, except in the very young. And, this is totally predictable. This is why in the past we have vaccinated to prevent the complications of influenza which occur mainly in the over 65-year-olds, those with chronic conditions and to a much less extent in the very young. We are realizing the very young are important now. We now have recommendations in the United States that all children under five to be vaccinated with influenza vaccine.

The influenza genome, you all know this, the key issues are eight segments of RNA to one coating for the hemagglutinin and another coating for the . . . and two surface antigens, to which antibody produces protection. So, we have this virus which is ready to change. This is the issue with our influenza viruses that they keep changing.

One of the changes we get, and we've talked about this before, is antigenic drift, which is a gradual change that all of our viruses take from year to year – sometimes big changes; sometimes small changes. A gradual change is referred to as drift and it is a result of mutations in the segments coating for the hemagglutinin and the . . . and many of the other segments coating for internal components are much more conserved which is why people now are looking towards them for universal vaccines.

We have our nomenclature which is a pretty unique for influenza. Basically we pay attention to the subtypes for type A because we have subtypes for type A. Everything else is laboratory numbers, isolation numbers. In the United States, the press really likes the geographic designations. So, one year we will have the Ceshuan flu and another year the Hong Kong flu because that is the virus that is in the vaccine. This is the virus that is in the vaccine right now. It is being changed. Next year, because again a gradual change in the virus. And, the inactivated vaccine – three kinds of antigens in the vaccine. I want to make a very important point is that we can't up to date it well unless we have good surveillance, good submission of specimens to WHO from the collaborating countries of the world will give us the best chance of having the right virus in the vaccine. This is why open reports and transmission will be the best for all of us because we all have the same problem. The reason we have a southern hemisphere and a northern hemisphere vaccine doesn't mean that the virus is different in the summer or the southern hemisphere than the northern hemisphere. It just gives us another opportunity to update. So, now we update twice a year rather than once a year because with a changing virus, one update is not enough.

Vaccine production – the timeline in the United States, in February WHO meets. In the United States, FDA now this year had a conference call to confirm for the U.S. the recommendations from WHO. The vaccine is being manufactured even before that. The manufacturers know what is going to be retained in the vaccine from the previous year.

The point is, it takes awhile for the vaccine to be produced. It takes awhile for the vaccine to be distributed and administered. When we talk about production for pandemics, we have to remember that it is not simply getting the vaccine produced. It is getting it distributed and

administered, especially with a pandemic vaccine, if we are going to need two inoculations. So, it takes awhile.

Vaccine efficacy – this is a classic slide. You may not be able to see it down at the bottom, but starting in 1943 and running to 1969, there is an interesting story behind these randomized trials with virologic end points, and that is they were conducted by the U.S. military. Why? Because during the Second World War, it was figured that in order to keep the troops ready at all times, 20%, 30% of them could not be sick with influenza. So, until 1969, this program was conducted by the Armed Forces. In 1969, it moved to NIH. There were all sorts of reasons. But, the real reason is the Department of Defense had their budget cut and couldn't continue to carry out these trials. So, they made it mandatory – all recruits and active personnel in the United States have to receive influenza vaccines.

Now, we also have – and I'm just going to spend a couple minutes since we're interested in genomics here – on the live attenuated vaccine. Again, we are taking the eight segments. The problem with a live attenuated flu vaccine is that we have to update it every year. We can't have the same vaccine year after year after year the way we do with measles or rubella or other live vaccines. What they do is they insert into what we call the master strain, the two-segments of the new wild virus, those coating for the hemagglutinin and the . . . and they know where the mutations are which are producing the attenuation. So, this is well worked out from a molecular standpoint.

How do they produce this? I'm showing this particularly because it is the same story that happens in nature with the production of some of our pandemic vaccines. They actually co-cultivate in eggs the two parents and with antibody pressure, and then now can do this with reverse genetics, they select out those viruses which have just the surface antigen coating segments coming from the wild type and the rest comes from the master attenuated viruses.

Now, for an avian virus, an avian vaccine, they are working on this at NIH. They are able to do this, but the problem is you can't start using this until the pandemic starts because you could have re-assortment in nature and you could actually introduce a humanized H5N1 into the

population. One of the first uses of this vaccine, I think, is going to be as a challenge to those who are vaccinated within an activated vaccine to see whether they are protected or not. Of course, it is attenuated so it isn't the same as being infected by a wild virus, but it is the best we got.

