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ALPHA-1 FOUNDATION

ALPHA-1 ANTITRYPSIN THERAPEUTICS DEVELOPMENT WORKSHOP

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Welcome and Introductory Remarks:

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Research
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ALPHA-1 FOUNDATION Representative:

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Update and Summary of Alpha-1 Therapeutics:

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The Patient Journey:

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Novel Targets for Alpha-1 Antitrypsin:

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Novel Therapeutic Approaches That Raise Levels of Alpha-1 Antitrypsin:

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PARTICIPANTS (CONT'D):

Implications for Other Plasma-Based Therapies:

SENATOR RICK SANTORUM
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Therapeutic Approaches to Liver Disease in AATD:

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Clinical Endpoints and Biomarkers in COPD Trials:

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CT Densitometry:

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Panel Discussion: Current Needs in Alpha-1 Antitrypsin Drug Development:

PETER MARKS, MD, Ph.D., Moderator
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Summary and Conclusion/Next Steps:

PETER MARKS, MD, Ph.D.
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P R O C E E D I N G S

(8:33 a.m.)

DR. MARKS: Good morning, everyone. Thank you everyone who is here at NIH at the auditorium and thank you everyone who is joining us on the web today. I am Peter Marks, Director of the Center for Biologics and Evaluation and Research at FDA and I want to welcome you to the Alpha-1 antitrypsin therapeutics development workshop. This is a workshop that is being co-sponsored by the Center for Biologics and Evaluation Research at FDA, The National Heart Fund and Blood Institute at NIH and the Alpha-1 Foundation. We are going to try to keep introductions relatively short.

Something I once learned is that running the trains on time at a meeting like this counts a lot so we are going to try to do that. You'll see me making wild motions at speakers if

they start to run over time. You'll see me with my hook if they really run overtime so if you ever see me get up and start approaching the stage as a speaker, it means you are really over time and that will be your cue to sum up.

So just to do a few housekeeping remarks before I say a couple of opening remarks, for anyone who needs them, there are restrooms just outside the doors in the back on both sides. Lunch today, there will be box lunches out in the back just outside the doors. You can take them up to the first floor where there is some seating. We ask that you do not have any open containers in this auditorium, regardless of what's inside those containers. Thanks for honoring that. And with that said, I just want to thank everyone for taking the time today. We have a really nice turnout from industry, from academics and patients as well so we are very happy to have this workshop.

I think from the FDA perspective, one of the really interesting things and I think it will be a really great discussion to have today is that Alpha-1 antitrypsin deficiency represents

kind of a model disease where the end points are quite challenging to get at in studies and yet it's really a model for any number of diseases where we have to find ways to do clinical development efficiently to come and help patients so we are really looking forward to discussions today.

We have a wide range of different topics and a transcript is being made of today's discussions, so you'll be able to refer to it after the meeting and I just want to thank you again for coming today. I am going to introduce Dr. James Kiley from NHLBI to say a couple of opening remarks and then Miriam O'Day from the Alpha-1 Foundation will say some opening remarks and we will try to keep things moving very much on time or ahead of schedule. We know we have a package and we'll get through it. Thanks very much. (Applause)

DR. KILEY: Okay, well I don't want to get on Peter's bad side just to get started and get him up and activated here right at the beginning but let me just extend a warm welcome to all of you from the NIH. You are on the main

campus of NIH, if you haven't figured that out and we are very, very pleased to have an opportunity to co-host this event, this meeting, with Alpha-1 and with the FDA. It's a really important meeting.

I think one of the first challenges, whenever you come up with a topic area for a meeting is to bring all the relevant stakeholders together and so we've made a really great first stride towards doing that, great attendance here. I see a lot of familiar faces but a lot of people that we don't know and that's really even better because now we can bring together a critical mass of people to address this topic and this is a critically important area. Looking for advances in how do we bring new therapy forward. I want to say for Alpha-1 but also for lung diseases as a whole. National Heartland and Blood Institute, Division of Blood Diseases has had a long history of supporting research in this area. Not just COPD but certainly around Alpha-1 antitrypsin deficiency.

We would like to be able to continue that so we look forward to hearing the dialog, the

exchange and all the information that may come from this and what I sort of urge you to do is to not lose sight of the fact that if there is research, if there are questions, if there are hypotheses that still require some additional data, more answers, it's important that we hear those because that's our lane, that's our swim lane, the research end of this.

So we are certainly very, very pleased to partners with FDA, to talk with all of you, to see you here, to hear the recommendations from this and hopefully there will be some follow up that will occur, whether it's at the FDA regulatory phase or whether it's really at the phase of identifying the research and bringing those forward so that we can have a role to continue to support it. We'd like to begin to sort of think about cures.

We'd like to be able to think about where we can really invest and advance the science so we can begin to start talking about cures and not so much palliative approaches. So, again, thank you all for coming. We are very, very pleased to see you all here. I now it's a very

busy time for all of you. You've taken time out of your schedule to come here and participate in this, so we thank you very much. Please feel free to be open with your comments. I know you're in a formal setting here; you're in NIH but this is a time that all of you should be speaking up and telling us what you are thinking about in terms of this space.

So again, warm welcomes, thank you. Hopefully you'll have a really productive day. We'll be here most of the day and we look forward to hearing the recommendations so thanks again. (Applause)

MS. O'DAY: Good morning. I am absolutely thrilled to be here and to be in full partnership with FDA and NHLBI and I want to thank Dr. Marks specifically for the invitation to have this workshop and to Dr. Kiley in NHLBI for supporting us.

You know, today is a day that could have great impact on patients with Alpha-1 antitrypsin deficiency. This is a rare, orphan disease. There are about 10,000 individuals who are identified and it's thrilling that we are here

with our industry partners, with researchers, with physicians, with the government and also with patients to be able to have a thorough discussion about next generation and new therapies.

We are in a very privileged position as the Alpha-1 foundation and the Alpha-1 community to be able to talk directly to decision makers and so thank you to everyone who took the time to be here today and thank you to the FDA and NHLBI for being in partnership with the foundation and giving us this opportunity. We are really excited about the day. Thank you. (Applause)

DR. MARKS: Okay, before I introduce our first speaker, I want to just make one more note of thanks that I missed doing before which is that today's workshop would not be possible without the work of the people who actually put it together that did actually the real work. We are lucky enough to just have to show up, but I need to thank both the folks from the Alpha-1 Foundation, Adriana De Arce who worked with the personal side, Debra Ellison to do large lion's share of getting coordinating done.

I also have to thank other staff members, Irene Caroll, Ann Bishop, Kai Orloff, Sherri Revell and Lonnie Warren-Anderson, all of whom really worked together to make this happen. When we started out, we did not have a venue and we now have a very nice venue and I think we'll have a wonderful workshop today so thank you very much.

Without further ado, I want to introduce Dr. Robert Sandhaus who is a professor of medicine at National Jewish Health and he is going to talk to us about Alpha-1 antitrypsin deficiency and augmentation therapy. (Applause).

DR. SANDHAUS: Thank you very much for inviting me to present here. I am going to do a very different kind of presentation than I would normally do. I am not going to do the whole spiel about Alpha-1 antitrypsin deficiency and its treatment with pictures of my son and things like that that have been in my slides for decades.

There are many people who have seen it too many times and, to those of you who are new to the community, I encourage you to listen to the

a lecture by Mark Brantley, myself, Charlie Strange, Gerry McElvaney or, if you're lucky enough, Gerry Turino, to get a more detailed view.

I want to really concentrate on the things that will set the stage for today's discussions. I am going to briefly outline the history of both Alpha-1 antitrypsin deficiency and its treatment with augmentation therapy, the reasons to focus on augmentation therapy for part of this discussion, problems with focusing on augmentation therapy, remind people that Alpha-1 antitrypsin deficiency is not only a lung disease but has other manifestations that are quite important to the patients that have them. And, in ending, talk about, the impasses to direct development posed by a rare genetic condition that causes slowly accumulating morbidity and mortality.

So, my one page history of Alpha-1 antitrypsin deficiency. As most of you know first described in Sweden by Eriksson and Laurell and that description included its association with familial emphysema and so lung disease has always been top in the consciousness of people who are

working in those early days with Alpha-1 antitrypsin deficiency.

By 1970, we had an appreciation that elastolytic proteases could lead to emphysema in laboratory animals. We have the first descriptions of human neutrophil elastase being able to cause these same changes in animals and that the fact that human neutrophil elastase was inhibited by Alpha-1 antitrypsin protein. And we also had an appreciation that the liver was a target as well as the lung.

By 1990, we had an understanding that the plasma deficiency of Alpha-1 antitrypsin protein was related to protein misfolding and polymerization within the hepatocytes of the liver and that that polymerization and accumulation of antitrypsin protein in the liver was a likely cause or at least contributor to the liver disease of Alpha-1.

At the NIH pulmonary division, the group there identified that augmentation of the circulating Alpha-1 protein might be of benefit to patients with lung disease due to Alpha-1 and they developed a purification process from human

plasma of this protein that was handed off essentially to the plasma industry and the first augmentation therapy was approved for marketing by the FDA based on biochemical efficacy — acceptable safety and acceptable blood levels and lung levels of the circulating alpha-1 antitrypsin protein. And in association with that initial marketing approval, the NIH cosponsored a registry of Alpha-1 antitrypsin patients to look at the natural history of lung disease and liver disease in Alpha-1 antitrypsin deficiency. 1,129 patients were enrolled, each followed for five years with repeat testing of lung function and liver function and there have been many publications that have come out of that NIH registry.

Augmentation therapy itself -- the history is that a single product was approved for marketing in December of 1987. That remained the only augmentation therapy product we had through 2003. It was administered intravenously on a weekly basis and was well accepted over the years that it was the drug of choice because it was the only drug we had.

In 2003, two additional plasma-derived Alpha-1 antitrypsin augmentation therapy products were approved based on a relatively small, non-inferiority biochemical efficacy and safety studies, and then a final fourth product entered the market using the same criteria for approval in 2010.

Clinical efficacy has always been the goal — the documentation of clinical efficacy. It has been documented in case-controlled study, several small randomized placebo controlled studies and one well powered placebo controlled study that unfortunately failed to meet its prespecified primary end point and I'll mention that bit and Ken Chapman will expand on that in his presentation about CT densitometry.

That trial, the RAPID trial, used CT densitometry as the primary end point to calculate loss of lung tissues due to pulmonary emphysema. It took a decade to enroll during which time the techniques for analyzing CT densitometry had progressed. The prespecified end point, which was combined CT densitometry at total lung capacity and at functional residual

capacity. By the time the study was unblinded this was not the preferred method for analysis, and I'll leave the rest of that story to Dr. Chapman.

Probably the most -- what I hope you will find the most interesting — in my talk is that we have completed the survival study -- excuse me, comparing matched Alpha-1 antitrypsin deficient patients with lung disease in the US and the UK.

The US has augmentation therapy approved; UK has augmentation therapy approved but not funded and so that -- individuals in the UK with their healthcare provided by the National Health Service can't get augmentation therapy and so the vast majority of patients diagnosed with lung disease due to Alpha-1 antitrypsin deficiency in the UK are not receiving augmentation therapy. The only difference in the matching of patients between the United States and UK is that the US patients all receive augmentation therapy in this analysis and matched patients from the UK were identified with similar characteristics who were not on augmentation therapy. We matched based on age, sex, lung function at diagnosis and lung function at the

time augmentation therapy would have been started or was started, genotype, and each of the patients in the group had been followed for at least 15 years or until death or lung transplant.

And our data sharing for this, which took two years to arrange conformed with both US and UK regulations, and you'll see how that affects the results I am going to present. So, I am going to show you two slides from this study. This study is under peer review right now so the data should be considered preliminary and may change.

Clearly, there are a lot of limitations to comparing the healthcare and the outcomes in two different health systems. We tried to minimize the differences as much as we can and I will say that with each of us having -- in the case of the US population, we had close to 7,000 patients to choose from for our matching. The UK had about half that number, and we were able to identify, as you'll see, about 650 patients on each side that met our matching criteria.

So, for the first slide, I'll show you a Kaplan-Meier survival curve over 16 years

comparing matched patients on augmentation therapy in blue with the control group, not on augmentation therapy in red. The line represents the mean. The shaded areas represent the 95 percent confidence interval and I think that the first thing you'll notice is that there's a divergence over the years in the survival in the group on augmentation therapy that reaches statistical significance by seven years.

It's highly statistically significant but I would point out the things that this tells us. It tells us that in order to do a survival study in Alpha-1, we have to follow between 500 and 1,000 patients per group over at least seven years in order to see this divergence in a statistically significant way in Alpha-1 antitrypsin deficiency.

It certainly is encouraging that the therapy appears to have benefit to patients but it also means that -- it reflects the fact that clinical endpoints in Alpha-1 antitrypsin deficiency, a rare disease, are very difficult to analyzing real world situations.

I'll show you one additional slide that

has even more caveats associated with it. This is the time to lung transplant between the two groups. Obviously, this adds the limitations that lung transplantation criteria and wait times and things like that are not the same in the US and in the UK but it is tantalizing to suggest that there is a significant delay in the need for lung transplants.

This is a smaller group of patients, with 56 per group, than we just saw because this is only patients who eventually received a lung transplant. You can see that the control group dropped to zero survival by 14 years in terms of survival being lung transplantation, whereas the US group still has far to go before all the patients that were eventually transplanted will wind up getting transplanted.

So, I hope you find this preliminary data to be stimulating, interesting, but also that it shows how difficult it could be to get the kind of meaningful clinical end point such as survival in a population whose lung disease -- in this case, lung disease progresses at such a slow rate.

I am actually ready to end my presentation and my goal is not only to get on to the more interesting and important discussions but also to leave as much time at the end for discussion, which is probably where the most information will be displayed but I do want to end with two additional slides.

When I summarize what I know, of other therapies moving their way through to treat Alpha-1 antitrypsin deficiency both lung and liver disease. There are other companies interested in moving forward with plasma derived therapies, both intravenously and inhaled. There are recombinant protein therapies that are moving into clinical trials or have moved to clinical trials. There are orally bio-available neutrophil elastase inhibitors that have been a long time coming but are now moving into the clinical trial arena in humans. There are liver directed therapies that include gene silencing molecules, protein folding and chaperone modifiers, gene therapies and potentially gene correction – and potentially many more. But the path to approval is quite difficult in Alpha-1

because of how well we have enrolled studies for rare disease, many times, investigators and companies feel that unreasonable expectations are placed on this rare condition. That the requirement for well-defined clinical end points can be very difficult in a disease state that progresses so slowly, and it's a rare disease. Wide acceptance of augmentation therapy, at least in the United States, as an effective therapy by both patients and physicians, makes placebo controlled trials quite difficult. And I think that when the final product of the mortality study is enrolled, I think that doubly so. And I believe that acceptance of augmentation therapy is not misplaced.

So, my view of the main areas of focus today includes the request by patients that we develop trial designs that don't include a placebo. Ways to do that: we have to have really creative study designs. Perhaps with the use of historical data, depending on the end point, or comparison of new drugs with existing therapy as opposed to placebo. Regulatory acceptance that augmentation therapy is clinically effective is

the only way we could use current augmentation therapy as the comparative and so far that's not the case.

In 2004, thanks to a joint liaison committee that was meeting — originally organized by Miriam O'Day — Industry, FDA, Alpha-1 Foundation got together to talk about novel end points for clinical trials, and both the drug division and the biologics division of the FDA accepted CT Densitometry as a clinical end point for emphysema directed studies in appropriate clinical trial designs.

That's kind of fallen off the list as an acceptable primary end point and we'd love to see that -- I know many people in the audience would love to see that — reinstated as a potential primary end point for drug approval.

It would also be attractive to evaluate and find appropriate biomarkers that could be used for the approval of therapies for lung and liver disease. This is a kind of a double-edged sword. Most biomarker evaluations have to -- are required to evaluate thousands and thousands of patients within various states of disease in

order to document the applicability of a particular biomarker and we are hopeful that with novel evaluation of biomarkers and using the data that we have, it might be possible to identify important biomarkers that reflect clinical change in Alpha-1 patients.

And with that, I am going to end my presentation and move on to the next. Thank you very much. (Applause).

DR. MARKS: Okay, thanks to people for staying way ahead of schedule. We have time for a couple of questions if there are any in the audience if someone wants to go to the microphone.

MR. PIERCE: Ross Pierce. The comparison between the rates of death in the UK and in the US, did you also control for smoking and past smoking?

MR. SANDHAUS: Yes. We did not match the patients for smoking, but we equalized the smokers and the non-smokers. I think we'll probably be asked to match based on smoking history in the review process and we can do that. [Note added in editing: The study did, in fact, match based on smoking history.]

I have to -- I can talk about other caveats that we could not match based on FEV-1 because a significant portion of the US patients did have FEV-1 because they were followed by physicians around the country whereas all the subjects in the UK are followed by the remarkably proficient and well-versed team of Rob Stockley's at the University of Birmingham. We actually thought that ding the results of the study since all of the patients in the UK are followed at a well-established Alpha-1 treatment center whereas the patients in the US were followed by physicians around the country, many of whom had only a single Alpha-1 patient as the only Alpha-1 patient they were following so we had -- we felt we might be weighing the slide in favor of the UK. So, I was glad to see the results that we found. Any other questions? Thank you very much. (Applause)

DR. MARKS: So, with that, I am going to invite Dr. Charlie Strange and Miriam O'Day to tell us about the Alpha-1 Foundation scientific infrastructure. Thanks very much.

