

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 suspension 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 hemoglutin 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 antivirus. 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 androgenic 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.