Now, this is a vaccine session, but I have to spend a few minutes on neuraminidase inhibitors and we have two of them. Amantadine is now worthless in much of the world because it has been used too much to treat influenza and it induces resistance very rapidly, unlike the neuraminidase inhibitors – and we have two: zanamivir, which is inhaled, and oseltamivir, which is a pro drug but it is taken orally. Why are we looking at oseltamivir? Because it is absorbed, whereas zanamivir is not absorbed, and as I'll get to in a few minutes, our avian influenza infections in humans suggest that they are systemic – that they are not confined to the respiratory tract. So, zanamivir may see a use for prophylaxis because the portal for entry is the respiratory tract. But, for treatment, it is probably oseltamivir, which is why the world is grasping for oseltamivir. A very unhappy situation – a single manufacturer producing a drug that everybody wants that a few years ago to treat seasonal influenza, when everybody should have wanted it, nobody wanted it.

Why is this going to be useful do we think? We prevent complications. In little children – I'm not showing you this – 40% of children treated with oseltamivir do not develop otitis media. There is a 40% preventive efficacy in children who get otitis media from all kinds of infections. Here in adults you prevent bronchitis, you prevent pneumonia, you may even prevent hospitalizations. This is the hope – if you treat with oseltamivir, you will prevent the more severe manifestations.

You've heard about our pandemic years and I won't elaborate on these because we are going to spend some time looking at where the viruses came from, looking at history as the predictor, we hope, of what may or may not happen. I will not concentrate on 1977 – a very strange episode where a virus which had been in the freezer since 1950 came back and took over and now circulates throughout the world – the mildest of our three types and subtypes.

As you all know and we heard about this yesterday, the wheel of influenza, shore birds, migratory birds, the source going to poultry, a solid line, and now going from poultry to people.

It used to be a broken line, but is now a solid line. We used to think that the pig was vital for this because humans have receptors for human viruses. Birds have receptors for bird viruses. And pigs have receptors for both. So, the idea was the pig was the mixing vessel. A pig doesn't seem to be that important for H5N1. We have tigers and cats and all sorts of other unexpected species with H5N1, but the pig doesn't seem to be that important.

And, we used to believe that this always happened to produce a new pandemic virus – that you have co-infection in a pig, let's say, of an avian virus and a human virus, and some of the genetic material was shared and a new virus on the right came out. Here one segment coating for the neuraminidase is shown as being shared. We'll talk about how many segments actually changed. And, in 1957, with the H2N2, we now know for sure that three segments, hemagglutinin, neuraminidase, PB1 a polymerized gene, came from the avian parent and a new virus with pandemic potential occurred. Two segments, hemagglutinin and PB1, not the neuraminidase, changed.

The big news is the 1918 virus because the 1918 virus has been recovered from archive specimens from specimens of lung and paraffin at the Walter Reed Institute. It has also been recovered from the permafrost of Alaska to confirm that this is the 1918 virus. And, it has been shown very clearly this is a fully avian virus of as-yet unknown origin. We don't know what bird. We're not even sure what time. That is important when you look at the 1918 story which I will not do in detail. But, whether there was a first wave that was a different virus or it changed or things of this sort. But, it is a fully avian virus and it occurred by mutation and in at least one of the viruses, the number of mutations that has to occur to make this able to infect humans, work with human receptors, is small. This is why we are worried about the H5N1 virus. If there are mutations in the hemagglutinin that allow it to attach to human cells, then we are in trouble and to me, I don't know how to predict chance events like that. That is why I feel that even though it may be of relatively low probability that we will have a pandemic of H5N1, we still have to prepare because it is possible.

The 1918 virus – I just want to show you this. This is relatively new information. The results of an excellent collaboration between CDC and Walter Reed and Mt. Sinai in which they

reconstructed the virus and then looked for virulence factors associated with the various gene segments. On the left is an ordinary H1N1 virus and the log titer in lungs. The second is the hemoglutin from the 1918 virus being put into this. So, you have hemoglutin in 1918 and the rest of the virus is just an ordinary H1N1 and look how the titer in lungs goes up. Then you have five segments from the 1918 virus that goes up further. But, the full virus is even worse. So, this is polygenic in origin unlike the avian influenza virus where not all but a lot of the virulence is associated with the hemoglutin – the multi-basic hemoglutin.