MS. ODAY: We are thrilled to be here and talk about the Alpha-1 Foundation and its role

in therapeutic development and trying to advance therapies for individuals. Dr. Charlie Strange has a list of disclosures. Personally, I have no disclosures, but I would note that the Foundation does receive support from industry for its programs.

In 1995, three individuals with Alpha-1 antitrypsin deficiency -- these individuals who refer to themselves as Alphas, established the Foundation with the primary objective of funding research to find a cure. They eventually merged the foundation with the patient organization and so now our mission is two-fold. It's to find the cure, fund research headed in that direction and also support the individuals that are affected with this disease and we do that through support, education, and advocacy.

We have a research registry and you heard Sandy introduce the registry that was run at the NIH. That registry then moved over to the University of Miami and then the Medical University of South Carolina and you'll hear more from Dr. Strange about the research registry and

some of the registry products that have been developed at MUSC, some of which are moving over to the Alpha-1 Foundation offices themselves.

We understand that there are a number of different types of registries and the foundation has its own IRB review of its own research protocol. Our PI for this project is Dr. Janine D'Armiento and we will be collecting contact registry information and clinical information. Hopefully we will be able to link in the future with other registries or databases.

We have a number of assets and resources, that we have paid to develop, and you'll hear more about that from Dr. Strange. The idea was in a rare disease, there were two things that John Walsh, our founder wanted to do. Through the establishment of Alpha Net, which is our sister organization, also a non-profit that provides disease management, John wanted to be able to come together and define what best practices were in the area of Alpha-1. As you know, there are about 7,000 rare diseases, the majority of which do not have FDA approved therapies but Alpha-1 is very fortunate and the

fact that you heard from Sandy, we have a number of therapies that have been FDA approved and that we consider efficacious but we also have a whole host of therapies that are on the horizon for us.

The work of Alpha Net was to put together a disease management program so we know best practices in this rare disease and for a rare disease, especially an ultra-rare disease like Alpha-1, this is significant because across the country, if you are a pulmonologist, you may, in your career, see one, two, three, maybe five patients that have Alpha-1 if you are testing for it and you can go through our open source data and find out what the best practices are all the way through the treatment and care of Alpha-1 including end stage, which as Dr. Sandhaus mentioned, is transplantation.

The Foundation does professional and patient education. We have established a DNA and tissue bank. We have a translational research lab. Clinical Resource Centers, there are about 80 of those centers around the country. One of our newest assets that we hope will advance therapeutic development is a partnership that we

are putting together through some of our clinical resource centers to create a biomarkers consortium.

Our clinical resource centers are dedicated and committed key opinion leaders. We have over 100 physicians at 80 sites and 26 of those are CTSA certified so we plan to expand our clinical resource centers into a therapeutic development network and the first step in that direction, as I mentioned is our biomarkers consortium. We'll be moving forward with a therapeutic development network, a cohort that will be available to expedite clinical trials.

To date, the Foundation has invested over 76 million dollars in medical research at 116 institutions and that's international. Our investments are peer reviewed grants. We go through an NIH style peer review on an annual basis and that accounts for over a million dollars' worth of grants. Our resources, as I mentioned, include our registry, a coded testing study, DNA and tissue bank, our biomaterials exchange.

We also have a series of critical issue workshops, and what we have done is we have found questions that are imminent, critical to the Alpha-1 community and we've convened single topic workshops and I know Dr. Pierce has attended a good number of our clinical issue workshop series in the past.

Our most recent one, focused on detection. We wanted to know what has been done? What's worked? What hasn't worked and we have a publication that is coming out on that.

Taking part in research is an important message that we talk to our community about and we know that they consider the Alpha-1 Foundation a place to come to find out about clinical research. Our community is very loyal to the foundation and we are grateful for that and we try to make sure that we use that loyalty appropriately when we talk to our community about research. We make sure that we are guiding them in the right direction. I am going to turn it over to Dr. Strange.

DR. STRANGE: Why thank you, Miriam. Welcome, everyone, and again I'd like to thank Dr.

Marks for holding this conference.

I have been the wonderful recipient of Alpha-1 Foundation funds to run the Alpha-1 Foundation Research Registry for the past 19 years and we are proud to say that we have 6,500 individuals that have enrolled in the registry. I wanted to make a few points, though…, that this took 19 years.

This is a rare disease entity and if you start thinking about mortality studies that Sandy alluded to, you'll see in the purple, the diseased members and the point is that mortality is not a good end point for this community just because it is so rare in the disease state.

You'll notice also some discontinued members in the yellow here. These are all the individuals that enrolled as children and then at age 18, have to resign that consent form that says "I want to be a member of the registry" and if you all think about your 18 year old high school graduate and their willingness to participate in research, that's not the first thing on their mind. So, this engagement with the community is real, it's living, it's work every day to get this

6,500 group of individuals that are geographically dispersed. The point for every pharma company in the room is that to enroll a trial in Alpha-1 needs significant travel funds, and engagement with the community at a very high level. The Alpha-1 Foundation program has been put together with the mindset to be able to let every individual in the United States know a trial is happening. We challenge them since they are uniquely able to participate in these trials. This is a rare disease and to think that we can treat this like COPD, or Hepatitis C, or cirrhosis is really a mistake in identity.

When we did the statistical analysis trying to figure out if there are little pockets of Alpha-1 patients hidden among America, we find statistically this population density is no different than that of the entire United States. So, we think that we have scattered Alpha-1 gene across America and that's where we need to go to enroll Alpha-1 trials. We are here on the NIH campus and I think Sandy alluded to one of the first large studies, which was the NHLBI registry of 1,129 individuals. The freezer space on the

NIH campus gets pretty crowded over time. So, about a decade ago, the serum samples left over from that registry that have been in the freezer now for 30 years were moved to MUSC in Charleston, if any individuals in the room want to take 30-year-old serum samples that have biological traceability in minus 80 freezers of that duration.

We've been part of both Quantum-1 and GRADS. These are two separate NIH endeavors in the space. The Quantum-1 study was a three-year serial CT densitometry study enrolling individuals with normal lung function, trying to understand those signatures of CT density before you drop your FEV-1. Almost everyone in that group has a bit of emphysema before lung function begins to drop. I will notice that SomaLogic is in the room and the 5,000 protein signatures from this technology from the Quantum-1 and GRADS cohorts are now in the process of being analyzed and they should come out soon.

More recently, the GRADS study was enrolled. Genomic Research in Alpha-1 antitrypsin Deficiency and Sarcoidosis studied

two rare diseases, Alpha-1 and Sarcoidosis in which bronchoscopy and serum samples, oral microbiome and stool microbiome were collected. Serum samples, CT scans, RNA transcriptomes from the lung and blood, and clinical characterizations are available for analysis.

Of the eight centers that participated in that study, there were two prominent Alpha-1 centers. Because the rest of these centers were mainly sarcoidosis researchers, we have a shortage of bandwidth to analyze the “omic” signatures of this wonderfully collected data. It's a Foundation resource, it's a resource to Alpha-1 affected individuals and the audience.

The last resource on this slide is a recently applied for rare disease cohort grant from the NHLBI that was announced and applied for by Alpha-1 Foundation individuals. The goal is to generate the next model of registries in which biomarkers are hopefully going to be developed out of the larger cohort. More deeply characterized patients will move into the next generation of the registry with biomarker analysis and be developed as much as possible.

I want to make a few comments about our home genetic testing study called ACT. Detection is a large part of the Alpha-1 Foundation mission and as part of this, we offer a test kit using a consent form that's online. We then mail the kit to a participant's home where they historically will prick their finger and put blood on a blood card anonymously. We return their genetic signature (Alpha-1 genotype) back to them. Then they can inform their doctors, should they so choose.

We've done follow up studies on people that are ZZ or severely deficient, who almost always tell their doctor. The other piece of this is that families that test in this program end up having a significantly higher hit rate for deficiency alleles. In ACT, 5.9 percent of the samples coming into this study of 36,000 tests find severe deficiency. This is a family disease and to think about brothers and sisters of affected individuals as being individuals that might participate in research trials is a very important part of our foundation mission.

We have a lot of questionnaire data that

can inform the severity of COPD, and presence of lung and liver disease. What this looks like over time is that we have 37,333 patients that have consented for us to re-contact them for life. Importantly, this includes a lot of MM individuals that did not have Alpha-1 as control populations. In testing a family, we instantly get large numbers of MZ individuals available. Therefore, my challenge to those in the community is to expand your studies, because we do know that MZ individuals who smoke will develop COPD at a higher prevalence and have more severe disease than MM cohorts. This is the number of patients tested over time; and not everyone can prick their finger themselves. We all realize that's the major reason that people don't complete their kits.

I am privileged to be one of the medical directors of AlphaNet. This is a program sponsored inside the walls of the Miami offices. There are about 6,500 active AlphaNet patients served since 1999 and all pharma companies with approved Alpha-1 drugs currently have contracts for disease management services. In the past

AlphaNet served as the CRO for FDA approved products. The results of disease management that happens inside AlphaNet have been published in peer reviewed papers. Currently, our results show high adherence rates and increased patient survival as Sandy showed you.

Just to show you what this looks like from 1994 up to the current dates of 2016, something is happening. The median survival age of the AlphaNet population is increasing; and we don't know how much of this is drug, how much of this is disease management, and how much is due to identifying and placing older individuals onto services. But you may notice that if our median survival now is 80 years old and the United States median survival is 82 years old. We've got challenges to be able to show that therapy makes the difference in a diseased population.

Part of that, as you know, is that COPD treatment is changing. We have non-pharmacological approaches, we have pharmacologic approaches and then we have all the assessment and education which is what we do with the phone calls that go out from our AlphaNet

coordinators to the patient population once a month.

So, these are conversations that are happening monthly. We think this helps the drug development effort by getting patients on a standard platform that improves Alpha-1 care. Now bring us your medications and see if we can show the difference once everybody has a very good disease management plan in place.

And to that end, our current 2019-2020 agenda is to develop some Alpha-1 improvement metrics or AIM metrics that are going to be passed out to all the 6,500 individuals on AlphaNet services. 10 of these are on the slide. AlphaNet will make sure you got your Hep C testing, got your pneumococcal vaccine, have an exacerbation plan, and are able to exercise and engage in the things that you should be. Many of these are HEDIS outcome measures; so, we can actually speak to payers as well as to the population. But the point is that this is the important piece of taking care of Alpha-1 patients that we think we can improve upon.

The other thing that we really need to

do (and we need partners to do this) is to develop some patient reported outcomes; and we think this is twofold. It's not just developing a tool to be able to take with you to the FDA; but it's also engaging patient focused outcomes in which patients are part of your drug development team. The Alpha-1 Foundation community, honestly in their heart believe we add value to your companies by doing that.

We've done the literature review. There is not much done on good PROs that are Alpha-1 specific. There are PROs specific for liver disease or lung disease with COPD; but we have an engaged community and we have had focus groups and patient engagement opportunities. We have an ongoing online engagement platform being developed in AlphaNet to improve our data collection. We now have 20 years from that database and the 33 publications that have come through that.

What we need are instruments to be developed and then validation of these tools. We need partners to do this with; and so, if you and your company think you might add value, we'll be

very happy to talk to you in future discussions.

Unfortunately, the lung disease COPD has exacerbations of coughing, mucus production and decreases in general quality of life. In liver disease the clinical outcomes are similarly nonspecific with fatigue and problems with cognition. The point is that these are not very specific. I think many of us in our clinical trials place these outcomes front and center and have few beneficial outcomes with the therapies we have currently available. Those are some of the limitations. We feel we can do better; the patients tell us we can do better.

They tell us we can do better in these focus groups exemplified by this word search. The size of each word is how often it was mentioned in a focus group. If you look here it's family, it's oxygen, it's diagnosis. These are very important things; but the point is it's all about the Alphas. I think we come at this from a very patient centric focus and I'll turn the podium back over to Miriam to finish out because "It's all about the Alphas."

MS. O'DAY: We'll go back to the

beginning for the -- so you know, the GRAD study was the result of a class that the Alpha-1 foundation made of the government to focus a specific study on Alpha-1 and to know also that the survival rate has changed, and we've actually moved the needle.

When I first started working for the Alpha-1 foundation, many, many years ago, the average life expectancy was 54 years old and I remember that we had a large birthday celebration for John Walsh because he turned 54 and now, we've moved the needle to 80 years old with appropriate therapy and appropriate care management.

So, this is an exciting time for us. Alphas have driven research to find a cure. Detection, as you heard from Dr. Strange is critical because families are affected by this disease and we need to average in detection, education, support and advocacy and to work with our partners to advance the mission.

And ultimately, our goal is to find a cure and there may be some people who are sitting in this room today who may make a contribution to what we define as a pure and we want to thank you

for being here and for your interest.

So, the Foundation's resources are broad and far- reaching and comprehensive. We are focused on the mission with particular interest in assured flexible end points for currently licensed products, restoring the next generation therapies to move forward with regulatory purpose that compromise commercial viability -- that don't compromise commercial viability in rare disease populations and we have a great appreciation that FDA and NIH are getting this single disease workshop focused all day on Alpha-1 antitrypsin deficiency.

You can reach us for more information at our website or the 800 number. We are happy to talk to you at any time about the resources that we have available and we appreciate you coming in here today so that we can discuss our goals and we so thank you very much. (Applause)

DR. MARKS: And since we are still doing very nicely on time, if there are any questions, let's have them --

DR. FLOTTE: Hi, it's Terry Flotte from UMass. Really a question more for Charlie, and I

thank you both for your presentations. This is about detecting more cases. Is there a process where individuals find out that they have one or more Z or S alleles from direct to consumer genomics if you get the extended health package? Is there a process where they can find you and call you and enter the registry?

DR. STRANGE: There is. As many of you know, 23 and me reported they have now done 2.3 million tests as of last fiscal quarter. Alpha-1 is one of the 14 products that were licensed by the FDA to report on. And so, we have added to our registry database how many individuals come in from that venue. It's quite interesting that early on, 23 and me came to the Foundation and our genetic counselling program actually helped them write a lot of the script on their website today. So, when your report comes back from 23 and me with your carrier single Z allele, the message of what this means and the support available from the Foundation is in there. I think it's a good report. In my opinion, if we tell everybody in America to go test with a single company, this is probably the wrong thing

to do because there are 50 of these companies out there that are moving in this direction. It's just this whole process of getting your whole genome and then certifying that you are doing it correctly and have accuracy for each of the 7,000 rare diseases that you might want to report on is the hard piece that FDA is engaged with (and I think is doing well personally).

DR. FLOTTE: So, I also, in a similar vein, if I may, to the second question with the time, maybe bring up what might be a more controversial question.

So, I read recently a longer-term outcome of the Swedish screening program from the 70s, from Thomas Sveger and others and it clearly indicated that individuals who were diagnosed from a newborn screening program were less likely to smoke and so I am actually probably one of the few pediatric pulmonologists who works on Alpha-1. I have felt that arming patients with the knowledge for a diagnosis and giving them the opportunity to avoid nicotine addiction early was an intervention that had reasonable evidence for implementation. But also, of course, in a more

pragmatic sense (actually I think quite pragmatic) this might impact more people. I think, understanding full detection of the population through a new test which is the only time that we really do test everybody, might be worth considering so I don't know if there has been any effort to consider pushing for that.

DR. STRANGE: No, it's a good question. I want to hear your answer, but I think there has been a large effort to develop a newborn screening program for Alpha-1 in the United States. As many of you know, Baby's First Test is a venue that now tests, state by state, on average, 50 different conditions.

When we've met with them, they've said well if you don't start smoking until the age of 10, what you really need is an age 10 genetic test and the bandwidth within the pediatric community to take on one new initiative at some later time has not been there. This is a big lift, I think for the community. So, you are still interested, we don't have a demonstration project that shows that we improve any aspect of care in that first 10 years of life although you could think about

pediatric liver disease as a condition that has potential treatment outcomes that might make a difference in delay of diagnosis. Miriam, do you want to say anything else?

MS. ODAY: What I am going to add to that, and thank you for your question, Dr. Flotte, what I would add to that is that newborn screening, as you know, is a state by state program. It's an old public health program that grew up organically. We tried for a number of years to put together a Federal legislation that came up with regulations and rated newborn screening tests based on the ability to do interventions.

I think that there is not a chance that Alpha-1 newborn testing will take place considering the program with the structure that looks at now.

I think that there are two things that will shift the paradigm. One is if we have new treatments and liver interventions so that there is something that impacts the pediatric population directly with treatment or therapy and I think the other thing is that we go to whole

genome testing.

DR. FLOTTE: Just to make a point, if anybody ever has an opportunity to make these points, in the first month of life, when patients are diagnosed because of liver disease and we counsel them. The probability of us finding MZ parents and getting them to stop smoking is greatly enhanced and also it is quite clear that secondhand smoking exposure is very important. It's probably the most important prognostic factor in young cystic fibrosis patients in terms of early progression. The outcome not only has evidence behind it, but those are rational, potential benefits in the first ten years to the family of diagnosed infants.

MS. ODAY: Thank you.

