The Science of Genomic Associations: Current Status and Future Directions
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DR. MANOLIO: Sure. Thank you very much. Good morning to you. You can see in your briefing books the questions that I was asked to address. This could probably take a week or so to go through.

[Laughter.]

DR. MANOLIO: I have but 20 minutes, so I will do my best.

This is an interesting time to be discussing this. I think we all recognize that as of, maybe, four or five years ago the genome was a pretty barren place when it came to associations with complex diseases. There were maybe a couple of associations that were known, actually through linkage studies. But it really wasn't until 2005 when this first finding on age-related macular degeneration was found by genome-wide association. In 2006 there were three more associations added, and then things really began to pick up. It has really been remarkable in the past year, 2007, and even into this year, to the point where we can barely get everything all on the same slide.

This has caused, in 2007, the general science to dub that year the Year of Genome-Wide Association Studies. This was the breakthrough of the year in human genetic variation.

This was based on, primarily, the HapMap, first building on the Human Genome Project and the sequence of the genome, and then the development of a haplotype map that basically showed the relationship between the various 10 million single nucleotide polymorphisms, or SNPs, the most common form of variation to date in the human genome, and the relationships among those so that one doesn't need to measure all 10 million of them but you can measure a smaller subset and then use that to infer relationships across the genome. The second generation map was just published last year.

The goals for the HapMap were to use just the density of SNPs needed to find associations between SNPs and disease, not to miss chromosomal regions with disease associations, and basically to make a tool to assist in finding genes, and really, what a tool it has been. Then, recognizing that one would need more SNPs for more complete coverage of populations such as those of recent African ancestry who have shorter stretches of linkages to equilibrium.

In parallel with the development of the HapMap, and in large part probably stimulated by it, the cost of genotyping has fallen dramatically. Shown here in this slide from my colleague Steven Chanock, in 2001 we thought we were getting a very good deal if we paid about $1 for a single genotype.

Those costs fell dramatically as the number of genotypes per test increased. In 2005 we were getting this for about a penny a genotype. Costs have continued to fall. This now is almost a two-year-old slide, and these costs now are at about the $400 range for about a million SNPs in a single individual. So, truly a remarkable reduction in cost.

This has led to a huge host, as I showed you on that previous slide, of diseases and traits that have had genome-wide associations done. As of last night there were 58. I haven't checked this morning, but there are probably another couple because they come out very, very rapidly.

This onslaught of information has been referred to as "drinking from the fire hose," from David Hunter and Peter Kraft, who observed that there have been few, if any, similar bursts of discovery in the history of medical research. I think we can all agree with this. This has really been an incredible time to be in genomics.

We have been trying to keep up with this at the Genome Institute. We have a genome-wide association catalog that is put together by my colleagues Lucia Hendorf and Heather Junkins. I look over their shoulders at times. You can find this on the genome website or you can just Google "GWAS catalog" and it should pop right up. This provides information on the study, the diseases under study, the number of people examined, the region of association, the gene, the odds ratios, P values, minor allele frequency, as much information as we can pull from these papers. This is just about a full-time job for two people to be able to keep up with this. It is really quite something.

But we would refer you to this if you are interested in knowing what are the most up-to-date findings. We are a little bit behind in keeping it updated.

In looking at this with the associations through about May, we were looking particularly at the SNPs that have been identified in terms of what they actually are or what they do in the genome. One would have expected, prior to about 2001, that most of these would have been in coding regions in the genome. In fact, there were big debates about one should only do the HapMap in the coding regions or one should only be sequencing in the coding regions.

In fact, the variance that one would have expected to be the most powerful in associations, those that change the amino acid that is used in putting together a protein, or that cause the protein to be truncated entirely, only 13, or about 6 percent, of these 284 were actually in those regions.

Another three of them were what are called synonymous SNPs, where there is no change in the amino acid but there may be a change in the speed with which that protein chain is produced.

Then, maybe about 40 percent of them [were] in the introns and not in particular splice regions where the exons are stuck together, but in the intronic regions of no known role.

Then, a few [are] in the untranslated regions of the messenger RNA, a few more in the five-prime promoter region of the genes and then the three-prime region that also may play a role in the speed of translation. Then, nearly half of them [are] in other or unknown regions. This, I think, has been one of the big surprises of this.