So, let's go quickly to some of the issues about 1918 and why was it so lethal. 20-40 year old people died. It was a very simple, although hard to explain observation that happened everywhere in the world. How many people died? I hate these estimates. Why did they go up from 20 million, which I heard about 20 years ago, to 40 million to 60 million? Most of the rest of the world, the non-developed world was left out. In India, which didn't have that good health care, but had good data collection, it has been documented through contemporary accounts that more than 12 million people died. You have China, you have other large countries that really didn't report during that period. That is why the numbers are staggering in terms of global numbers.

How did it spread in the United States? Rapidly. Once it got going, it covered the country in about six weeks. I'll just go over the history of some of these outbreaks. In the city of Philadelphia, look how fast it went through the city – ran out of coffins. All sorts of problems associated with very hard and rapid spread of the virus in the cities.

In 1957, this is a very interesting story and you probably can't read the numbers. This was downloaded from a contemporary document. I'll tell you what the story is. It appeared in Guan Dong province in February. By May it was in Hong Kong and that is where the viruses came from that were used to prepare the vaccine. It started to cause outbreaks in the United States when schools opened in the south where they open early in August. It was pretty clear it was going to behave like ordinary influenza, mainly high attack rates in children. But, deaths only in the typical risk populations – older individuals, chronic conditions and very young children. But, there was a second wave. The first wave occurred early -- out of our typical influenza

season peaking in October and then February March we had a second wave. But, we had vaccine coming in at the time this was going on because the virus got from Hong Kong to the manufacturers in May. Japan, by the way, was having major outbreaks in May and into June. The important thing for controlling avian influenza and preventing its transmission to humans in a country is that means you will not be the start of the pandemic. You will have more time to prepare because what you want to do is delay the appearance of the pandemic so you can have some vaccine. So, it is critical to try – and now that we have a global situation, it could come from anywhere. It doesn't have to come from Asia because it is everywhere. At least outside of the Americas, it is spreading everywhere with deaths occurring in various countries and outbreaks occurring all over.

In 1968, again Hong Kong in July. In the United States, it took about six months to get to the U.S. So, you can have a delay even with the planes going in those days.

Finally, a few words about avian flu. Again, this is the bad virus – the H5N1. You all know about 1997. In Hong Kong, the wake-up call – six cases; 18 cases; six deaths. There, Amantadine did work. Where are we now? This is 16 March – the numbers you can find on the web. If you look at all the countries involved, India on CNN this morning, big outbreaks in India. India is a country which has backyard flocks everywhere. India could be the source of the pandemic virus if it decides to mutate. The number of deaths – again recent reported WHO, and it is still a very uncommon event – transmission to humans. But, look what happens if you do get cases? ... fatality is high.

We have three interventions to consider. I'm going to very briefly go over anti-virals. I will say nothing about non-pharmaceutical interventions which is the subject of a Department of Defense meeting next week in Washington because that is all a lot of the world is going to have during most of the pandemic and most places for at least the first part of a pandemic – social distancing, closing schools. We've never looked at this because we've never really had to if we had vaccines and anti-virals.

So, vaccines – how quickly can they be produced? How much will be available? Only countries that produce inter-pandemic vaccines will be able to supply vaccines to their own people, vaccines for pandemics. No country is going to be allowed to send vaccine out of its borders until the country has enough and that is not going to happen rapidly. So, we need to look at various . . . Other issues - -this is reverse genetics and this is how we produce the seed and you know about this procedure. It has been referred to before. Taking out the multi-basic cleavage site, reconstructing the virus. But, the problem is this is not a good antigen. In order to use less antigen, you need an . . . studies with MF59. There are also studies with H2N2 which is another potential pandemic virus in healthy adults, and it seems that alum allows for what we call antigen sparing – giving less antigen because if you can give a sixth of the antigen to one person, you can vaccinate another five people. It is all very simple.