DR. STRANGE: And I would only add that in our ACT program, which is sponsored by the Foundation, we have testers that have blood spots from one hour of age to 99 years of age so it's an opportunity to test within families your Alpha-1 genetics at time of birth.

DR. MARKS: Any other questions?

DR. STRANGE: Okay, thanks so much.

(Applause).

DR. MARKS: I thank everyone for keeping us ahead of schedule which is really wonderful so with that, I'd like to invite Dr. Ross Pierce. He's a medical officer in the Office of Advanced Therapies in FDA to talk about an update and Summary of Alpha-1 therapeutics.

DR. PIERCE: Okay. I'd like to thank the sponsors very much for the opportunity to speak to you today about this important topic of a class of therapeutic agents, the only specific agents for Alpha-1 antitrypsin deficiency. I am going to move quickly through the first few slides, as much of it is introduction which was already covered by Drs. Sandhaus and Strange and is familiar, no doubt, to the many members of the audience.

So, we have five products presently available in the United States that are in the class of Alpha-One Protease Inhibitor [A1-PI] products.

These products, as was mentioned, are targeting the lung disease and do not affect AATD liver disease. The Alpha-1 Protease Inhibitor

protein is a multi-functional protein but the key attribute is the inhibition of neutrophil elastase. Normal individuals have low levels of neutrophil elastase but it's very important to note that individuals with a severe deficiency of AATD have increased neutrophil elastase levels, so just raising the level of the A1-PI protein in the lungs and the blood to those of normal individuals may not be sufficient to reestablish protease / anti-protease balance because of the increased burden of neutrophil elastase seen in patients with a severe deficiency.

We've gone over the symptoms and exacerbations are a very key aspect of the morbidity of this disease. The therapy -- again, we have this one specific class of therapy for this disease -- the augmentation therapy with Alpha-1 PI, but many very important supportive measures.

So, the theory would say that if we can increase enough in the lungs, the levels of Alpha-1 antitrypsin, we should be able to stop the accelerated destruction of lung disease and slow the progression of emphysema to that of normal.

But the currently recommended doses of Alpha-1 Protease Inhibitor at 60 milligrams per kilo per week intravenously may not be sufficient to completely inhibit this excess neutrophil elastase that we see in patients with a severe deficiency, and, in part due to variability between patients in the actual levels achieved at the same dose of 60 milligrams per kilo, the present dosage for these products may be little more than a placebo for many patients.

If we look at the levels that are characteristic of the different phenotypes in Alpha-1 antitrypsin deficiency, we see that the normal levels range from 20 to 53 micromolar. A severely deficient patient, the most common phenotype is the PIZZ and they have levels below 11, typically 2-7 micromolar, and the SZs have an intermediate risk of emphysema and a range between 9 and 23 micromolar, so the SZs straddle the historical target of 11 micromolar.

The innovator product Prolastin was approved 32 years ago and the clinical evidence from the Phase 3 clinical trial was a demonstration in 19 subjects that A1-PI

functional trough levels were maintained over 11 micromolar over six months and a demonstration of a statistically significant rise from pre-treatment baseline in lung epithelial lining fluid levels of antigenic and functional Alpha-1 PI, but where did that 11 micromolar target for the serum levels come from?

Well, in a meeting like this, sponsored by FDA and NIH in 1985, two years before the approval of Prolastin, it was recommended to actually target the levels of MZ heterozygotes.

At that time, it was thought that MZs did not have an increased risk of emphysema; now we know they do have, particularly if they are smoking, so the level of MZ heterozygotes ranges from about 17 to 33 micromolar. The midpoint of that is about 25 micromolar, but instead, FDA accepted the lower target of showing that the levels in the blood were greater than 11 micromolar.

The follow-on products were approved as was mentioned by demonstrating non-inferiority of the serum trough levels at steady state in head-to-head randomized comparisons against

Prolastin, as well as the demonstration of the rise from pre-treatment baseline (or after a washout) in lung epithelial and antigenic and functional Alpha-1 PI levels.

So, reviewing the approved products, the innovator product, Prolastin, approved in 1987 was followed by Aralast in 2002. It was discovered post-approval that Aralast was actually missing the C-terminal amino acid in the sequence and was replaced in 2007 with Aralast MP. Zemaira was then approved, and Glassia was approved as the first liquid alpha-1 protease inhibitor deficiency product in 2010.More recently, in 2009, Prolastin-C was approved and in 2017, Prolastin-C liquid.

In this slide, we have the levels obtained in the blood [during trials]. The antigenic levels in the second and third columns and the functional levels, anti- neutrophil elastase capacity, in the column in the far right rose with different products and in different clinical trials for registration, and we see that mean antigenic A1-PI levels in these different trials have varied from about 15 to 19 micromolar,

often around 17 micromolar.

However, in the next column to the right, the range in different individuals enrolled in these relatively small clinical studies has been from just over 11 micromolar to as high as 23 micromolar in different individuals, and you can see that the functional levels, arguably more important, are actually about 15 percent lower on average in terms of mean, than the mean antigenic levels in these studies.

So why do we think now that the originally-recommended dosage of 60 milligrams for per kilo per week intravenously may be suboptimal? While the trough levels as reviewed achieved a mean of 15 to 19 micromolar, in a range of 10 to 23, they are often less than normal levels of 22-53 micromolar. They are also less than those MZ heterozygotes that they originally recommended targeting of 17-33 micromolar, who have some increased emphysema risk.

Very importantly, the PIZZ patients have been shown to have increased neutrophils and increased burden of neutrophil elastase in their

lungs compared to normal, so it may require supraphysiologic levels in order to adequately restore protease/anti-protease balance.

The SZ subjects in two data sets have shown increased emphysema risk whether their Alpha-1 PI levels were above or below the historical target of 11 micromolar.

An exposure - response analysis of the RAPID trial data suggests that higher Alpha-1 PI levels are associated with lower rates of lung density decline by CT and there was no threshold effect, there was no plateau effect, there was no inflection around 11 micromolar, and no clinical trial data or epidemiologic data actually exist to support 11 micromolar as an appropriate and optimal therapeutic target. This point is also agreed to by the European Medicines Agency.

So, what do we really know in 2019 about augmentation therapy with Alpha-1 PI? We know that it increases blood levels of Alpha-1 PI. It's generally well tolerated short term. There's a very low risk of viral transmission, but it's inconvenient, requiring regular weekly intravenous administration.

There are many things that we don't know and many areas of residual uncertainty with respect to augmentation therapy:

We don't know the optimal dose or serum level for inhibition of neutrophil elastase 32 years after the approval of the innovator product. We really don't completely understand whether the recommended dose slows progression of emphysema and is a disease-modifying therapy, although this is suggested by serial CT lung density measurements. Whether the effects are different at different stages of lung disease is unclear. Whether the effects consistently differ for men versus women [is unknown]. This definitely deserves more scrutiny including using existing data sets. Long term function, effects on function are unclear, as are effects on exacerbation frequency and severity, symptoms, quality of life, and mortality, notwithstanding the epidemiology data, we've seen -- we know that epidemiology studies provide a different level of evidence compared to those of randomized controlled clinical trials because of the potential for confounding variables that

may not be captured.

So, what are the long-term effects of augmentation therapy on airways obstruction in AATD emphysema? Epidemiology studies have suggested consistently, but did not prove, that augmentation therapy might slow FEV1 in patients with moderate airways obstruction, FEV1, ranging from about 35 percent to about 65 percent, but this finding has not been supported by three randomized placebo-controlled trials of limited size.

Each of those three placebo-controlled randomized double-blind trials has actually showed a non-statistically significant somewhat fasterdecline, on average of FEV-1 with the treatment group compared to the placebo group, which seems a bit surprising.

I'd like to go over in some detail the results of NHLBI registry study, which is the largest epidemiological study that's been done on this disease. The authors on the NHLBI registry study earlier published a paper discussing the design of the study and in that paper, they say that there were actually two primary end points,

and they differed and were to be applied to different sub-populations.

For mortality, that was expected to be highest in patients with an FEV-1 percent predicted less than 30 percent, so the primary endpoint for mortality was actually supposed to be in patients with FEV-1 less than 30 percent, according to the protocol of the Registry study.

For FEV1 that primary endpoint was in the remaining patients with FEV-1 greater than 30 percent. Well how do we do? In those patients who had an FEV1 less than 35 percent, augmentation therapy with A1-PI did not improve the rate of decline of FEV-1. The average reduction -- the average differencein decline of FEV-1 between patients on augmentation therapy versus those that were not was aonly 2.6 mL/year and nowhere near clinical significance, as you can see – or statistical significance, as you can see from the confidence intervals listed in the right-hand column of minus 11.3 to plus 16.5.

For all of the subjects in the trial taken together, the trial failed and the difference between augmentation therapy and

non-augmentation therapy with 368 subjects being on augmentation therapy; the difference was just 4.2 milliliters per year, and you see the confidence intervals there straddling zero for the difference.

However, for the patients with moderate airways obstruction, FEV1 from 35 to 49 percent, there were 141 subjects in the augmentation therapy subgroup and in the comparison group that did not receive augmentation therapy there were 29 subjects, so a total of about 170 subjects, and you saw a statistically significant difference of 27 milliliters per year, approximately.

Now this is actually using FEV1 averaged throughout the entire trial [rather than baseline FEV-1 to define this subgroup]. If you look at this subgroup by FEV-1 at baseline, the difference [in FEV1 decline] between those on augmentation therapy and not on augmentation therapy in this stratum of intermediate airways obstruction was closer to 22 or 23 milliliters per year.

The P values that were reported in the paper are not adjusted for multiple end points,

which is an important point. When analyzing trials, if you don't see an effect in the overall population but you do in a subgroup, experience has shown that when you try to replicate those studies, you very often fail to show that significant effect in the subgroup. But an argument to believe that positive FEV-1 result in patients with intermediate degrees of airways obstruction, that augmentation therapy may be helpful, is the consistency that's been seen across different smaller epidemiology studies in identifying a similar subgroup of patients with intermediate degrees of airways obstruction that shows statistically significant superiority and a slower rate of FEV1 decline as compared to patients not on augmentation therapy.

Now again, I want to emphasize that this subgroup of FEV1 35 to 49 percent with a total of about 177 subjects did achieve statistical significance without adjusting for multiple end points. So, to those that say that you need to study patients over 1,000 in number in order to achieve a favorable result for FEV1, I would point this out and say it might not necessarily be the

case. This was a very unequal distribution between those on augmentation therapy and not on augmentation therapy, 140 on augmentation therapy and 29 or thereabouts not on augmentation therapy, and we know that if we had had an equal number in both of those groups with the total being 170, that would have given you more statistical power.

So, the mortality -- again, the primary endpoint that was pre-specified, and this we emphasize in medicine as being important to enhance our confidence in results when you analyze according to your pre-specified plan, that was to the examine the subgroup with an FEV1 less than 30 percent.

Well, for those with FEV-1, less than 35 percent, the authors reported there was only a 17 percent reduction in mortality but this -- you can see that the confidence intervals straddle one for the relative risk of death -- this was not significant in those with the most severe airways obstruction.

So, it appears that they failed this primary end point [mortality] and going back to

the FEV1, I should mention that that magnitude of the 27 milliliters per year difference in patients with FEV1 35 – 49% -- that was a disappointment. It was far smaller than the difference between the treatment groups that was used in the power calculations, and if you compare it to the normal rate of FEV-1 decline with aging, the therapy didn't come anywhere near close to normalizing the rate of FEV-1 decline, so I would describe the results of that epidemiologic study as somewhat mediocre even for the subgroup of patients with intermediate levels of airways obstruction.

So overallin the Registry study with mortality examined for over 1,000 subjects, there was a statistically significant and clinically meaningful reduction of 36 percent in mortality and again, in that subgroup of patients with intermediate airway disease, a statistically significant result was obtained and there was no adjustment for multiple end points, but with a striking 79 percent reduction in mortality.

I should mention also that in the stratum of FEV-1 35 to 65 percent actually gave

you just as low a P value as the stratum of 35 to 49 percent with a confidence interval ranging from about 2 to 34 mL/year in terms of the difference between augmentation therapy and those not on augmentation therapy.

So, what about CT lung density? A very intriguing endpoint and a lot going for it, but there haven't been any FDA approvals based on CT lung density to date in emphysema.

FDA does not consider CT lung density to be fully validated to reflect clinical benefit by CBER or by the Center for Drugs in the sense that there's not yet been an interventional study that has shown statistically significant results for both lung density rate of decline and any other clinical end point.

There are limited data on how exacerbations affect CT lung density. There is one publication on it. There is potential that they may be confounding by fluid shifts as in congestive heart failure, and we don't yet know how we should handle that the best way in the analysis.

There have been positive correlations

between lung density rate of change and FEV1 rate of change which is encouraging in two randomized control trials if you include the RAPID extension trial data, but in all three of those [RCT] studies, FEV1 actually did better in the placebo group than it did in the group randomized to receive augmentation therapy with A1-PI. And very importantly, the minimum clinically relevant difference in lung density rate of change has not been established and attempts to work backwards to establish a minimum clinically relevant difference, using data from the RAPID trial and doing a straight line extrapolation into the future over a decade, I don't think it's scientifically justified, because we know, for example, that with FEV-1 progression in this disease, it is not a linear function over the lifetime of the patients.

Personally, I think it's unlikely to be a linear function over the lifetime of the patients for the lung density.

We've had a number of meetings at FDA on Alpha-1 Proteinase inhibitor and AATD including in 2009 at a workshop with NIH to

discuss clinical trial end points, and then later that year we had an advisory committee discussing clinical and biochemical end points. There were a number of recommendations that came out of the BPAC, as we call it, meeting. Some of them justifiably controversial, including to stop relying on serum and epithelial lining fluid to provide evidence of effectiveness for new intravenously-administered Alpha-1 PI products. To use instead clinically meaningful endpoints to study higher doses of intravenous Alpha-1 PI for effectiveness and at that time, which was prior to the publication of the RAPID trial results, the sense of the committee was that CT lung density may be a reasonably predictive end point to predict clinical benefit for aerosol Alpha-1 PI products, for example, but the efficacy needed to be confirmed by conventional end points such as FEV-1.

And they were thinking kind of in terms of an accelerated approval approach, but for intravenous A1-PI products, accelerated approval is not an option because we already have products on the market, so we would saythat there is no

unmet medical need for more products of the same class.

We had, in 2015, a patient-focused drug development workshops on AATD and we saw -- we heard directly from the patients and I'd like to thank again the Alpha-1 Foundation for the tremendous support they gave for that meeting --we saw that the patients were most bothered by shortness of breath, that they were very wary of conditions which may lead to exacerbation. They curtailed activities due to their disease. They want more autonomy in their treatment of their disease. They are enthusiastic for participating in clinical trials, but they are not enthusiastic for going off existing augmentation therapy for a prolonged period of time in order to participate in studies.

So, what are the long-term effects of augmentation therapy on the progression of emphysema as opposed to airway obstruction. Well serial, lung density measurements using CT scans in three randomized placebo-controlled trials suggest that decline in lung density may be slowed, and this is certainly supported by

meta-analysis, but none of the trials, as an individual trial, was statistically conclusive, as was mentioned by Dr. Sandhaus in the case of the RAPID trial. It did not quite make statistical significance for its pre- specified primary end point, and no clinical benefits were associated with the observed changes in lung density -- none of those changes in lung density were observed to be associated with positive changes in measures of clinical benefit.

The largest trial also showed a not-statistically- significant but sizeable 26 percent excess in serious exacerbations in the Alpha-1 PI group compared to the placebo group.

It showed confidence intervals that are very wide. FEV-1 did not show statistical significance but based on the epidemiology studies, we had thought that the RAPID trial was big enough that we were expecting to see a change in terms of the point estimate, a trend that favored the product -- we didn't see that -- it was a bit of a surprise.

The smaller EXACTLE trial also actually had nominally a higherrate of exacerbations in

the Prolastin group than it did in the placebo group but the Prolastin group had fewer severe exacerbations, so it’s kind of difficult to interpret.

So, we've [FDA] entered into agreements with all of the manufacturers of licensed Alpha-1 Proteinase Inhibitor (Human) products to conduct postmarking studies to get to this question of clinical benefit, and the intent has been to look not just at the currently recommended dose but at higher doses, and this was originally seen as a two-stage process.

First, do a pilot trial to get an estimate of the effect size using a clinically meaningful end point, and then do a fully-powered follow-up study. We have one study ongoing right now which is sponsored by Grifols. It's a three-year, three- arm, randomized, placebo-controlled, multi-national –trial comparing two doses of Prolastin-C - the recommended dose in the package and a double dose - every week in about 330 subjects using CT and other clinical end points, and I’m really excited about what kind of outcome we may get to help

settle some of these residual uncertainties that we have in this disease to help patients.

The RAPID trial is the largest randomized control trial that's been completed in Alpha-1 antitrypsin deficiency and included about 90 subjects per arm -- the primary endpoint was the rate of decline in lung density by CT. I would describe the outcome of the primary endpoint as borderline. The pre-specified primary endpoint, which as it combined together, CT lung density measurements made at FRC and at total lung capacity actually showed, after adjustment for lung volume, a little bit less variability than the lung density measurements only made at total lung capacity. That's an important point. The non-CT-related clinical endpoints favored placebo in this trial in terms of trend; none that were listed in the publication achieved statistical significance.