So, lessons learned from these initial studies. Genes that wouldn't have been on anybody's candidate gene list are now popping up for these diseases. Macular degeneration everyone thought was an ischemic disease. It is actually very strongly related to complement factor H, an inflammatory disease-related factor. Coronary disease with a cell cycle variant, actually previously related to melanoma. Childhood asthma, type II diabetes with a cell cycle gene, QT interval prolongation with nitric oxide synthase.

Prostate cancer has been shown to be in what is called a "gene desert." Finding after finding after finding, beginning with the Decode Group, showing associations in this region where there aren't any genes at all. Also, in Crohn's disease, a similar kind of thing, with multiple regions without known genes.

Then, signals in common across diseases. You might have thought that these two were similar, but boy, CHD and diabetes don't have a lot in common with melanoma and they all share this strong association. All these forms of breast cancer, Crohn's disease, and psoriasis are related to each other, but Crohn's disease and type I diabetes are not, and yet they share a strong association. At least they were not known to be. Rheumatoid arthritis and type I. So there are lots of surprises in here, and many more surprises to come.

In addressing the first question, what are the recent advances and how have these facilitated the emergence of personal genomic services, probably the low-cost, high-throughput genotyping is now within reach of large-scale population research studies. That has then generated all these incredible findings.

Over 150, probably more like 170 now, such studies are completed, with over 180 well replicated loci in nearly 60 diseases and traits. So, in just three years, really an unbelievable bounty of findings.

Genotyping costs are now also within the reach of at least perhaps well-to-do consumers. That has been a considerable change and one that we would not have seen just a few years ago.

Things that I don't have time to talk about but are on the horizon and you should keep on your radar screens are associations with copy number variants; next generation sequencing, which you will hear a little bit about, I suspect; the Thousand Genomes Project, an international project involving the NIH and many other groups to identify even rarer sequence variants and alleles that might be associated with disease.

Then, DNA methylation, epigenetic changes, catalogs of gene expression. All of these are things that probably will provide even more valuable information about genetic associations.

The second question I was asked to address is for which diseases are strong genetic associations and/or markers established. I guess I would turn back to you and say define "strong." One could have a lot of debates about what metric one should use. Is it a large odds ratio, so people who carry the risk allele have a five-fold increased risk of disease than people who don't carry it, or a two-fold, or a 20-fold.

Is it a very small P value. The association that you observe is very, very, very unlikely to have occurred by chance because, remember, we are testing millions of SNPs in many, many, many studies. So, is that a good metric.

Is it a risk allele that is very common. Instead of being 5 percent of the population or 10 percent, is it in 80 percent or 90 percent, as some of these variants are.

Is a large proportion of the disease attributable to the risk allele. There are ways of estimating this based on the prevalence of the risk allele and the odds ratio, the population attributable risk. There are certain assumptions that go into that that really don't apply to genome-wide case control studies. I won't go into it further, but you will see that metric used.

Or, do they explain a large proportion of the genetic variance and therefore would be expected to play a large role in the disease.

Let's look at a few of these metrics, again from the catalog. Here are the odds ratios that have been detected for a variety of variants, just a number of associations. You see that the vast

majority of them are below about an odds ratio of 1.3 to 1.4. The only reason this drops off here is that the power to detect these loci is very low. One needs very, very large sample sizes. Probably, this is a distribution that goes up through the ceiling at these low ranges.

But there are a few that are much larger than this and one or two that are considerably greater than that. Maybe those are ones that you would want to focus on.

Similarly, for P values, I have plotted here the negative log-10 of the P value. Here is the number of associations, here at 10-to-the-minus 10th. Most of them are at this level because 10-to-the-minus 7th is about what is generally used for genome-wide significance. But there are some that are less unlikely than that, even out to 10-to-the-minus 80 or 10-to-the-minus 100. Are those much more believable? Probably. They might be ones that you would want to focus on.

How about the frequency of the risk allele. You can see there is quite a range of these. Most of them cluster around the 30 to 40 or 20 to 40 percent range. Some of them are much rarer, but some are much more common. Some are associated with fairly large odds ratios. These, again, might be risk variants that would be particularly important to focus on.

I was asked for which diseases are there lots of genetic associations shown. Crohn's disease has probably been the big winner. This paper from Barrett, et al. in last week's Nature Genetics showed 32 Crohn's disease loci. Many of them do not have associated genes with them. You can get this from the paper much better than from my drawing, but it makes a visual impact.