NIH is now conducting a trial with regular vaccine without angovin; another arm with MF59; another arm with alum, which is not patented and which is easily available. The disease, as I said, is in younger individual. It looks systemic and it looks to me like acute respiratory distress syndrome which is very familiar here with what happened with SARS – a very different kind of virus but with the same effects on the lung. Then we have oseltamavir and these are studies which a doctoral student of ours, Wheeling Yen, carried at out St. Judes where they have facilities for containment. If you are going to work with live H5N1, you have to have containment. What was done here was to show that you need at least longer duration of the antiviral and maybe higher doses. This is pretty clear from animal studies, especially studies that are ongoing in ferrets.

What would 1918 be like in the U.S.? These are just extrapolations looking at the 1918 numbers and increasing them to the U.S. population. This is not saying that antivirals will work or not, and not saying that antibiotics are present because we don't know how much of this is really bacterial. And, in the U.S. we would say that if exactly the same thing occurred now as in 1918, we would have two million deaths. What does that mean for China? 7 million. Very difficult problem because we don't know that is going to occur like this.

So, inevitable pandemic – we don't know if it is going to be H5N1, but that is the one that is everywhere and we have to prepare. Antivirals, vaccine is important, and probably we all ought to learn more about non-pharmaceutical interventions.

Thank you.

Moderator – Thank you very much. We do have a few minutes for questions, but we only have two of our panelists left, but I'm sure that they will be able to handle everything that we would like to discuss. While Dr. Monto is coming back to his seat, one question that I have heard from several people in the audience and because I want to encourage, especially people in the back row, to ask questions – but one question is for Dr. Zhan to tell us how you came from China to the United States to study vaccines?

Zhan – About 13 years ago, actually the exact same day, March 26th, the day I fly from U.S. to here to come to this workshop – 13 years ago exact same day, I flied to the U.S. At that time, I got a Rockefeller Foundation fellowship to study the . . . marker biology for a year at he university. . . . 13 years . . . lot of other labs and supervisors, and I just learn a lot and really appreciate everybody give me the help

(Tape 10)

Moderator - . . . ask if we have any other questions from the audience?

Question – I ask question to Dr. Xiaoyan Zhan. **(not transcribed)**

Zhan – **(not transcribed)**

Question – I have a question for Dr. Wang Youchun and in your presentation you mentioned one . . . vaccine have been approved in China. You mentioned the three . . . just for the doses. I would like to know how many . . . for and for each . . . , how many peoples participate? This is the first question. Second question is does every volunteer know what they have been

injected? Do they know what will be the benefit or maybe they haven't any benefit from this? Because some studies show that most HIV vaccines failed and maybe after you use the vaccine and . . . HIV virus each person, maybe this person will develop HIV more quickly.

Youchun – So far we have eight groups for the . . . clinical trials. Total about . . . subjected with . . . But each group is different. I didn't remember exactly how many person in each group.

Question – How many groups?

Youchun – Eight groups that had different doses.

Question – I'm not asking you for the doses. I ask you for the volunteer groups such as the – all of them are . . . or just no more control.

Youchun – Each group we have the vaccine group and the placebo. In each group we separate two groups. So, totally we have eight groups. In each group of eight groups, we have the vaccine group and the placebo group.

Question – All of them are volunteers?

Youchun – For . . . clinical trial, all are volunteers from the normal populations – not from the high risk population. So, because we of the vaccine so we must chose normal population . . . clinical trial. So, you ask me about whether one . . . know about whether they get the benefit or whether they know . . . vaccine efficacies. I think so far we choose all volunteers. Most come from the university. So, they get the education about . . . and they know the background of the HIV vaccine so far. So, they know. So far no HIV vaccine is effective to prevent the transmission of the HIV vaccine. So, they volunteer to do this work. I don't know whether you watch the TV program . . . , the . . . very nice talk. We don't like the media to join on this work, but the . . . and at the first immunization date. So, they volunteer It is the university student so they talk about this vaccine is properly . . . according to the knowledge so far about 80% is not effective. But, we want to be volunteer. For the objectives, we have the three

objectives in the clinical trials. The last objective is to . . . social impact . . . important because we must investigate the social impact and to decide whether to phase 2 or phase 3 can go on.