So, FEV-1, diffusion capacity of carbon monoxide, exercise capacity, dyspnea reported as an adverse event, exacerbations, and serious exacerbations all favored the placebo group in this trial of 180 subjects followed over two

years.

So, there also was a RAPID extension trial just for the patients who were in non-US sites, they could enroll to receive open-label Zemaira for years three and four, two additional years. So, we looked at a post-hoc analysis (and I should mention that analyses that we likely see from Dr. Chapman with respect to the Rapid extension study are also [mostly] post-hoc analyses. But post-doc analyses for exacerbation rates show that if you were originally randomized to Zemaira in years one and two and you stayed on Zemaira in years three and four, your exacerbation rate more than doubled. The confidence intervals for that were ranging, for the relative risk, from 1.2 to 3.7, statistically significant -- again, post-doc analysis, not adjusted for multiple comparisons, but rather strange and not completely explainable by the natural progression of the disease.

In placebo subjects, you had the patients -- again, we are only looking at people who completed the four-year extension as well as the original two-year RAPID trial, who were

originally randomized to placebo, who then crossed over to open-label Zemaira for years three and four, they experienced a 60 percent rise in their serious exacerbation rate [after switching to Zemaira].

This is all described in the Zemaira package insert. To just look at it graphically, – (standard error bars are not shown here; they could be large) in the left panel, in the blue and orange bars, we see the early-start group, which is those patients in the trial who were randomized to Zemaira, and you see the exacerbation rate, and here is years one and two and then it doubles in years three and four and that was statistically significant.

The late-start group, starting on placebo during the first two years, double blind, and then switching to open-label Zemaira for years three and four, had a 60 percent relative increase in serious exacerbation rate.

So, this table describes most of the secondary endpoints that are described in the publication of the RAPID trial, and you can see the absolute magnitude of these changes, of the

differences between the intervention group and the placebo group. They are not large. They lack statistical significance, but all those listed in this table, in terms of trend, are going the wrong way, except for the last one, the St. George's Respiratory [SGRQ] Symptom score, but then another recorded outcome of the functional score of SGRQ actually favored the placebo group.

So again, this is shown graphically where the first pair of bars on the left shows FEV-1 percent predicted; again, not huge differences here, not statistically significant, but the trend going in the wrong direction here to what we had hoped for FEV-1. For diffusion capacity of carbon monoxide, in the middle, with Alpha-1 PI in blue and placebo in orange, over the two-year period of the RAPID trial itself, and then exacerbations being modestly higher in terms of absolute terms in the treated group compared to the placebo group.

And if we look at the changes from baseline at 24 months, also in the shuttle walk test on the left, we see that subjects randomized to placebo increased their shuttle walk distance

at the end of the two year period compared to those randomized to Zemaira; and in the pair of bars on the right, the duration of exacerbations was also shorter in those randomized to placebo. So, from all these clinical points of view, you actually were better off if you had been randomized to the placebo group, notwithstanding the borderline statistically significant result with respect to lung density rate of change. But now let's go back into context.

I would argue that the absolute difference between the rate of lung density decline in the intervention group and the placebo group in the RAPID trial was small or modest. And on the left, you see the treatment effect, the difference in the rate of lung density decline between the Zemaira group and the placebo group, that's the yearly decline multiplied by the two-year duration of the study and it's 1.48 grams per liter of lung density difference between the randomized treatment groups over those two years.

On the right, you see the randomly-created baseline imbalance in the lung density values between the intervention group

receiving Zemaira and the placebo group; so the the randomization was not successful in achieving perfect balance. The lung density baseline difference was actually more than twice the size of the effect size that was seen from the intervention. So, it's just is a way to help put the relatively modest absolute changes that we are seeing between augmentation therapy and placebo over the two-year randomized, controlled trial into some context.

So, FDA's conclusion from the RAPID trial was actually, in view of the lack of direction and concordance of CT and other clinical endpoints, that CBER no longer considers CT lung density to be a clinically meaningful endpoint suitable for confirming the efficacy of augmentation therapy with A1-PI in AATD emphysema.

Based on the NHLBI Registry study, and the other epidemiology studies, we had expected that we would probably see a trend, at least, even in FEV-1 in the intervention group [compared to placebo], which we didn't see, notwithstanding the fact that the NHLBI registry study lasted on

average about five years, whereas this [RAPID] was a two-year study. Also, now in retrospect, too short to better inform us on the important other clinical endpoints beyond CT lung density and, strangely, a possible safety signal was identified with respect to increased serious exacerbations and total exacerbations during Zemaira treatment compared to placebo. We don't have an identified possible mechanism for that.

So, FDA's perspective on future clinical trial designs: Given that AATD is a rare disease, we want to be flexible with criteria to provide substantial evidence of clinical effectiveness, realizing that we can't enroll tens of thousands of patients in these clinical trials. So pre- licensure, FDA may accept a demonstration of a positive pre-defined trend in one or more clinical end points, such as FEV-1, serious exacerbations, exercise capacity, symptoms, provided that no adverse trends are seen in other clinical endpoints.

In other words, we are going to look at the totality of the data in making a decision. To confirm efficacy post-licensure, FDA may accept

a demonstration of a positive pre- defined trend in one or multiple clinical end points such as FEV-1 or the other points mentioned above with a two-sided p value of less than 0.10, which is more relaxed than the conventional level of statistical significance of $P < 0.05$.

Again, provided that there are no worrisome trends seen in other clinical endpoints. This represents our current thinking.

So, without categorizing endpoints into primary endpoints or secondary end points, here is just a list of things that we think are important to consider when doing trials, clinical trials in AATD, and we've gone over most of these except for the possibility of the combined endpoints.

And it was notable in the slide presented earlier [by Dr. Sandhaus] that survival seemed to diverge earlier at two-anda-half years in the comparison between US patients and UK patients, whereas the statistically significant difference in the countries' mortality curves did not occur until seven years.

Also, a change in BODE index and some other type of combined endpoint would be something that we may want to consider, as well as health related quality of life.

So, confirmatory trial designs -- that is to say post-approval trial designs for intravenous products: We would recommend that they be randomized dose-comparison studies. We are beyond the point of having placebo control groups I think for studies of intravenous A1-PI, and I would like to emphasize that FDA has never required a placebo group in any Alpha-1 PI study! There have been studies under IND that have been done with placebo groups. Those were always proposed by industry; we did not ask for it.

Reducing the maximum entry value for FEV-1 at baseline from 80 to 65 percent we think will potentially boost the study power; it's more in alignment with the sweet spot from the epidemiology studies; and is consistent with an Alpha-1 PI treatment guideline. We suggest an initial sample size of 400 for post-marketing confirmatory studies and an adaptive design with the possibility of sample size re-estimation.

We'd like that to be examined carefully as an option, and we recommend a four-year trial duration to assess FEV-1 nand exacerbation rate and frequency, and other non-CT endpoints.

Now, where did that four-year endpoint come from? In the RAPID/RAPID extension trial, it took four years to see a correlation between FEV-1 rate of change and CT lung density rate of change. And in looking at the results and looking at the other trials, we think a four-year trial duration and a sample size of 400 would be very informative with respect to the clinical endpoints beyond just looking at lung density.

So, in conclusion, AATD can be a serious rare disease characterized by progressive lung and/or liver disease that may ultimately require transplantation. FDA has approved seven forms of plasma derived A1-PI for augmentation therapy, of which five are currently on the market in the US.

Augmentation therapy is the only specific therapy for emphysema in this disease, but at the currently recommended dose, its effects on symptoms, exacerbations, quality of

life, progression of airways obstruction, and mortality remain somewhat uncertain and again, because of thepharmacokinetic variability between patients, some patients on the current dose may not be receiving a benefit that much different from a placebo.

Results of the largest placebo-controlled, randomized trial to date and its extension have actually somewhat undermined FDA's confidence in lung density by CT as an appropriate sole primary endpoint for Phase 3 and post-marketing studies, because we literally didn't see a whiff of efficacy in the clinical endpoints beyond lung density, and we had been anticipating or hoping for a trend at least with respect to FEV-1, based on epidemiology, given the sample size of that study.

Randomized controlled clinical trials using traditional clinically-meaningful endpoints (FEV-1, etc.) provide opportunities to determine whether higher doses of Alpha-1 PI administered IV and/or by inhalation improve symptoms and functions.

Here is contact information at FDA, and

I'd like to emphasize that we really need to better understand how to use our existing products, as well develop new products to better address the obviously tremendous unmet medical need of patients with this disease. Thank you very much. (Applause)

DR. MARKS: We have some time for questions. Can I just ask a quick question? Could you remind us of the dates of enrollment when the RAPID trial was enrolled?

DR. PIERCE: The RAPID trial was completed several years ago. I actually would ask a representative from the audience to provide information in that regard, if one would care to.

DR. MARKS: The only reason why I ask that is I am not a pulmonologist. I am a hematologist and oncologist but I am very aware that the advances in CT technology made it so that in 2005, I couldn't read a CT scan to save my life but by 2010, 2012, it was becoming very easy to read CT scans so there was a change in that technology so I was just wondering about --

DR. PIERCE: Yeah, I think Dr. Chapman, during his presentation could share some -- lend

some insight into any differences in the CT techniques for assessing lung density between then and now. I don't think there's been a big change in the way that we adjust the measurements for lung volume, for example, which is very key in reducing variability.

DR. MARKS: Thank you.

SPEAKER: Thanks. That was a great review of a whole lot of data and I realized I don't have to speak here at all. Everybody else is presenting. It's a challenge to go through all of the data including end points that weren't primarily looking for trends.

DR. STRANGE: Sure.

DR CHAPMAN: And I flagged one that I may have missed you mentioning but at the end of the two-year double-blind trial, there was quite a difference in deaths and withdrawals. That is, there were more people who died on the placebo arm and more people who were withdrawn early from the placebo one.

Again, those numbers were not subject to statistical assessment and so we can't say that they were significant but if there is a skew, if

sicker people leave the two-year trial early because they were on placebo, it may explain some of the other numbers, not lining up the way we expect.

DR. PIERCE: Now the deaths were just a handful; about three or so in the whole study. As you know, that is not enough to really analyze. Withdraws due to adverse events, however were not tremendously increased in the placebo compared to the control arm but it is true that there was a differential dropout rate in the trial withdrawals for all reasons were greater in the placebo group due to the intervention group. Perhaps that has a role in the directionality of the small trends that we see in these clinical endpoints that again were not large in magnitude but after you thought it was a bit peculiar that all of them seemed to go in the wrong direction.

DR CHAPMAN: Thanks.

DR. PIERCE: I think the more important point is that the RAPID trial didn't provide any indication from the other [non-CT] endpoints that were studied that the small -- the relatively modest difference that was -- seen in absolute

terms, in lung density decline translated into a difference that we can measure in terms of how patients felt, how they functioned, or how they survived in the clinical trial over two-to-four years (if you include the extension) and it started with 180 subjects.

SPEAKER: Dr. Pierce, excellent presentation again. Thank you. Do you think that the imbalance observed could have been addressed by appropriate stratification and should the future trial incorporating CT be stratified during randomization for baseline lung density to avoid such an imbalance, and could that lead to a different interpretation instead of dismissing the asymmetry all together?

DR. PIERCE: I think that's something that certainly could be considered. The Sparta trial that is ongoing with the CSL product does have baseline stratification according to three strata of FEV-1 at baseline but some consideration to stratification by baseline lung density is something that perhaps should be explored.

SPEAKER: So, in your opinion then is the dismissal of this end point premature?

DR. PIERCE: I am sorry, I missed your -- the dismissal of --

SPEAKER: The dismissal of the CT densitometry as a valid end point --

DR. PIERCE: So we are not dismissing the utility of CT density as an endpoint of interest, we just don't think that it stands alone in terms of getting us where we want to be to understand because -- it is a very sensitive endpoint. It may be too sensitive, and you may be able to do a trial in which you see a statistically significant change [in lung density], but it's of questionable clinical meaningfulness to patients, so you want to get that second part of it, so we can convince not just the people in this room, but the people over in the UK, so they'll start paying for this therapy at a higher appropriate dose if in fact that's of benefit to patients.

And I should mention that where we are on the dose, we may be so far onto the left of the dose response curve that when you finally get data on comparing higher doses to the dose we are recommending now, those effect sizes might even

be bigger than what we have seen to date with the currently recommended dose versus a placebo. We believe that will be the case.

SPEAKER: Thank you very much.

DR. SANDHAUS: So, I wanted to say one thing supportive and one thing that I would like to challenge. So I think of your list of reasons for considering higher dose, one that you didn't say is that the fact that in response to infections, inflammation and things like that, in MM individuals with normal Alpha-1, the individuals boost their levels 2, 3, 4 fold and that doesn't happen in patients who are on augmentation therapy and those are times when much of the lung destruction may be taking place.

On the other side, and I think a key element, is we are looking at FEV-1 because that's easy to measure and has a long history of being an end point for various therapies for lung disease but those of us that treat many Alpha-1 patients are very well aware of the fact that patients can have significant emphysema and normal FEV-1s or significant emphysema progression without the FEV-1 changing.

We measure it because it is easy to measure, and it's done everywhere but I think that as the gold standard comparison for these trials in an emphysema producing disease aims too far away from the primary process that's going on. It's a shame that the things that we think are more directly related, like diffusing capacity are so difficult to standardize in a multi-center trial.

And so, I think that's where a lot of our efforts should be is to find things that actually correlate with the disease.

The other issue that affects this are the treatments for COPD in general have improved over the course of these long trials and that exacerbation rates are greatly affected by those new therapies and may not be reflected on a study that's going around for a decade or something along those lines.

DR. PIERCE: Right, and I think that goes to the importance of using concurrent controls rather than historical data because of the improvement [in concomitant therapies] and of course with the excellent work that the Alpha One Foundation has done with respect to patient

education, we have seen improvements in terms of how patients daily manage their disease.

DR STRANGE: Ross, I also had a question around FEV- and I think when we look at the Copenhagen Lung Cancer Screening Trial and some of the other cohorts that have come, we see a very poor correlation between FEV-1 and CT densitometry. As you know this also holds for SGRQ. The point is these are shotgun blasts around FEV-1 in an emphysema producing disease. We've watched and reviewed FEV-1 for 20 years. It has this historical basis, but I think most of us in this field think FEV-1's day of prime was 20 years ago. Yet I share with you that we are not having the broad correlations with other clinical endpoints that we'd like to see in these therapies. So, I just wanted to make that comment. Your response?

DR. PIERCE: Thank you.

MS. GOFRAM: Hi, I am Maria Gofram in London and we are looking potentially to some molecule for alpha-1 lung disease and seeing the potential trial designs that you put forward, I

am interested to know a couple of things. So approximately 400 patients. Is that based on an empowering of the potential end points or is it your gestalt of what sort of number is needed?

DR. PIERCE: So for FEV-1, there was a paper published by the authors of the NHLBI Registry Study describing power calculations for FEV-1 as a primary endpoint in placebo controlled trials that suggests that if you cut out the top FEV-1 study entry limit at 65 percent, that the number of around 400 subjects in a two-arm trial may be appropriate with a four year total trial duration, and that [four years] includes the screening [and enrollment] period. But we are talking about a four-year duration for even the last subject entered, so the power for a placebo-controlled trial of that duration would be even greater.

DR. MARKS: I am going to suggest we take your question up later this afternoon because I think -- just my prerogative would be that we can't compare due modalities with therapy to plasma derived therapies. I think that's probably -- and I think we are going to have to

think differently before we take one thing and paint with a broad brush. Just one last question so we get to break here.

SPEAKER: There were biomarker measurements made in the RAPID trial. What is your position on the role of biomarkers and showing efficacy?

DR. PIERCE: Personally, I am excited about the emerging elastin breakdown products' (desmosine and isodesmosine) data and we encourage sponsors to explore this and to have discussions with us about their appropriate role and particularly in exploring their possible use in Phase 2 trials –to potentially help with dose selection for further study. There may well be a role for that but it's something that we need to look at carefully together with our industry partners.

SPEAKER: Thank you.

DR. MARKS: Thank you very much. So, thank you everyone for -- and thanks to our speakers for staying pretty much on time. We will take a -- just under a 15-minute break. We will come back at 10:35 promptly and we will get

moving again. Thanks very much. (Applause)

(Recess)

DR. MARKS: If everyone would take their seats, we're going to try to get started again. Unfortunately, as always happens with breaks, they expand, and we'll try to keep it from expanding too much. So, if everyone would come back in. Usually when they see the train parked at the station, they run toward it.

Thanks so much. We're going to move on now to Dr. John Scott from the FDA Office of Biostatistics and Epidemiology at CBER. He is going to talk to us about the approach the clinical trials. Thanks so much.

DR. SCOTT: The organizers clearly thought it had to provide everyone coffee prior to the stats talk and I'm very grateful. There is actually very little statistics content in my talk. I'm going to be speaking pretty generally about clinical trial approaches for rare disease. I'm afraid I really can't speak with much specificity to designs for Alpha-1 because it's not an area of particular expertise, but I find a lot of workshops so far today and I know there

are a lot of people here. There is Dr. Ross and Dr. Pearson who can address those questions. So, it's sort of a whirlwind tour of things to consider for clinical trials for rare diseases and kind of a menu of options in building a trial. First, I'm going to give some background and some recent developments or occurrences with FDA's rare disease regulation. I'm going to talk about some design features to consider for various trials; then in a little bit more detail adaptive clinical trial design and Bayesian clinical trial design; and then finally a little bit about a new program we have on interacting with FDA for a complex innovative trial design proposal.