In that paper they also show the proportion of variants explained, another metric that one could use. You can see that, again, the vast majority of these loci explain 0.1 to 0.2 percent of all of the genetic variability that one can estimate from family studies. That is not very much. There are a few that explain a little bit more, but you will also notice that this drops off a fair amount.

This dotted line is the power to detect risk loci. You will notice that it drops off quite steeply below about 0.1 percent of the variance explained. So, as we are able to detect more of the variance we probably will find more of the variants, with a T.

What those mean at the very low proportion of variance explained is, again, another matter for debate and may be very important in terms of identifying pathways or mechanisms or druggable targets, et cetera.

Next, I was asked what criteria should be used to determine whether associations between a marker and a phenotype is strong enough for the marker to be included in genetic testing. Here again we have that "strong enough" that I was stumbling over previously.

Because we are in the District, perhaps like a politician I will say I don't like this question. I would rather answer a question that I would pose.

[Laughter.]

DR. MANOLIO: What criteria should be considered in determining whether a particular variant should be included in genetic testing. Then, also like a politician, I won't answer it but say that it depends to a very large degree on the purpose of the testing.

So, what would the purposes of genetic testing be. You will say, silly thing, of course what we want to do is improve health and prevent disease, but how best to do that. One could provide

targeted, proven risk reduction strategies to those identified to be at greatest risk. We currently do this with non-genetic factors. It makes sense to do it with genetics.

We could identify persons at high risk for later rapid implementation of newly proven interventions. This is being done in eye disease. We don't yet have interventions for these diseases, but wouldn't it be wonderful if you had these people already identified and could just pull them in and start giving them whatever intervention was necessary as soon as you had proven it.

To improve the cost efficiency of non-genetic risk reduction strategies. So, other ways of reducing risk may be targeted in people at higher genetic risk.

Possibly to facilitate reproductive choices, or even to provide information that may be of personal value to individuals regardless of whether we might consider that to be actionable or valid or useful. People can make their own choices, and that may be an appropriate reason as well.

I'm sure many people around this table could provide much better criteria than I could, but just to throw some things out for you in terms of things to consider, obviously here we are at strength again. But, whatever the strength of the evidence is for an association with risk.

Availability and acceptability of proven risk reduction interventions. If you don't have a way of reducing this risk, one can debate how valuable it is to identify it, although there are those who would argue it still is useful.

The validity, availability, and cost of the test. The potential anxiety, stigma, cost, additional testing, or other harms from receiving the results to an individual. The confusion that it may give to their physician, et cetera.

Trade-offs in other testing or care that cannot be paid for within a fixed budget. So if we paid for this kind of testing or this kind of targeted work, we may not be able to pay for something else.

This was explored in great depth in a recent paper by Paul Feero, et al., from the U.K., where they do have, clearly, a fixed budget for health care, in the recent New England Journal. [It shows] what they estimated to be the distribution of genetic risk in the U.K. population for breast cancer using only seven known loci related to breast cancer, which explains, actually, less than 10 percent of disease. But that is not bad when it comes to these complex diseases.

What they estimated was taking those seven genes, you have two alleles at each locus. Basically, you could have 2,200 different combinations of genotypes of those three genotypes in each of those seven loci.

You could look at women who have none of the risk alleles at any of those seven loci, and they would be at this lower end of the distribution of risk. For those who don't think in logs, this is a relative risk of 0.4. So, in the U.K., the risk of breast cancer for women at age 50 within 10 years is about 23 per thousand women. For these women, it would be 40 percent of that, or about 10 or 9 per thousand women. There are only about 60 such women per 10 million in the U.K. population. This is somewhat population-specific because of allele frequency differences.

At the other end of the distribution, if you had the risk alleles for all seven, two copies of the risk allele at all seven loci, you would have a relative risk of about 2.5, which means that instead of a

23 per thousand risk you would be at about a 60 per thousand risk, which is a considerable increase. Only about seven to 10 women would be in this group according to these estimates.

What is shown here is a nice estimate. This heavy black line is based on the current loci. One could identify 50 percent in the highest risk group. So, here is the proportion of the population based on genetic risk. It would include about 60 percent of the cases. Down here at 20 percent of the highest risk would include about 28 percent of the cases. So you have almost enriched your population by about 50 percent.