Question – My question to Dr. Xiaoyan Zhan. Actually, you did a very good presentation. But, my question is again about the Sendai virus. The Sendai virus actually is a murine derived virus and is a negative strain antiviral. My question is, because you said the virus is not . . . to humans so far, my question is, did you have any evidence to prove that this one would have not have any pathogenic . . . to humans in the future?

Zhan – I understand your concern. I think this was also the question for us. But, just based on the so far evidence, it didn't show any possibility of this. But, I think whenever you use a live attenuated virus and the vector and all kind of virus have the possibility to mutate into something else, especially for the . . . virus. So, no matter what kind of vector you used, have to be concerned this possibility.

Question - . . . **(not transcribed)**

Zhan – I think you bring a very good point and actually yesterday, just before the session, I talked to Dr. Atkinson about this and I mentioned virus is SARS, HIV, and the flu, but I think we still have to pay attention to some other viruses, especially the . . . virus. . . . and before SARS happened, this virus . . . and nobody care about it and very few research, but just overnight SARS break out. So, I think it is a caution. But, I think the live attenuated virus is a very good boost . . . because some virus like the flu and . . . could be a very good vaccine, but for some virus like HIV, if you just give a . . . , you will not see any antibody come out. So, I think you have to use something to boost these. So, it cannot be avoided and you have to use some live attenuated virus. I think Sendai is a good candidate, but I cannot 100% guarantee that something will happen one day.

Question – I had a question for Dr. Monto and I think a number of the other speakers would also be interested in weighing in on this one. By the way, thank you very much for your talk – I

thought that was one of the most concise and informative descriptions of avian influenza I've heard in the dozens of lectures I've heard in the last few months. But, the question I have for you in relation to the genomics revolution is where is molecular biology and genomics going to take us in the next number of years? What is the hope or the promise they afford us in terms of generalized global vaccines you mentioned, but also cell free and cell-based vaccines which you did not, I believe, get into so much? Or, if you prefer, you could also talk about the antivirals.

Monto – Well, I think right now in terms of the genomic revolution and influenza, we are sorting ourselves out. We are basically getting to a point that if we don't watch out, we are going to be drowning in data which we don't understand because we have a great deal of diversity. We really don't know what this diversity means and I'll give you a very direct example because we are in the process of conducting an NIH-sponsored clinical trial of an activated versus live attenuated vaccine, again seasonal influenza, and last year was a drifted year. As confirmed by both the genomic and the phenotypic characteristics, we find to our surprise that the inactivated vaccine worked quite well, which would not be predicted. So, I think we are on the road, but we are not there yet.

I think also down the road, we will be able to better tailor our vaccines, better select those viruses for the vaccine, assuming we don't have a universal vaccine which will produce the best protection against both vaccine-like and drifted strains. So, I think that is the direction we're going in.

I'm not sure about the cell-culture based platform. I think the cell-culture based platform is going to be at least initially just like the egg-based platform using the same kind of viruses and as a matter of fact, I think because of FDA regulations and the rest, they are going to be egg-based viruses because in order to have . . . and there are some issues about whether there are antigenic and genotypic changes that occur in egg adaptation. But, the trouble is getting the virus in with a history of being an acceptable cell substrate. So, it is going to be a long road.

The most exciting, and I think the most problematic in terms of whether we will be able to do it, approach now is the issue of universal vaccines – trying to go for something with a conserved

components. Many people will say if conserved components protected, we wouldn't have pandemics because the conserved components by definition are conserved and are still present in the pandemic virus, and yet we see very little protection. But, we have things that we don't understand yet.

One thing I did not show in 1918 is that if you look at the data carefully, older people were spared. If older people were spared, and this is a new virus, fully avian, where did that protection come from? We usually think it is from past experience from a similar virus, 60-80 years before, but we don't understand a lot. I think if we all have good communication, which has been emphasized, between the applied people like me, and the people working in the molecular genomics side, then we are really going to see some major advances in controlled influenza, which is tough now. Our current vaccine at 70-90% efficacy is a good vaccine; it's not a great vaccine. It is not 95%. We have a long way to go. I think now we are getting to tools to do this.

Moderator – I'd like to thank all of our speakers one more time. Now, please join us for a tea break and we will see you back here in 10 minutes.