So, for rare diseases, it's highly recognized that you can't get the same level of evidence for a very rare disease that you can for a very common disease. They are harder to study, for a variety of reasons, primarily the size of the patient population. Despite that, the criminal standards by law for rare and non-rare diseases are the same. To approve a product, FDA requires a substantial evidence of effectiveness, sustain adequate and low control

investigations, also evidence of safety. Usually that's a term that's to mean adequate and well controlled clinical trial, but there are cases where that's not the case. However, despite the fact that we have this standard for all approvals for drugs by logics; we have a lot of flexibility in applying the standard to individual products.

I'm going to highlight a few things we've done recently with rare diseases, starting with this guidance. This is a revision of a guidance that was released in 2015. It's a guidance registry on common issues in drug development for various diseases. It covers a lot of relevant topics, most of them not in a lot of detail, but it does provide a good framework to think about these trials. It talks a bit about natural history studies. We also have a separate recently released guidance specifically about natural history studies. It talks about end points to vital records and how you go about validating a new vital record and serving at that point. It talks about evidence of safety and effectiveness for rare diseases, somewhat about

non-clinical considerations and pharmaceutical quality for CMC considerations. It talks about patient, caregiver and provider participation in drug development and about FDA expediting programs such as breakthrough designation for accelerated.

A lot of our recent sort of policy and outreach initiatives have been driven by 2 main places, 2 main documents. One is the 21st Century Cures Act which was passed by Congress at the end of 2016 and the other is the most recent iteration of the Prescription Drug User Fees Act which is the act that described the circumstances under which FDA collects application fees from industry. Both of those singled out in some detail rare disease development for a particular focus. There is a lot of recognition, both from industry and Congress that we could be doing a lot more to promote development in rare diseases. They also both called on FDA to promote the use of novel innovative complex trial designs and analysis.

Last year in March we held a public workshop through the Duke Margolis Foundation called Utilizing Innovative Statistical Methods

and Trial Designs in Rare Disease Drug Development. This was a really good workshop. It's all archives and presentations and I think there is reporting off of that website. The focus of discussion was on 3 main topics.

One was Bayesian methods for leveraging prior trial data in a Phase III trial which I'll talk a bit about in this talk. There was a lot of discussion on natural history in registering data and there was a lot of discussion on master protocols and platform assignments.

A couple of years before that, I'm never sure if this is IRDIRC or IRDIC work. It's not a lot of fun to pronounce. This is an international consortium that had a task force looking at small population clinical trials. This included representatives from FDA and regulatory bodies throughout the world as well as industry and academics throughout the world. They put out a report that suggested potential design features to consider for rare diseases and general consideration, sort of things you might miss in planning a rare disease trial.

I'm going to go through these pretty

quickly. So, I'm not going to talk about them in any great depth. Several of them I'll also discuss a little bit when I get to adapting trial designs, but some things to consider for rare diseases according to the IDIRC report were adaptive randomization, Bayesian methods, crossover designs, re-randomization designs. These are where you have, for example, you treat everyone initially with placebo or with controlled -- I'm talking about placebo or controlled trials in most settings here. In the case of placebo, people who respond to placebo are then re- randomized to treatment for control and the people who don't respond to placebo are given placebo.

They talked about factorial designs where you can study multiple treatments in the same trial. Sequential, which is a form of adapted design. Platform designs, which can be a couple of different things. It could be multiple candidate products in the same trial, or it could be one candidate product for different sub-populations for different indications. And of one trial, where you intensely evaluate what

happens to a few numbers of subjects. Preference based application, it's situations where randomization is really considered impossible. You can provide such as the opportunity to choose which treatment you get. Randomized for all studies where everyone is initially treated with an experimental therapy and then randomized to be discontinued to see what happens to said use over time. Such re-estimation is where you get adapted design in smart trials, which are sort of complicated algorithmic designs for studying sequences of treatments when that's an option.

In terms of design features, but general considerations, the task force recommended you see much general data, whenever it's available, as opposed to looking at a single time. We've recommended avoiding dichotomizations for power reasons, extended follow up beyond what you would consider for less rare diseases, use of a cola or other statistical approaches to control for baseline proviants or prognostic time periods. We talked about considering the appropriate use of historical data and analyzing data on trial. We do collect

adequate safety data. You need to consider alternate data sources, especially for safety, alternate data sources for the registry trials but trying to close records, close marketing trials and so on. There was a lot of discussion about the use of decision analysis and rational approaches to adjusting the level of evidence. So, this is something that we always have a lot of robust discussions, internally or externally by clinic approach. Dr. Pierce mentioned potential post-marketing design where the Alpha level would be.10 instead of.05. Decision here is sort of a collection of techniques that try to establish expressions and other principals.

Earlier we talked about extrapolation, for example, extrapolating from an adult to pediatric subjects or from clinical subjects down there. It talks about the need for a patient to engage in one, which I don't think is something we need to sell this to. Okay. Let's talk about the trial designs. A little background, back in 2010, FDA at the request of Congress, published a tract guidance on adapting designs in clinical trials for drugs and biologics. In text, the

guidance was to encourage the use of adaptive design, whatever fits well in regulatory decision making. What that guidance did was it sort of provided a catalogue of potential adapted designs and said some of these are well understood by FDA and some of them are less well-understood by FDA. A side effect of that is that it was received by trialist unity as meaning that less well understood designs should not be used, which was not really the intent. We want to take what we don't understand in 2010 may be understood in 2019.

We recently published a new tract guidance to replace that tract. It no longer categorizes designs that are well understood or are less well understood. Instead it focuses on key principles in design conduct analyst and reporting of adapting design trials. It expands discussion on technical aspects such as estimation of treatment effects, the use of trial simulations to estimate operating characteristics and basic methods and adds some clarity on how to interact with the FDA, what documents need to be submitted for an adapting

assignment.

For adapting assignment, why do you want to do an adapting design? There is sort of 3 general categories of motivations that are cited to suggest the use of adapting design. One is that in many cases, there can be advantages in statistical conditions. Basically, what we mean by that is you need fewer subjects to detect the fact of a given size, or you can detect an effect or estimate the effect with more precision in the same number of subjects. In some cases, there can be ethical advantages to adaptive designs. With sequential designs, it lets you stop the trial early, if there is really evidence that the investigational product does not work, or it lets you stop the trial early if there is overwhelming evidence that it works very well. It can prevent too many trial subjects in the former case, subjecting trial subjects to an ineffective treatment and in the latter case, it can lead to providing the patient population effecting treatment faster. People talk about ethical advantages, in some cases, with techniques like response to developing application where, as the

trial goes on, if one arm appears to be superior to the other arm, you start to randomize more people into the superior arm. That's a little bit more controversial from the ethicist perspective. Then finally, adapting designs in some cases helps us to understand the drug matter. They are letting us do more nuance testing in those response relationships, for example, if a study has adapted just functional.

This is classified into sort of 2 broad categories. Those are adapted to science through based on non-comparative interim results or adapted science based on your character results. So, the non-comparative, people often call this blinded or masked. For assorted technical reasons, we're trying to call it non-comparative instead. These are typically based on pool's data where you don't know which are the subjects were assigned to. They often have a negligible effect on the false positive rate of the trial. So, these are, in some sense, kind of a free lunch. The type of designs that are based on a comparative intro results are called unblinded or unmasked analyses. These are usually

based on intro estimates on the treatment effect itself. You stop to look positive at the trial in the middle. You actually compare the two arms and then you make the decision for how to change the design and trial based on what you saw there. These typically do affect operating characteristics of the trial, including the false positive probability type of error grade. They require a specialist just to get that. It doesn't mean they can't be used. You just have to make sure you're using them right.

So, in terms of adaptation with non-comparative interim results, there are a few kinds of these, but the one that people actually use is sometimes called applying sample size re-estimation. This is using pool data where you don't know what arm people were assigned to, to re-estimate your sample size. The reason you do that is when you start a trial, your sample size compilation depends on several factors. It depends on significance level, power, the effect size you're targeting and what we call usage parameters which are things like variance of -- I'm not sure of what is of primary interest. Yet, at

the design stage of a trial, there is a lot of uncertainty in some of those factors. You have your significance level and you might have what power you're targeting, but typically you don't know what the true effect size or about the true value of the nuisance parameters since the beginning. So, the goal is to use the cumulating information about the usage parameters to modify the sample size to maintain a desired outcome.

So, without knowing what treatment our people are assigned to, you can't estimate the treatment difference, but you can get an estimate of the variance of the metric and if that variance is higher than you thought, you might want to enroll more subjects than you initially planned or vice versa. Oh, sort of a minor class of adaptations, are the unblighted or the application is based on comparative interim results. These include group sequential designs which are probably familiar to most people. A sequential analysis is where it sorts of is just starting the trial, finishing it, and then doing an analysis. Your analysis it as you go. Group sequential means to do it in sort of batches. You

don't analyze it after every subject. You may want to analyze pm every hundred subjects. These can provide a lot of advantages, both ethically and in terms of omissions either, probably the most widely used design technique.

There are other adaptations to sample size, sample size re-estimation. Where again if you look at the treatment effect, midway through the trial or halfway through the trial, and if it is lower than you expect at the design stage, you might want to enroll more subjects than you initially planned. Less commonly if treatment effect is higher than you expected, you might enroll fewer subjects than you planned. That is not as widely done. It's possible that you adapted to decided terms to population of patients enrolled into the trial. Adapted enrichment is one example. What adapted enrichment is - enrichment is a general approach to trials where you try to involve people who you think are more likely to benefit from the investigation therapy. Adapted enrichment is where you use accumulative trial data to get a better sense of who is more likely to benefit in

terms of medical suffering of short people with certain baseline prognostic factors. There are annotations to treatment on selection, especially in multi-arm trials or dose selection trials, whatever you might start a trial with 3 or 4 different treatment arms and as you go through, the arms that are to a less swelled get taken out of the trial.

Adaptations to patient allocation, we call this adapted allocation or adapted randomization. There are 2 different brands of this. There's Covariate adaptive and response adaptive allocation. These two still can work together. The covariate adaptive allocation is that as the trial goes on, if you start to get invalid sense between treatment arms, if they support baseline covariance, you change the way you allocate subjects to try to correct those imbalances.

Then response adaptive allocation is where you look at the outcomes for subjects throughout the trial and use that to decide to allocate future subjects in the trial. Response adapted, in particular, generally relies on very

short term and is probably not very reliable.

Adaptations to end point selection, this is something more notional that applied. People talk about it whenever they talk about adaptive design. I have seen it once, I think. The idea of this is that you might have potential, they need you in analysis halfway through the trial to decide which end point is being affected most strongly by the product. Then proceed to use that as the primary endpoint. This of course requires special statistical handling because there is definitely a Type 1 error inflation on this stage.

Then finally, we talk about complex adaptive designs which usually means a design that provides multiple different adaptive technique. I'll mention that the new guidance talks about new principles for adaptive designs instead of saying which ones are good and which ones are bad. It just says if you're going to do one, please follow these principles. The principles are the chance for erroneous conclusions should be adequately controlled. So, the focus there is often on Type 1 error,

incorrectly concluding that an ineffective product is effective, but we're also worried about inflating the Type 2 error, the probability of erroneously concluding that it effected drugs, not effective. We're also worried about getting accurate and unbiased estimates of treatment effects. So, it also speaks to estimation, because those treatment effects go into risk benefits patients.

The estimation treatment effects should be sufficiently reliable, and we pull that out as a separate principle. The details of the design should be completely re- specified. I'm going to put an asterisk on this bullet. This went out as a draft guidance and we got public comments. The final guidance should be out in the not too distant future. One of the major comments we got is that people were not happy with the word, completely pre-specified. That seemed a little too all encompassing. So, in a revision, we are going to try to be more specific about that, but generally speaking, the important aspects of a statistical and trial design need to be pre-specified to ensure that you're not relying

on post-hoc or cherry-picked analyses.

Then finally, the trial that is produced should be appropriately maintained. This speaks mostly to unblinded analyses, making sure that there are appropriate firewalls in place so that people who need to know, unblinded case knows it. No one else knows the unblinded.

Okay. Onto Bayesian trial designs. So, I'm guess that everyone, or almost everyone in the room is familiar with phase 3, sort of a buzz word. I obviously don't have time to talk in any detail about what phase 3 statistics is, but just sort of a 1 or 2 slide primer. When we're talking about baseline statistics, we're talking about a whole wide range of things that tend to have a few things in common, but it's sort of a loose thread. The things that they tend to have in common is the idea that evidence should be synthesized so when you make a decision or you making an inference, that should base on using all the relevant data in that decision of inference. In basis, just when we talk about prior probability distributions as encompassing, what we know about a question before we have new data

and then we'll divide that with new data to get an updated posterior constraint.

The second principle is that inference should be based on the conditional probability, that my hypothesis is true, given the observed data. That's a lot of words, but it's very straightforward. The ideal is to have a hypothesis. The hypothesis is this new product is effective for such and such an inference. You collect data and your inference should be based on what is the probability that the hypothesis is true, based on the data observed. That sounds like common sense. You might think that we always do that. In fact, traditional statistical inference does the opposite of that. What P-values measure are the probability that you would see the data you saw if the hypothesis were true, which is very backwards and the source of a lot of confusion about in staff development.

The final principle is that the emphasis should generally be on decision making, not hypothesis testing. So, you might still make a binary decision about something works or not, but what you're really headed toward is an

approval setting, where you're trying to make a decision about approval and a Bayesian approach, we try to synthesize all of the information we have and what do you value to make a decision for obvious reasons.

So, in late phase 3 clinical drug development, there are a few different reasons people look to Bayesian statistics and all of these are becoming more and more common in sufficient testing. One is too much external information which might be incorporating prior clinical data in Phase 3 analyses or extrapolating from adult populations to pediatric populations. It is a very popular important topic right now. Another to use phase is mathematical convenience, which is beyond the scope of this talk, but it is actually easier to be a Bayesian statistician than not to be a Bayesian statistician. It makes the math and a lot of the analyses a lot more straightforward.

Then the final motivation is to the interpretability and the decision making and this gets to what I was saying before about the probability that the hypothesis is true, given

the data rather than vice versa. Bayes helps you express the uncertainty on a directly interpretable scale, and it cites different inputs into making a decision. So, in rare disease, there are a few key areas where people want to talk about the application of Bayes. All of these were discussed, for example, at the workshop I mentioned.

The first are Bayesian mechanistic models, then Bayesian effect logical models that base the trial size. Then explicitly borrowing external data from the patient power from the precision and then finally extrapolating and cross populations. In terms of what I mean by basic pharmacological models, it's obviously the case that a lot of the disease products are expensive to produce, expensive for patients and providers, but for insurance sake, or in some cases, they are scare. They are a limited resource. It would be critical to get the optimal dose, both for clinical reasons for optimally treating a patient and also for cost or resource allocation reasons. So, at CBER we've reviewed multiple proposals that use Bayesian population

pharmacokinetic modeling to try to personalize the dose of the product or something. In terms of basing adapting design, this is been pretty basic and has been and statistical literature for a while. What this is in most cases is actually tradition frequentist kind of P value statistics, using Bayesian mathematics because, as I mentioned, the math is easier. What this can do is provide a lot of flexibility in design. If you want to combine multiple adaptive design features in a single trial, this is very often the way to go. If you try to just be a pure and frequentist statistician and do that, you would need many dissertations to get to where you need to go, whereas with Bayes, you can just write a little computer program for the answer. These do tend to rely on extensive clinical trial simulations to establish the trial operating characteristics, with capability and power, things like that.

Onto the use of external data in late phase trials, just generally speaking, when we talk about using external data in late phase trials, there is kind of a spectrum of use, kind of the bottom of the pyramid to use external data

for planning future trials. Everyone does this all the time. You should always do this whenever you have different. Another thing that can be done is if you have a single arm study, the external data can sometimes be used as a comparator. We're again not generally talking about single arm studies, but again, late stage oncology. This is a situation where randomization is simply impossible. Then what I'm going to talk about most in the next couple of slides is partial borrowing of external control data into a trial. You can also talk about partial borrowing of both external control data and external investigational data. Finally, the sort of ultimate phasing approach would be the complete evidence of the sequence of trials. This generally requires planning from the very beginning. Say, we're going to run 3 different trials and we're going to sequentially combine them to get a synthesized answer.