Interestingly, they project if you were able to identify all of the genetic loci that were associated with disease, you actually in the top 50 percent would have 88 percent of the cases. In the top 20 percent you would have 64 percent of the cases, a three-fold enrichment. So you could really do quite well in terms of targeting screening.

Then, how could one improve the efficiency of these screening strategies. A 50-year-old woman in the U.K., as I said, has a 2.3 percent risk, or 23 in 1,000, of breast cancer in the next 10 years. Currently, the U.K. recommendations are to offer mammography to all women over 50 each year.

One could say perhaps one should offer screening to all women at that particular risk level and possibly not to offer screening to women who are not at that risk level.

Women in the 40th percentile of current risk, just based on those seven loci, have a 10-year risk at age 50 of 2.1 percent. Now, this risk is somewhat age-dependent. So, maybe you don't want to test them at age 50. You might want to wait until age 60 or age 55, or whatever.

This one is not all that different, though, from 2.3, so you might not get excited about that. But how about the women in the 5th percentile population risk. They have a 10-year risk of 1.5 percent, and they actually never reach a 2.3 percent risk because they die from other things they are at such low risk. At least these are all estimates, and the kind of estimates that one could expect to see.

This, again, is based on only seven loci. Would one not offer mammography to these women? Again, a difficult decision to make but one that we should be considering if what we are talking about is targeted screening.

Lastly, what are the limitations in risk assessment for disease. Most markers, I think as you have heard repeatedly, are not deterministic. Many people who don't have the markers will develop the disease, and many people who do have the markers will not develop the disease.

Much of the genetic risk remains unexplained. At best, we are getting about 10 percent of the variance explained in many of these complex diseases. In many diseases we are not even close to that.

Plus, there is little or no evidence to date that interventions based on genotype will actually improve outcome. We need that kind of evidence.

Genetic markers may, though, provide additional risk information so that you could target more aggressive risk management and carriers of those variants. But again, there is little evidence to support this.

Some have likened this kind of risk information to a cholesterol level. They carry about the same risk, 1.3 to 1.5 in some cases. One would never not measure a cholesterol level even if you knew many other risk factors. It is something that would be, perhaps, equally useful to do with genetics.

Remember that cholesterol is quite different. There is a huge body of evidence of the effectiveness of cholesterol lowering in preventing heart disease. In just a smattering of the recent papers plus the most recent, the third adult treatment panel, the consensus panel in the U.S. and many consensus panels abroad, this evidence took 30 to 40 years to put together. Do we want to wait that long to develop that kind of evidence for genetic variance. Probably not. Do we want to bypass this step entirely. Also, probably not.

So, what kind of research is needed. There is lots. I think we will be talking much more about that today. This is just one example. It is cited on page 8 of your brief. The multiplex initiative from my colleagues at NHGRI, Collene McBride and Larry Brody, is designed to test a number of risk variants, about 15 or so, for common complex diseases in basically healthy people and then provide that risk information back to these folks and see what changes they make in their lifestyle, their health behaviors, et cetera, and also to create an infrastructure to facilitate this kind of research.

I realize that you are probably used to researchers coming to you and saying what we need is more research, and that does seem a little self-serving. Indeed it is. But we have to recognize we are very early in this technology. Like this early microscope, it is a mammoth. We have a fair amount to do before we learn how to refine these techniques and to apply them so that we can improve health and prevent disease. Thank you.

[Applause.]

Question-and-Answer Session

MS. AU: Thank you, Dr. Manolio. Do we have questions from the Committee? Julio.

DR. LICINIO: I have a question. I think this type of work is extremely important of course, and I think coming up with new candidates is fundamental. One thing that people often forget is that in the issue of cholesterol lowering the drugs were discovered through work in very specific families that were very rare. From those rare genetic findings you can generate the most commonly used drugs in all of medicine that apply to everybody, mostly people who don't have the genetic problem. So I think discovering new targets is crucial.

But I think what people out there don't understand very much, and I wonder how we can improve that, is the concept of risk. If you have a 2 percent higher or lower risk of having diabetes or having higher weight or whatever, you could have that genetic component and not have the risk at all. If you have a 1 percent chance of dying as a result of something, you could be the one in 100 and die or you could be one of the 99. This gives a prediction.