So, borrowing into Phase 3. There is a lot of interest in the trialist community in borrowing external data into Phase 3 analyses. Most often people are talking about Phase 2

clinical data for this borrowing. It's possible to borrow both the controlling data or the investigational data or both, but when both kinds of data are borrowed, it raises significant type 1 error inflation issues and buyers hesitate to make sure of inferences. There are a lot of different methods, logical approaches to this, and it's especially popular or of interest in sort of data poor settings, including rare diseases. When we're talking about this partial borrowing, some considerations are, first of all, the scientific comparability of data sources, where the prior data and current data are collected in the same population, same end points. Same site is ideal. Same kind of background character, as the discussion this morning about the background character or pulmonary conditions changing over time. That would be a series of pediment borrowing alter data into a contemporary analysis. Perspective planning is important; was the external data collected? Was it intended to be used in this way? That's not generally required, but it does help address the comfortability issue and helps avoid carrying the good data, to use an

analysis, when there might be other good data available. That's what the next bullet point also says. Is it possible to use all the relevant external data? Are they all being appropriately used? This again, speaks to cherry picking. Did you have one good Phase 2 trial, and do you want to use that good Phase 2 trial data, but you're ignoring fewer good data. Are you using all the relevant data that's available? Then finally, one statistical method that should be used, we talked about 2 different kinds of partial borrowing. I'm not actually sure I was clear on this. By partial borrowing, what we're talking about is a control trial where part of the control arm is sort of borrowed from data from an external source. The chief general methods for this are fixed discounting where you say, okay, we have this Phase 2 data, but we're only going to count is 50 percent as much as the Phase 3 data or 20 percent as much or 75 percent as much. Those percentages are very arbitrary. They're very hard to come to, which is a complaint against those mechanisms. Then there's dynamics discounting methods where statistical method

itself based on how similar priority contemporary data sort of automatically determines how much to borrow, if there is evidence that the data is heterogeneous.

Finally, FDA is complexing the data to try to design another program. We've pointed to this term CID. I'm not sure why there's not a "T" in there, for complex innovative trial design, to encompass a pretty big range of things, including complex adaptive designs, phasing designs, extrapolation, master protocols, all kinds of stuff. In order to be successful with innovative proposals, it's been noted that sponsors might need robust regulatory feedback. They often need sort of hybrid level buying than the review team. They might need assurance that a review division or office is comfortable with the design imposed. So, and also to encourage the use of innovative designs, FDA needs case studies to be able to talk about it. Part of the problem we have is, for example, we've reviewed a lot of partial borrowing Phase 3 trial proposals under IND, but I can't talk in any detail about any of them because they're under IND and they're priority

information. So, part of the problem we have is having case studies that we can talk about.

Under 21st Century cures, we've launched a pilot program to try to address these things and to promote the use of CID designs. This is a joint effort between the Center for Drugs and Biologics. The way it works is sponsors submit an innovative design proposal and they have the opportunity to engage with regulatory staff, including senior level regulatory staff, on those assigned proposals in 2 interactions with FDA beyond what would ordinarily be granted in the review process. The agency selects up to 2 of these designs sufficient each quarter and then will use those designs as case studies for continuing education and information sharing. These meetings are usually led by the biostatistics groups and FDA recommends them on clinical or other disciplines, but all relevant disciplines participated. The grant money for this study is for 5 years. It started in 2017, so it's running into 2023.

To be eligible for the program, you have to have an IND or a pre-IND number for the medical

product to do the proposal. The proposed design is meant to provide substantial evidence of effectiveness to support approval of the medical product. The trial can't be a first human study and there has to be sufficient clinical information available to inform the proposed design. Finally, the sponsor and FDA have to be able to reach an agreement on aspects of the trial design that can be publicly disclosed if it gets to that case. So, here's the process for that. Basically, sponsors submit a meeting request. FDA evaluates those meeting requests and notifies the sponsor whether they will proceed to the disclosure discussions. Those disclosure discussions are where we hash out what elements we'll be talking about publicly once the design is accepted. Then we go find a sponsor, whether the CID meeting is granted and provide dates. Then there are 2 CID meetings which occur -- I think by statute they occur 120 days apart. So, the idea is you come in for a meeting. You get detailed feedback from FDA on the innovative proposal and then you have about 3 months to incorporate that feedback and modify design and have a sample made.

So, just for all conclusions, FDA is substantially focused on ensuring the availability of safe and effective therapies for rare diseases. We spend a lot of time thinking about this and working on various of these problems. There are a lot of opportunities for applying innovative approaches for various means of development, including conventional design possibilities that go beyond simple placebo control, double blind, minimized, sample size trials. They can include adaptable designs for Bayesian restrictions. Also, these innovative approaches often need more intensive feedback from FDA, so the CID pilot meeting program I described and other meeting mechanisms can be used to get advice from FDA. That is it.

(Applause)

DR. SCOTT: Questions?

MR. KOZNIAK: Hi, Mike, Kozniak from Decatur. When using external data from maybe a prior trial, and trying to be using it as prepared, are there any hints of what the sponsor's mistakes are and to try to avoid using external comparative data?

DR. SCOTT: Yeah. That's a good question. Are you talking about using the data as a control arm in an uncontrolled trial or using it to sort of bolster a control arm in a concurrent trial?

MR. KOZNIAK: Sort of using it as a fake placebo arm with 2 treatment arms.

DR. SCOTT: Right, so rather than talking about problems that sponsors get into, I guess I would talk about some of the things that definitely come up during the human process with those types of questions. First of all, if it's at all possible to have a concurring control, that's always preferable, but when it's not possible, the things that people look at are the clinical comfortability of the populations, especially are they close enough in time to be reasonably sure that they are treated in a similar way. There is a collection of methods for kind of quasi-contribute comfortabilities which is propensity scores. People love to use those methods to try to do a kind of matching between the concurrent data and the external sort of involvement. Pitfalls, I think it's usually the

case that when these kinds of submissions come in, they don't have enough information the first time for FDA to really understand what is planned and what the rationale is for borrowing. So, just providing a lot of detail in the developing process can be very useful.

MR. KOZNIAK: Okay. Thank you so much, Mr. Scott.

DR. MARKS: Okay. So, we're getting back on time here. We're good. I want to welcome Peggy Iverson, who is an Alpha I patient to give the Alpha I patient prospective.

MS. IVERSON: Good morning. My name is Peggy Iverson, and I'm from Des Moines, Iowa. I'm grateful for the opportunity to be here today to tell you what it's like living with Alpha-1 Antitrypsin Deficiency. I understand everyone in this room has the opportunity to make a difference for those of us suffering from this rare disease. While I may look like a healthy 66-year-old, I suffer from a chronic rare genetic condition that affects my lungs and may affect my liver. I am one of the few lucky “Alphas”, as we call ourselves, because I am not currently

dependent on oxygen. I'm not tethered to a large oxygen tank in my home or forced to lug one behind me to perform my day-to-day activities, as so many in our Alpha-1 community do. I am not living with painful, frightening liver issues, as so many in our community do. I have not received a lung or a liver transplant as so many in our community have required.

I am one of the healthier Alphas and I'm here today to represent the Alpha-1 community, all the individuals who can't stand here before you today. You see many of their faces on the image behind me, unable to tell you how much they have suffered from this genetic disorder. I'm deeply saddened to see how many of these amazing people are no longer alive today, due to this devastating condition.

My journey and early diagnosis are not typical. Most Alphas receive a correct Alpha-1 diagnosis after seeing an average of 3 to 5 doctors. By then, significant lung damage has already occurred. Because Alpha-1 is a genetic condition, it's also a family condition. My uncle, a smoker, died at age 40 of severe

emphysema because he didn't know he was an Alpha. My beloved mom, who never smoked, died at only 54 years old, in the prime of her life. She died while I was pregnant with my first child. She died never getting to meet her grandson who was born only 8 days later. My mom was the 36th Alpha diagnosed at the Mayo clinic in Rochester, Minnesota, in 1974. Because of her correct diagnosis, they knew to test my brother and myself. I was correctly diagnosed at age 21 while I still had completely normal lung function.

After my mom's death, I enrolled in the first Alpha-1 NIH study. So being here today feels a bit like having come full circle to me. At approximately 34 years ago, I was here in that very first study. I was then followed by a clinical resource doctor in Iowa. He knew 14 years ago when my lung function began to decline to start augmentation therapy for me. I never had to lose significant lung function. I am one of the lucky ones.

As a parent of two sons who carry one deficient Alpha-1 gene, I understand the fears parents of Alpha-1 liver effected children

suffer. I have witnessed the agonized faces of parents who learn their child has been diagnosed with something they've never heard about. Imagine being a parent of a child born with Alpha-1 Antitrypsin Deficiency and having only two options. One, to wait and see if the child gets better, or two, wait for an organ transplant to receive a new liver.

In Iowa, I told an annual Alpha-1 fundraising event. Several liver effected children and their families participate and share their fears about what the future will hold for their precious children. I have seen in the eyes of the Alpha-1 kids they already have fears and anxieties. They want nothing more than to have normal, healthy childhoods.

Alphas face fears every day when they are diagnosed with Alpha-1. What does it mean to have a rare chronic condition that has no cure? Will I be able to afford the treatments that I need to keep me healthy? Will my health insurance cover the cost? What if I get sick? I am an Alpha-1 peer guide that listens to Alphas in various stages of their disease.

An Alpha-1 diagnosis typically strikes in the prime years of your life, during the fourth decade when you're in the peak of your career, with young children and a bright future ahead. Many of you in the room may be in your peak years right now. You might be an Alpha and don't know it. Many Alphas become disabled and are devastated by this debilitating diagnosis.

My life has centered around infusions to keep my lungs as healthy as possible. Once a week I receive my therapy and hope there is not a shortage or a recall and that the plasma-based product entering my blood is safe. I pray that my veins will stay strong and I will not have to resort to a port in my chest. I hope that my insurance company will continue to provide coverage.

It is extremely challenging to afford the high cost of weekly IV augmentation. Because we have a rare disease, the companies who provide our therapies experience cost challenges to generate revenue. Cost and access to care challenges affect the entire Alpha-1 population.

For the past 14 years, I've received my

weekly infusions. That's 52 infusions a year. 52 multiplied by 14 years, for a total of 728 infusions. 728 augmentation therapies thanks to the plasma donated by countless donors.

I was able to know when my lung function began to decrease and get on treatment immediately. I was able to protect my lungs because of my early diagnosis, but most are not as lucky. Some Alphas are lucky enough to have a home health care nurse come to their home to administer treatment. Many of us travel to infusion centers which adds extra exposure risk during flu and cold season from other patients. We are very grateful for the current therapies we do have. We know that not all rare diseases have specific FDA approved treatments. We have the benefit of the Alpha-1 Foundation who has organized a scientific infrastructure to support expediting the review of new therapies and put us in a position, so I am able to be in front of you today.

We need new therapies in the rare disease space. We need change for our families and for our future generations, an answer leading us

closer to a cure. The Alpha-1 community is a family of strong advocates fighting for each breath. We're a dynamic, motivated group that will participate in clinical trials to ensure that we are working together to find a cure.

We understand that the FDA has the opportunity to make a difference. As an individual personally affected, I have come to ask you to take responsibility in insuring new and innovative therapeutic development is available for Alpha-1 Antitrypsin Deficiency. We ask you to give Alpha-1 patients that suffer from this horrible disease access to new, safe, next generation products that enter the marketplace.

When you can't breathe normally, everything becomes challenging. The quality of your breath equals the quality of your life. From walking up steps or hills, exercising, taking a shower, carrying groceries, traveling with an overall lower energy makes life hard. You always have to consider the environment when making any plans to avoid risks from pollutants, smoke, and anything that will further damage your lungs.

Or when you are liver affected, and

faced with ascites, intestinal bleeding or simply waiting to be sick enough to even go on a transplant list. Imagine that - hoping to become sick enough to qualify for a transplant, and then waiting to receive one before you are too sick.

My journey may be different, but my future is the same. I am an Alpha fighting for the next breath. I rely on my family, friends, and our Alpha-1 community for support and inspiration every day. We are in this fight together. I am relying on you to help change the future of Alphas. We need better therapies. Alphas are dying. I have lost family and many dear friends to this debilitating disease.

Through support group meetings, education days, national conferences and mentoring, the community is strong, and we are highly motivated to be part of the steps to get us to better answers. We are all empowered to live our best lives and fight for that each and every day. Decisions made here today impact my life, my family and every individual with Alpha-1. We are counting on you to do the right thing. Thank you so much.

DR. MARKS: Thank you for that perspective. With that, I think we don't necessarily have questions for that. Why don't we have Dr. Wanner come up and we'll get his slides up here and hear about novel therapies for Alpha-1 Antitrypsin and novel targets.

DR. WANNER: Thank you very much for the invitation to be here with you and talk about novel targets for Alpha Antitrypsin. It could be rephrased as using Alpha Antitrypsin for treating patients who do not have Alpha-1 Antitrypsin deficiency. I have no conflict and I'm going to talk about an aspect of Alpha-1 Antitrypsin that I think goes way beyond Alpha-1 Antitrypsin deficiency that has the great potential for use in the future.

What do we know about Alpha-1 Antitrypsin? It's a positive acute phase reactant which means that in response to a tissue injury and inflammation and infection, the serum levels of this protein increase. It is a potent serine protease inhibitor and it has been used therapeutically because of that action. However, it is also a caspase inhibitor and it has some

anti-apoptotic effects because of that. Then finally, it has immune modulatory effect and anti- inflammatory effect that are purely defined in terms of mechanisms, but I think there is now ample evidence to suggest or show that these broad anti-inflammatory actions exist and may actually raise the possibility of using alpha antitrypsin as a therapeutic agent for conditions not associated with Alpha-1 Antitrypsin deficiency.

How about the acute phase reactant response? I'm showing you 2 examples of what this actually looks like. On the left-hand side of the slide, I'm showing a study by Sanford and colleagues who looked at patients with open heart surgery and obtained serum levels of Alpha-1 Antitrypsin before and 45 days after surgery. I show that was about a two-fold increase in serum levels after surgery.

Going to inflammatory conditions, I'm just showing you data from a relatively small study that is how we looked at Alpha-1 Antitrypsin levels and serum in stable patients with COPD and patients who had acute exacerbation of COPD. There you can see the levels are higher on stable

COPD and significantly higher in acutely exacerbated COPD than in healthy controls. The investigators also looked at Alpha-1 Antitrypsin concentrations in the exhaled breath in the same subjects. You can see that there was no difference noted between healthy subjects and stable COPD patients, but in the acute exacerbated COPD patients, there was a wide range of increased and actually the majority of the patients showed an increase in the level and the mean was different from the stable COPD patients Alpha Antitrypsin as a therapeutic agent in Alpha Antitrypsin repeat individuals, then you should know something about the frontal genetics. Unfortunately, such data does not exist at the present time. The only data we have is in patients with Alpha-1 Antitrypsin deficiency and one such study is just shown here that looked at 2 formulations of plastid in patients with severe Alpha Antitrypsin deficiency. It is shown that after infusion of the protein there is a 3- fold increase in the serum level of Alpha Antitrypsin is a subsequent explanation decline and reaching the baseline level of pre-administration level

after that week. This is actually the basis also for current therapy for this Alpha Antitrypsin in patients with Alpha Antitrypsin deficiency.

We do not know if the curve will look the same in individuals who have normal resting Alpha Antitrypsin levels. It clearly would be different, but we need this data before we really embark before using Alpha Antitrypsin as therapy patients' individuals who do not have Alpha Antitrypsin deficiency.

Now, what is the evidence that Alpha Antitrypsin is an anti-inflammatory agent? I've got to show you the data and the sediment level and the tissue level and finally, the whole organ level. In this study, they looked at the effects of Alpha Antitrypsin on the expression and release of TNF Alpha, the propit inflammatory cycle can and the anti- inflammatory cycle can in healthy human models. Looking at the 6-hour time point, you can see that LPS increased what you had trial for. It also increased aisle 10 and this increase for both was generated in the presence of Alpha Antitrypsin. That's the expression. We went looking and the release of Alpha

Antitrypsin - of these site proteins. Again, I am focusing on the 6-hour point. She is in the middle of the panel on the right. It's the 18-hour time point. You can see that LPS increased the level of Antitrypsin Alpha and to a less extent, the level of IL-10 at 6 hours and this was sustained for 18 hours. Interestingly, when looking at IL-10, the combination of Alpha-1 with the LPS actually lets to an increase of this anti-inflammatory cycle. In contrast, what will show with the pro-inflammatory cycle of TLS Alpha.

Now, moving to the tissue level and here I'm considering whole blood as tissue, these investigators looked at the effect of Alpha Antitrypsin on the side of concentration of whole blood, which was activated with heat, deactivated scratched at the epidermis. They looked at several site occurrences again. You can see in the top part of the slide that for those site occurrences listed there, the type of situations of Alpha Antitrypsin, there was a dilution of the concentration. Interestingly, in this study IL-10 was not affected by Alpha-1 Antitrypsin.

Then moving to the Morgan level, they

have to go back to the patients with Alpha Antitrypsin deficiency because they formally have no data on the effects of Alpha Antitrypsin on these inflammatory markers in individuals who are not deficient in Alpha Antitrypsin. So, Michael Turnquest conducted this pilot study where he treated patients with Alpha Antitrypsin deficiency for 4 weeks with 60 milligrams per kilogram per week, followed by 4 beeps of a double dose and then going back to 4 weeks of the usual 60 milligrams per kilogram per week dose. The obtained trust levels of Alpha Antitrypsin are shown on the left-hand side of this slide. As you can see, there was a marked significant increase in the trust level during the top of those and that returned as expected back as a pre-topical period after the double dose was discontinued and replaced as the single dose.

This study is quite remarkable in my opinion because it really shows the profound effect of topping the dose of Alpha Antitrypsin infused into this patient on a whole variety of sediments. Almost every second time they measured; it shows a marked increase through the

double dose period. Also, very impressive as far as my interpretation of the state of the concern is that this effect was sustained during the subsequent 4-week period when the dose was reduced to the usual low dose. In this study, IL-10 was also decreased by the doctors. We don't know whether or not this would also be observed in individuals who are deficient in the Alpha Antitrypsin, but it certainly suggests that there is an anti-inflammatory effect of Alpha Antitrypsin in these people.