It is very hard to communicate that to people and for people to understand it. We had another meeting yesterday that I'm sure you are all aware of. Someone came from the audience. The person got tested in one of these consumer-based genetic companies and was told that she had a very low risk of colon cancer, but her father had colon cancer, she had the polyps, and she went on to take care of herself and look at that very closely as opposed to just ignoring it.

So you could have a 1 percent risk of developing something and you could develop it, and you could have a 99 percent risk of developing something and be that one person that doesn't have it. How does that get transmitted to patients, to doctors, and to the healthcare system.

DR. MANOLIO: I don't have a good answer for that. I'm a cardiovascular epidemiologist. We were just thrilled in the Framingham Study when we came up with a risk score that would tell people that you are not just at the usual 0.5 or 1 percent risk of disease, you are at 10 percent risk of disease, we need to do something about that. People would say, "Well, that is only one in 10. Heck, what does that mean?"

There are others around the table that I think can probably comment on that better than I.

DR. KHOURY: I wanted to ask a question, and this a very clear expose, I guess, of the field of genetic associations and the GWAS era right now. Thank you for your comments.

I want to make one comment and then ask a question. I think what I heard you say is that the low P values we are seeing and the low odds ratios are not necessarily translating into clinical validation yet because we have predictive values, probabilistic information, increase your risk and decrease by a certain amount.

More importantly, I think I heard you say something about clinical utility. If you take the example of cholesterol, which took years in the making before widespread population screening was adopted, I think you said do we want to wait that long? Perhaps not. But, do we want to implement right away? Perhaps not. So, where is that balance? That is the first question.

The second one is, in your estimation where does the field of genetic association and its clinical utility lie compared to more traditional ways of stratifying risks such as using traditional risk factors and/or family history.

I can cite you data from [the] cardiovascular [field.] In the State of Utah, people like Roger Williams many years ago found that 15 percent of the families in Utah cluster about 50 percent of all cases of heart attacks in the whole State of Utah based on their genealogies, and they didn't do any genetic testing. It was only based on a family history score.

So, what is the value added of genes vis-a-vis pure family history and other existing risk factors that we know of today?

DR. MANOLIO: That is a critical question, Muin. Nobody really has looked at that very well. There have been a couple of estimates in diabetes that I'm aware of looking at the proportion of disease that is explained by genes. At that time only two or three were known. It may be a 58 percent area under a receiver-operator curve, which you are familiar with. Basically, 50 percent is dead even. You don't do much better than chance. Fifty-eight percent is not all that much better than that. If you use things like age, sex, BMI, and family history, you get it up to 88 percent, and no genetic information at all.

Family history for Crohn's disease. If you have a first-year relative with Crohn's disease you are at a 25- to 30-fold increased risk. Do you need genetic testing if you have a family history of that. Perhaps not. On the other hand, as the example of cholesterol illustrated, the LDL receptor variants that were identified in people with familial hypercholesterolemia are in less than 1 percent of the population.

If you did a genome-wide association study, you probably wouldn't even see that, or it would be at the 1.05 level. But in certain people those are very high-risk alleles and they lead to a pathway or a mechanism that then works for everybody, except, ironically, in the people who are homozygous for that defect, when the drugs don't work at all.

So there are trade-offs here that one has to make. But I agree; the research really needs to be done to prove that this information adds to what we currently know.

MS. AU: Paul.

DR. BILLINGS: Thank you. I thought that was very interesting. I wanted to pick up on one line of argument that you raised, and it is colored by my experience during my training over the battles at establishing mammographic standards for screening of women.

So, as I understand your argument, it is conceivable that there would be a recommendation not to provide mammographic screening for a subset of women who might have none of the risk alleles that were identified. What kind of study would have to be done to undo what is a blanket and relatively well evidence-based standard of mammographic screening that is now recommended for all women above the age of 50, let's say?

DR. MANOLIO: No, it is an interesting question and a very challenging one. Probably, the only way really to nail that would be a randomized trial, where you screen some women and you don't screen others based on their genetic variants. That would be a very large trial, and it would take a long time to conduct.

Could one do this from observational data. Probably not in the U.S. because mammography still is not universally applied. It is something that goes along with a whole host of other health behaviors. There may be other places where mammography really is universally applied. In the military, possibly, or in other controlled populations in the U.K. or Canada or other places where the healthcare system is really much more organized and standardized. Those might be places to do it. But, really, probably a randomized trial is what would convince most people.