So, based on all of this, what conditions have been considered for Alpha Antitrypsin therapy? Type 1 diabetes, organ and cell transplantation, cystic fibrosis, inflammatory bowel disease, rheumatoid arthritis, acute myocardial infarction, I don't think this is necessarily a complete list, but it certainly is already a very long list. I would like to just show you some data on Type 1 diabetes, organ and cell transplantation and cystic fibrosis. In Type 1 diabetes, we have animal studies and human studies. I would like to show you one representative study in each of these

species. This study, which was published in 2008, I think was the first definitive study that showed that Alpha Antitrypsin is effective in controlling blood sugar in new onset diabetics. The study was conducted such that the treated mice were given 2 milligrams per kilogram intraperitoneal Alpha Antitrypsin every 3 days for the duration of the study, which as you can see was 270 days. The two groups were either insulin treatment plus Alpha Antitrypsin or only insulin treatment. You can see that the percent of euglycemia rapidly declined in the control group and despite the fact that these animals were treated with insulin, they eventually died because the blood sugar could no longer be controlled presumably due to insulin resistance. Whereas those animals represented in the red line had no animal directly became infused at glycemic levels up to 90 percent of them. So, this really shows that there is some kind of anti-inflammatory in the new modulatory effect of Alpha Antitrypsin with this particular model of Type 1 diabetes.

Moving to humans, there is this small study that was conducted by direct mail in 2016

and they used Alpha Antitrypsin in children and adolescents with recent onset Type 1 diabetes and they treated them with 45 milligrams per kilogram per week for 6 weeks, followed by 90 milligrams per kilogram per week for 6 weeks. When you look at the outcomes on the left-hand side of the slide, the total days for which they received therapy was between 24 and 20 days. In other words, all the ages are left of the grant. The 3 outcomes that they looked at were the 2-hour C peptide response, the A1C level and insulin use. If you look at the end of the treatment period, its kind of coincides with a decrease in A1C and the decrease in insulin use. This was the case in the adolescents as seen on the left-hand side, as well as the children with recent onset Type 1 diabetes.

Again, we have animal studies here and human studies as well. This animal study looked at acute graph versus host disease in bone marrow transplantation and the treatment consisted of 2 milligrams Alpha Antitrypsin intraperitoneal daily from Day 2 to Day 13. The outcomes are shown on this graph. Now, if bone marrow from B-6 mice was transplanted into B-6 mice, there was an

almost complete - I will mention the top across on this slide. On the other hand, if bone marrow from reasons different strength was injected into B-7 mice.

And the mice were treated with placebo which was albumin. There was a marked decrease in survival and after 60 days, the survival was only 40 percent. On the other hand, if the albumin was replaced with Alpha Antitrypsin, the survival was about 75 percent. So, again there was a difference here between the target ones that Alpha Antitrypsin treated and the placebo treated animals. We are so lucky we made our reservations back in the spring.

How about human studies? Just one study that I'm showing you here, this one looked at steroid resistance acute graph versus host disease in human bone marrow transplantation. They looked at 40 patients and the patients received 8 spaced doses of 60 milligrams per kilogram Alpha Antitrypsin over 4 weeks. The graph shows the responses over that 4-week period. They looked at these patients at terms of either complete response, partial response, no response

or progression of disease. If you go from the beginning to the end of the study period, you can see that complete responders increased. Partial responders also increased, of course, as expected. Therefore, non-responders decreased, but there was also a small group that actually had progression of disease despite the augmentation of the therapy given to them. This study is not placebo controlled. So, obviously, it has to be looked at with some reservation, but I think again, it suggests there is a potential role for Alpha Antitrypsin as an anti-inflammatory and a new modular entry agent in a condition such as bone marrow transplantation.

How about cystic fibrosis? No animal studies. There is one human study that is a positive study. I would like to show that one for you. This was conducted by colleagues and they had the hypothesis underlying this study that I lL8 is released from lung cells. It recruits nutria fills into the lung and the nutria fills in the last days and that will damage and contribute to the pathophysiology of CF. Then they hypothesized also that Alpha Antitrypsin would

inhibit the release of IL8 in the lungs and would also antagonize Nutra fill elastics. So, again, 25 milligrams per kilogram Alpha Antitrypsin by inhalation daily for 4 weeks. No placebo control was included in this study. I looked at the sputum age concentration, neutrophils and sputum in the last days activity and you can see here as a result of the treatment, they found that there were significant decreases in all of these Alpha ranges.

So, the beat goes on. There are still current trials with Alpha Antitrypsin for conditions not associated with Alpha Antitrypsin deficiency. Of 24 trials, I actually found 20 such studies listed. The conditions are shown on the left- hand side. This is Type 1 diabetes. This is host disease, organ transplantation, CF and even HIV. The concept on the right, using Alpha Antitrypsin in HIV is that viral entry into the cell requires the serum protein. That serum protein can be antagonized or attributed by Alpha Antitrypsin that would actually reduce the infection of this disease.

The phases of these studies are shown

in the next column and then moving to the right, you can see that 4 studies are still recruiting at the present time. 11 studies have been completed, and 5 studies have been terminated, either because of futility or maybe for some other reasons, but I don't think any of these studies were terminated because of adverse events associated with the administration of Alpha Antitrypsin. So, again, a total of 20 studies that are recent or current.

There are issues that obviously should be considered, that maybe a topic to discuss back in a panel discussion that would take place at the end of this conference. One of them is the availability of product. So, if a broader group of patients, actually available or can be elected for this kind of therapy, the supply at the present time is human plasma device, may have to go some stretch. This raises some question of maybe reactivation of efforts for big companies' product or maybe even transparent to go into plasma to become successful. Finally, the issue of aerosol should be discussed as well.

So, in summary, Alpha Antitrypsin is an

acute phase reactant, is broad anti-inflammatory and has huge monetary reactions. I think there is evidence for that. Raising Alpha Antitrypsin levels in serum and tissues both normal, is a valid therapeutic goal in several Alpha Antitrypsin repeat clinical conditions. Exploratory studies are ongoing, as I have shown you. Again, to my view, product availability may become an issue in the future.

Thank you very much for your attention.

DR. MARKS: Questions?

SPEAKER: All right. Thank you. Yes, there is the implication that neither of the diseases that are known here. This could also be a week on the common readings of the Alpha. There is no proof or an example, if the glucose control operating off the Alpha-1 Antitrypsin patient. Do we know that? Is diabetes more frequent there?

DR. WANNER: I'm sorry. I can't understand the question. There is an echo.

DR. MARKS: The question was could this open some of the pathogenesis such as Alpha-1 Antitrypsin conditions like glucose regulations for abnormalities.

DR. WANNER: I think so. All of this will have to be shown in the future. I mean right now we have very minimum data, as you saw, in terms of understanding how Alpha Antitrypsin would work beyond its division. That could apply to Alpha Antitrypsin deficiency as well. So, I don't think we have to think that serum protein is deficient in the only way Alpha Antitrypsin works in patients with Alpha Antitrypsin deficiency. I think some of these studies being ongoing will hopefully shed some light on how Alpha Antitrypsin works in deficient individuals.

DR. MARKS: Thank you. Any more questions? With that, we are actually pretty much on time. Just a couple of quick things. Correction on the eating arrangements. We have conference rooms, J, H, and C, too. They're down on this level to eat in. Apparently, there are multiple conferences going on, but there are lunches out back. Thank you to Alpha-1 response right now. Enjoy lunch. Maybe everyone will try to aim. It's just noontime just now. Let's try to aim to be back here about 10 minutes of 1:00. That way we'll actually depart on time. 1:00.

This afternoon we have a kind of tight agenda and we'll try to stay on time. It will be a little fluid on how we run things this afternoon, because we have quite a number of talks packed into a short time. Thanks very much and enjoy lunch.

(Recess)

DR. MARKS: Okay. This audience gets the compliance award which is saying something coming from FDA. So, thank you for coming back very promptly. We're going to go ahead and get started. This next segment of five presentations on novel therapeutic approaches the grays levels of Alpha-1 Antitrypsin. We have a variety of different presentations here and I think they should be very thought provoking.

We may run a little, teeny bit long here, but we'll make it up between the break and other places in the afternoon, and I promise you we'll be done by 5:00. So, without further ado, I'm going to introduce Dr. Terry Flotte who's going to talk to us about gene therapy.

(Applause)

DR. FLOTTE: I will try my best to stay on time. So, these are my disclosures I do have

two relationships that relate to the editing, but that's not what I'm going to emphasize today. So, I'm not going to spend much time on this slide since we are talking to a very educated audience in this case. But just to -- the two things I really want to point out here about the pathobiology of this disease is that it's really two different diseases, right.

So, there's a lung disease due, we think, generally to the unopposed action of various proteases and other pro-inflammatory substances which in the absence in the absence of Antitrypsin will exert its effect and cause damage to lung elastin, a loss of lung elastic recoil which can be measured in various ways. And this is really a loss of function.

It is co-dominant, of course, because there is an increased risk in heterozygotes, but it's a loss of function. And the liver disease is generally attributed to being due a gain of function. So, there are two different considerations here, they don't actually act entirely independently, but we will come back to that later on. I think individuals more

conversant and learned than myself have already addressed this issue of what has been set as the target level on which previous molecular therapy approaches and augmentation have been approved.

And the only point I want to make here is that most of the territory in here is compound heterozygous individuals. And so really this scenario of gene therapy, where one is augmenting wild-type Alpha-1 antitrypsin continuously through continuous release against a background of ZZ has really not -- really isn't a natural experiment, or a national parallel to that.

So, the thorny issues we face -- so, I've been working on gene therapy with -- for a long time. Been in the field since the late '80s, started on this campus mostly all in genetic diseases. And the thorny issue for gene therapy in this disease is really what is the end point? And particularly, because at the very least we can agree, a very important pharmacokinetic and pharmacodynamic influence, the plasma level.

But the one issue is, what is the significance of the plasma level? And I'm going to present some new data here from Chris Mueller's

lab. He is here and could comment on it later. There's a discussion, but evidence from the Null mouse produced in his lab was in collaboration from us and others with CRISPR, that was published, but there's now correction here that's not published, but I'll show it.

The second piece of this is that if we are trying a gene therapy that would achieve a high plasma level, as an initial approximate point at the very least, how do we achieve that, and how in particular do we achieve that while avoiding injuries of the liver. That is really the focus of our NHLBI funded program project manned at UMass. And I'm really presenting on behalf of everyone whose part of the UMass team that I have the privilege to work with.

All right, so this issue number one. What about the plasma level? I'm going to be present clinical data just to find that the plasma level -- we'll present data from the Null mouse and what we see, or what Chris' lab has seen, with 20 percent achieving roughly 20 percent or even a little less than 20 percent of the target level, namely, correction of the protease imbalance and

correction in the inflammatory phenotype.

So, this is the Null mouse, again, I'm trying to stay on time. This was published in PNAS last year, but basically, CRISPR technology has allowed for the knockout actually on both mouse chromosomes of all five of the Serpinal 1-A genes. And, in fact, that confirmed really going all the way, you can confirm by the absolute absence of Null phenotype of Alpha-1 Antitrypsin protein.

These mice develop a lung inflammatory state and emphysema, either with age, or the pro-inflammatory stimuli. So, you can see with age here you start to see a difference between wild type and normal and various cell counts in the BAL lymphocytes, monocytes, macrophages, at the 42-week age.

You also see, as expected, even at the beginning, you see an increase in the free elastase activity. But that goes up over age, obviously, there is also this increased burden of cellularity. It's the anatomical emphysema and this was measured morphometrically in the, and you can read it, but I think just scanning the

average is very nice to see it.

And then, physiologically we have established at UMass, a pulmonary physiology core for mice. So, we can do all kinds of different physiologic testing in mice. But really the hallmark here if you look at the pressure volume loops, these are the younger mice and then after introduce, or you can see something similar with inflammation induced emphysema. If the black is the normal type pressure volume in relationship, you see an abnormally increased compliance, which is really the -- within the definitional aspects of emphysema. You're losing lung elastic recoil, having an abnormally increased compliance shown here in the knockouts over time. So, yes, this is a model, a Null model of against emphysema. Also, gets emphysema with various other stimuli and they're working on smoking and the like. And Chris from here, are also there in the fourth row can answer questions about that, but the key thing for gene therapy and the level is the end point is that you can take a basic vector here and put it into the mouse IV they get expression. When you get the expression of

around 100 micrograms per mil, which -- but it's all wild-type. That level, we say our target is about 570 micrograms per mil, or at least that's the micro molar target. This is what you get from the gene therapy. And if you look at the free elastase activity in the serum, obviously, its high in the nulls, and the treated come down to a level that's not statistically different in normal.

And then very importantly, on that physiological test of emphysema, what you see is the age related change between 35 and 50 weeks of age in the mice; the age-related change that you see in the untreated mice where they're continually increasing this abnormal increasing in compliance that is really halted, or largely halted by the AAV gene therapy.

The point again, is that the level that's even lower, at least, it's a mouse model, but a level that's even lower than what we have been targeting for in our gene therapy programs is resulting in both biochemical, physiologic, and I'm sure we're going to get anatomic confirmation of that as well.

Now, I have, in timing myself here, I think I'm going -- I'm going to have to really speed through this, but just to say, NHLBI has funded us with three different ways to put the gene in that we think will be liver sparing. We think this is very important, because if this is the liver of your basic PiZ mouse, this is not the Null mouse now, the PiZ mouse, if you put an over expressing Alpha-1, normal Alpha-1 Antitrypsin expression back into this liver, its subclinical liver disease is exacerbated. So, you can see Apoptosis and large, super large accumulations of the protein in which we see interaction between the wild type and the mutant protein. And you see increasing liver enzymes.

There's a timing relationship with age here, but the point is, we want to avoid the liver. The simple way that we started with was targeting the muscle. Again, I really have to run through this. But it seems overly simplistic, right. You put the gene in a muscle and the protein is secreted. But it actually is. I mean, why this seems simple to take an AB- 1, inject it IM to secrete a protein, this was actually the first --

the platform of the first gene therapy approved by the EMA in Europe. But the AAV1-lipoprotein lipase, it was never really marketed. I think one patient was treated commercially. But this can be done. The problem is getting enough of the protein. And it ideally puts -- makes one injection replace the weekly infusions.

We show a very nice post response with this. The problem is to put that much vector in IM, we had to get up to 100 IM injections. This is the so-called pincushion method. Again, complex reasons this vector really can't be concentrated into a smaller volume, and by injecting 1.35 mils per injection site, we were able to get with 100 of those, 135 mils of vector. We were able to get up to a level that's conservatively we say two and a half, it actually peaks at 5 percent in this steady state here is more like 3 percent. This is the top dose cohort.

Now, just a point here. These patients were not treated with any immune suppression, unlike what is being done in the hemophilia trials, et cetera. And another thing we published back in 2013, but when we used this

vector this way we see a predominant T-reg response. These are AAV capsid specific T-regs. And I refer you to the paper for details.

But we just now published the five new data and the same patients, this is a lower dose cohort. These same patients are still going strong except for the ones who opted to go on protein replacement, they're still going strong here at five years.

You see continued immunostaining showing brown in the muscle. The cellularity of the muscle goes down, but the proportion of T-regs, which is the yellow bar, stays the same at 10 percent of the cells. Interestingly at least one of the patients who was in this cohort, if you look at the antineutrophil elastase capacity for the interval between gene therapy and when they went back on protein replacement therapy had effective normalization of their free elastase activity.

This is different again in looking at SC patients or even MZ patients, because you're overexpressing the wild type protein on the back end. So, 2.5 percent, 3 percent, let's say you

want to do 30 times the dose, you can't give 3,000 IM injections. Alicia Gruntman (phonetic), in our group, a very hardworking veterinary physician-scientist, has developed a venous limb perfusion method. I won't go through this in detail, because I am, I think basically over my time. But just to say, if you give the vector, you can effectively get with this limb perfusion method, you can effectively show improved expression. Most of the vector will stay in the muscle, not go to the liver, get higher expression, per vector genome, and thus, we're planning to increase the dose response. We're going to bridge this, we've had an INTERACT meeting and we're going to do this pharma tox study.

So, I do want to talk about the other liver-sparing approaches just briefly. So, largely with Chris's lab now, licensed from UMass to ApicBio, we have a dual function technology that can be given two ways, either with a straight AAV vector, or knocked-in to the albumin locus.

What I mean by a dual functioning vector, is the vector that is given as an AAV has

a synthetic microRNA in it, and it's been retargeted to knock down endogenous Alpha-1 inhibitor or mutant endogenous Antitrypsin in this case, and an augmentation allele, basically an allele that's rendered resistant to the micro RNA.

Back in 2012, just then in my lab published that one injection of this guy, you can see a substantial and stable increase in augmentation from here well ending in 80 to 95 percent, citing 85 to 90 percent knocked down stable over time. Based on this proof of concept, Apic scaled this up to the primate level. The primate vectors a little different because it has a myc tag to look at this. But basically, you can get levels here that are getting back up to that 20 percent of the level micro molar target even a primate.