Please understand, I'm not advocating that we not screen people based on their genetic risk. I'm just saying that that is one possible conclusion you could draw.

MS. AU: Any other questions? Francis.

DR. COLLINS: Just one comment in terms of the state of the art at the present time. Teri has nicely summarized what this deluge of discovery has offered us in the last couple of years, which is enormously exciting in terms of the insight it provides in terms of pathways involved in disease that we really didn't suspect, with most of these loci being completely unexpected in terms of exactly what their function is. It opens up entirely new directions in terms of therapeutics, which is a wonderful aspect of all of this.

But in terms of the heritability, as Teri has said, we actually are not yet in the position of being able to identify more than a small percentage of the heritability even for a disease where we have several loci identified. When you add it all up, there is still a huge missing heritability factor in there. There is much debate about exactly where is all the rest of that.

Is it going to turn out that those are rare alleles of large effect which you would not find by a genome-wide association study but you would find by sequencing. Much effort is going into applying sequencing for just that purpose.

Are these copy number variants, which are not particularly well assayed by the SNP chips that most people are using. These are, of course, large segments of DNA that may include entire genes or even whole sets of genes that are present in more or fewer copies depending upon your particular inherited version of that copy number variant. You can imagine that could have pretty interesting effects. People are rigorously now trying to look at that, although the technology is still tricky, to be able to say you have scanned the whole genome for those things.

Have we missed on some kind of gene-gene interaction so that individual effects don't look that impressive but if you actually had enough power you would be able to see that when you have a combination of a certain set of risk alleles it is not just additive. Things actually go substantially higher. So far, really not much evidence for the people that have looked at it to show that.

But I think it is fair to predict that in the next couple of years this whole question of where the rest of the heritability is, is going to get pushed pretty hard. As this Committee is deliberating about where this is going, you should expect that that percent that is accountable is going to go up. It is going to go up to a degree whereas, in the very nice example of the Feero, et al. study in the New England Journal, you are going to start to see that curve rising up more and more in the direction of being able to make more and more predictions about the place where the risk is most apparent.

This is obviously a bit of an unpredictable trajectory scientifically, but I think it is fair to say that is the direction we are. Whatever plans people are thinking about making in terms of the application of this to public health are going to have to be integrated with the sense that this is a moving target and that there is a lot more information just around the corner.

We are just starting down a path that is likely to have all kinds of interesting twists and turns and put us, potentially, in a few more years, in a more powerful position to make those predictions than the rather weak evidence that we have right now with these small odds ratios only adding up to a few percent of the heritability. That is going to change.

DR. TEUTSCH: I think our last question goes to Mike.

DR. AMOS: Teri, thank you very much for that. I think it is really important to get beyond the press and get to the real science of the issues.

Steve and I were at a conference last year at the Mayo Clinic where you gave part of this presentation. You have added a lot to it. I don't mean to put you on the spot, but somebody asked you the question, is it even worth doing whole genome analysis studies anymore. You said you guys were thinking about that a little bit.

Where is your thinking now, and where does it fit into the context of the broader research? I see this as a part of the complete disease signature. History, physical, and all those things come into play, but also many other biochemical and anatomical parameters.

DR. MANOLIO: Sure. Genome-wide association is all the rage, but it really is today's technology. Probably, tomorrow's technology is whole-genome sequencing, and that is really coming within reach, as you will hear from some of the later speakers.

My personal prediction, and there are others who share this view, is that genome-wide association will probably not be the tool of choice for research and discovery in the next few years, maybe five or so, maybe a little bit longer than that. In whole genome sequencing one gets the entire genome. Now one has to figure out how to analyze the entire genome, and we are not quite there yet.

But it may be of great value in terms of assessing an individual's risk not just for one disease but for, maybe, a hundred diseases. If you look at multiple diseases across an entire individual, you are probably going to find each of us is going to be at risk for at least one, probably three or five. We may be at very high risk for those diseases. Wouldn't you like to know that. You can probably capture that with a genome-wide association study without doing whole genome sequencing.

But, as Francis pointed out, things are changing very, very rapidly. We will just have to see where it goes.

MS. AU: Thank you, Dr. Manolio.