And this can also be given with a technology that uses AAV homology arms, to knock into the albumin locus, again this dual function with an Alpha-1 Antitrypsin and the synthetic micro RNA. And so low efficiency, but you see, we do see expansion over time. This is also

published, I'll refer you out to that.

I'll just say a word to say Wen Xue (phonetic), in our group, is also doing a more classical gene editing, a Cas9 mediated HDR, to reconstitute a wild type. This has also been pursued by some commercial interests. And that product is obviously at an earlier stage. Both of the dual functioning constructs are actually approaching clinical stage and certainly with five years in the clinic AAV-1 with a phase two of the AAV-1 IM, but we are bridging it so there's no confusion, we hope we'll go soon.

So, our have a hope for today, it's a modest hope, is to know what's the advocacy end point that we're shooting for? What we have seen -- I'm in the working Alpha-1 for quite a while, but I also work on gene therapy for a lot of other genetic diseases.

And people with these rare genetic diseases are seeing the gene therapy technology because of breakthrough benefits to them. That's certainly been the case with Luxturna for patients with inherited retinal dystrophy. And we're seeing that in our work with patients with

Tay Sachs disease.

And in those gene therapies, it seems like it's a simple genetic condition. And the efficacy end points seem to be viewed in a little more straightforward way, but I guess from my point of view, this is a genetic disease that certainly deserves to have the benefits of an AAV therapy, at least try, relative to the therapy.

And we need to know where the goal line is to pursue these therapies. So, I'll end right here. Our whole illustrious UMass group and I presented all their data, so I have to credit all of you. Thank you. (Applause)

DR. MARKS: We'll going to take questions for all five people at the end. That will help us get through this, so you get to hear all the presentations. So, now, thank you, Dr. Marciniak will present.

DR. MARCINIAK: Over the next few minutes, I'm going to talk about the complicated title of my talk. But basically, can we target the endoplasmic reticulum, which is where Antitrypsin is laid in order to fix or at least alleviate part of the problems.

Although, I'm not aware of having any financial interests in these. I don't know about my pension scheme, but I'm going to say -- I'd like to say that I am going to present some public, unpublished data. The slide initially says, so please don't share it on social media, and then I found out we're webcasting it, so that one is already gone. Okay, so just to remind you, I'm going to be the nerdy sub-biologist to just remind you that Antitrypsin like all secreted proteins are still made in the cytosol. So, the ribosomes exist in the cytosol, and as the new protein is made, it is extruded through a core protein into the ER, the endoplasmic reticulum, which is where the secretion proteins are folded.

These proteins don't fold spontaneously, they need to interact with complex machinery of chaperones. And are the enzymes that we're interested in. Alpha-1 Antitrypsin is a glycoprotein and has glycan added in the ER, and after appearing in the ER it is glycosylated, and then these like glycans are manipulated slightly to make -- to make it visible to these so called lectin chaperones. There is trimming of

these glycans.

They work with other chaperons, co-chaperons, the names of which I won't go into today. But then this might lead to folding. There is then trimming of these glucoses that morph, these glycans, or the glucose is lopped off and it can no longer interact with those chaperons.

But it might not be folded. If it's not folded it has to be bound by other chaperons. And probably the most abundant chaperon in the ER is called bed, it's basically HPS of the ER and it binds to exposed hydrophobic residues that are folded to the protein.

If it's not folded another enzyme identifies it and it goes back to that and it cycles between these and other chaperons several times until it is folded into the correct form. If anything should fold seven cycles, it passes quality control checkpoints and leaves the ER with secretion. And that's what it should do.

But even, it's always never that simple. And even a while time type Antitrypsin has a chance of misfolding. If it's cycling in

this chaperon cycle for too long, the cell gets fed up and targets it for degradation. If misfolded protein which will accumulate within the ER, this is very toxic for this. It's called an ER stressed. And it's recognized by a number of ER stress sensing enzymes.

But most misfolded proteins, or our Antitrypsin which hasn't folded properly, or many of our mutant proteins are recognized by this complex assortment of enzymes, which then return the protein back to the cycle from whence it came for degradation. This is called ERAD, or ER associated degradation.

It's probably the most important way of getting rid of even the Z allele, the Z allele, because that protein before it polymerizes, even though it doesn't directly cause ER stress it recognizes it's no folding well enough, and it's returned to the site of the degradation.

However, some of the antitrypsin can polymerize and form larger structures, which are very unusual to any of our biologists like me, because they look folded to these sensors, they don't cause ER stress, but they don't pass the

quality control checkpoint for secretion, and they build up. And we saw that beautiful picture just now of if you express too much of the wrong sort of antitrypsin, it expands the ER to all those massive inclusion bodies.

But we create very large structures with processes such as autophagy. Autophagy is where the cell actually eats part of itself. It wraps a target, the damaged organelle, it's a membrane causing to them something of an autophagosome which then fuses with an elastase for degradation.

So, we've ERAD and autogenesis, this is just in more detail. What's interesting is that these things, they accumulate, they don't cause ER stress, but they make the cell more vulnerable to stress. And this is probably the origin of the liver disease in alpha-1 antitrypsin. And why it doesn't happen to everybody? It sets the liver up to be sensitive to a second hit. So, we do want to get rid of those to protect the liver.

Now, this is the work or lots of work that's all rising just to put a slides, and there's lots of people. But we know if you purify

protein in the lab, it just hasn't, it just tends not to be very stable in solution. So, you add to various chemicals, crowding agents, like glycerol, which keep proteins in solution when we're freezing them.

Remarkably, none of these chemicals, just as a cluster of cells, it will stabilize proteins within them, and you get increased antitrypsin folding. There's a whole host of small molecules that you can add to cells in culture that will promote protein folding. And these fall under the name chemical chaperons, but I would argue this is a really terrible name for them because we know that not all of them interact with their client protein. The most used, most studies, this one 4phenolbutyric acid (PBA) asset which increases the folding all sorts of proteins like CFTR and antitrypsin, it probably doesn't bind to the target protein, it probably regulates antitrypsin. It's been called the chemical tractor. Sadly, it's been tried in humans and it doesn't work. At least on a small number of humans for only a two-week study, there was no increase in the antitrypsin in circulation, and

there was never improvement in metabolic markers over that development. Very small study, very short period of time, but it's -- there were side effects and so this is probably not going to be revisited.

And there are many, many of the molecules that do this. So, there is -- it is worth pharmaceutical companies revisiting the possibility of could they find a chemical chaperon, that whatever we call them, that they can treat cells which should increase protein folding that won't be toxic to humans.

We could do it in even a cleverer way. Bill Bulger's group has taken the approach of sort of inhibiting histone deacetylase 7. If you inhibit, he showed, if you inhibit this in th cell, you increase the expression of at least seven chaperons inside the ER, at least in cells you increase protein secretion. So, that's great. Again, I'm not aware if it's been tried in humans.

We might say that we can't improve protein folding and gene therapy is going to fix -- going to provide us with enough antitrypsin,

let's just get rid of the damaging Zed-antitrypsin that's accumulated in our cells. Well, one thing we can do is stimulate these degradation pathways. Autophagy is a very druggable pathway. We know that drugs like Rapamycin can activate autophagy and early studies suggested that if you treat cells with -- with rapamycin we could clear more Zed-antitrypsin. Those papers were published by others. It might be a cell time specific issue because we can't reproduce the finding in certain cells.

However, there are large numbers of small molecule screenings using cells and more animal models looking for molecules that reduce the accumulation of misfolded proteins. For example, these two moods stabilizing drugs were shown to reduce the amount of Zed- antitrypsin polymers in cells and in a model system. So, this really may be a druggable pathway.

I'm very excited. We know that the ERAD is probably more important than autophagy in getting rid of Zed- antitrypsin. And David Paul Martin's group showed almost 10 years ago, that

he could activate both autophagy and ERAD with carbamazepine, which we all use, which we're commonly using.

It also clears antitrypsin polymers from the liver of the mouse. Unfortunately, he needed far higher levels of drugs than we can safely give to humans. So, although those aren't the chemicals -- the drugs that we're going to be giving to patients to cure antitrypsin deficiency, or at least clear the polymers from the liver, it does suggest a path forward. Now, I'm not going to mention just now about all of the work that's being done to try and find drugs that directly block antitrypsin glamorization, but maybe that will come up in the Q&A. What I'd like to do is in the couple of minutes of the slides is just revisit, what is the problem we're trying to fix? We all say that we want to get of antitrypsin into the site -- into circulation. And we want to stop it from accumulating as polymers in ER, but what are those polymers doing?

These are two images, this is a cell expressing antitrypsin, which is also a yellow marker there. And this is cell its expressing

Zed-antitrypsin which is a yellow marker there. And I highlighted the ER with this red protein. You can see that in all, this is rather low-resolution image. You can see the ER has this beautiful lace-like pattern. It's made up of innumerable interconnected tubules. And that's all the orange color is where we got both ER and the antitrypsin.

We see some antitrypsin by itself in the because this is normal antitrypsin which is folded properly and exited to the ER. If we look at a cell expressing high level, this is a cell in culture, this is not your hindsight, but this is a model where we express high levels of Zed-antitrypsin. You see we completely wrecked the ER. The antitrypsin polymerizes, it expands the ER, it loses its beautiful tubular structure.

These were originally thought by the degradation way for antitrypsin, this is actually the endoplasmic reticulum. So, we've lost the structure. So, we can't diffuse proteins from one side of the cell to the other, which is a normal process of protein folding.

We were interested in why those two

working on what's going on inside one of these inclusions. So, this is a single cell with the yellow antitrypsin and the red ER model. Well, we wanted to look at protein ability within these inclusions. What's happening?

And this is a really fun experiment because we bleached some of the yellow antitrypsin with a high-powered laser. And if the protein is mobile, we'd expect it to mix very quickly. But you can see what we bleached a shade with the antitrypsin, it stays bleached. We basically have a solid that's formed within the endoplasmic reticulum.

I sometimes sloppily call this gel, but my biophysicist friends say, no it's not a gel, because we're not cross linking it's a soft glass. So, if you learned one thing today, antitrypsin forms a soft glass with the endoplasmic reticulum.

It's not a simple barrier like glass on your wind screen, because proteins can diffuse within it. We show that lots of proteins can diffuse through this large matrix. So, is it slowing things down; is it increasing the

viscosity in the ER? There was no way of measuring the viscosity of the ER, so fortunately, with funding from the Alpha-1 Foundation we invented one.

We took advantage of these fluorescent molecules called molecular rotors, they have internal axis of rotation. And if you put them in different solutions of different viscosity, it changes their fluorescent characteristics. And to cut a long story short, we were able to target these fluorophores specifically to different organelles. And when we -- when that was published, but now, the unpublished work is if we look at a cell expressing antitrypsin, or just low levels of Zed-antitrypsin, and we look at the -- its colors so that the warm colors should hide mobility, low viscosity, and the cold colors showed increase viscosity.

So, antitrypsin expression within the endoplasmic reticulum makes the environment unusually viscous. This isn't simply to generate quick pictures, it's a highly reproducible assay. And so, we now have a toxic effect of antitrypsin, on the endoplasmic

reticulum for which we can search for small molecules to fix, because we think this is important.

We think this is important because it alters protein mobility. We -- how we measure individual proteins moving through the ER, this is a high resolution, the nature of a bit in the ER. And what we can do using the current generation of high-resolution microscopes is to take pictures at a rate of 250 images per second.

We can image individual molecules. What the computers do -- is doing here, is its mapping out the trajectory of individual molecules moving along these tubules in the ER. So, if I left this running long enough and I better not -- if I let this run, you would actually see, you'll recapitulate the map of the ER by the movement of its contents.

But we can extract the mobility from each one of those proteins. And this is really hot off the press data that I'm really excited to share with you. Because if we look at the cell expressing M-antitrypsin, this is a close up of the ER, we see that most of these small proteins

are moving really fast, about 60 micrometers per second, in cellular terms, which is really quick.

But if we express Zed-antitrypsin, even low levels of haven’t much distorted ER lumen, we see that we start seeing the proteins are moving more and more slowly. I haven't got time to show today, I really must finish the talk, but when we do this experiment with the rest of these chaperons, the effects are even more dramatic. And we're really excited to know whether there is some sort of side effects and there were some loan chaperons that can't even move through this soft glass.

So, let us stop. What I said today about summarize the data on small molecules, which is set to be chemical chaperons, but what they're doing is they're just increasing protein folding efficiency inside the ER. I said an alternative approach is to look at all those drugs that come from mode degradation of the polymers. So, at least we might be increasing antitrypsin in circulation, but we will be reducing the toxicity profile.

I've shown you probably what you

already knew, that Zed-antitrypsin often damages the integrity of the endoplasmic reticulum, but the very new data and this is two projects, both funded by the Alpha-1 Foundation and I've shown we now know that antitrypsin increases the viscosity of the ER to reduce protein morbidity.

So, I'd like to thank -- and most of this work was actually done by two post docs who did the early work, Jenny and Adrianna, Joe King's is doing all the current viscosity work. I thank -- big, big, big thank you to the Alpha-1 Foundation for making this possible. Thank you.

(Applause)

DR. MARKS: Thank you, again, thank you to all for being on time. It's so nice that everyone's being so good about the time, so thank you so much. So, now, Dr. Brantly is going to come up to speak with us.

DR. BRANTLY: Thank you very much. So, I thank you Peter.

So, since alpha-1-antitrypsin was purified, and we started giving it in IV and thinking about giving it inhaled. So, we've been doing this since the early '80s actually and

there's been a lot of effort about that. But I'd like to talk just briefly about programming standpoint, thinking what needs to be done. This is a different way of acting, and obviously going directly to the lung, which is a primary target in this particular case.

Number one, normally, antitrypsin travels from -- to the lung from the blood into the interstitium, into the air space. And held up with travel in the opposite direction. So, it's that opposite direction. And for antitrypsin to get to the alveoli, the particles to be less than three microns from the target area.

The answer to lung damage, I think we all know but I'm going to state the obvious. Lung damage, emphysema from doses of bronchiectasis, et cetera, inflammation is mediated by cells and neutrophils primarily in the lungs and its airways, but there's greater knowledge about that also macrophage's role. And more cells than any cause damage to the lung.

Now, requirements for an inhaled alpha-1 antitrypsin, number one, it's got get into the alveoli, first challenge. It has to be

functional when it gets there. It must inactivate neutral elastic believed to a central cause of damage. And it really must eliminate or reduce the number of neutrophils in the lower respiratory tract, so we know that that correlates with lung damage as well.

Two, stop lung damage from this standpoint. Now, the potential of Alveolar space by itself is, we now know from a lot of data for a long time IV Alpha-1 Antitrypsin is not a magic bullet, and the problem requires larger doses in some circumstances. Most Alpha-1 Antitrypsin that's given IV does not reach the lung compartment and an IV does do -- it reached the lung is in lower levels than epithelial lining fluid.

Inhaled antitrypsin has some potential advantage. It can be easily used, in other words, it can be inhaled rather than getting IV. It's direct delivery to the lung and theoretically the potential to having higher doses of alpha antitrypsin and the possibility of using alpha antitrypsin in other diseases just as Adam Warner suggested in the past as well.

Now, one of the components necessary to get into the clinic, number one, we need highly purified Alpha-1 antitrypsin when the early development programs of antitrypsin were done, early elastin, it wasn't purified enough and it wasn't active enough, but we now have several drugs on the market that are highly purified. We need high efficiency delivery devices as well because patients can't be available for an hour or so. We need to determine the safe and appropriate dose. We importantly, if we've been talking -- we'll probably talk more, we need robust outcome variables appropriate for rare disease studies. There has to be low toxicity, robust circuit markers to try to figure out what the dose would be and then just demonstrate with Alpha-1 antitrypsin that reaches the interstitial space, which is thought to be at least part of where the damage occurs.

So, the robust outcome variables of some of these things have been, we really have come up with them. So, we do have some better outcome variables, including CP and other things. We have highly purified Alpha-1 antitrypsin and

we have some great devices now that are available to deliver highly concentrated amounts of Alpha-1 antitrypsin into the lung from that standpoint. Indeed, there's been several studies, and I'm not going to belabor we've looked at inhaled studies with plasma purified material and with common Alpha-1 antitrypsin as well and I'm going to send a little data, with permission from Carla on the latest aerosol study that's been done just to tell you about some of the possibilities that might be done from that standpoint.

So, the first one of the major questions is, does it get into the interstitium and that's a difficult question to ask because basically you have going from plasma into the interstitial fluid and then finally, into the alveolar space and nobody knows what the normal range is for the interstitial space from that for that matter, from that standpoint. And so, does it get into the interstitium, and what I'd like to present this data is, that using an alpha, an antibody that specifically recognizes M, but not C alpha antitrypsin. We can do ELISAs which show, this is in one aerosol study, where we have baseline

Alpha-1 antitrypsin level of around 20 micromolar and with inhaled Alpha-1 antitrypsin, you get substantial increases in the blood for this. So, that really demonstrates that M Alpha-1 antitrypsin has gone from inhaled compartment into the interstitium, but into the blood and presumably had to transit to the interstatium from that standpoint.

So, let's talk about this most recent study on API and this particular, the study assigned was 36 subjects from two different sites at two different doses. Eighty milligrams once a day and 80 milligrams twice a day and each group were matched and there were two separate sites, Univ of Florida and Baylor, Texas, coming from that standpoint.

Six individuals were -- it was a two-to-one placebo-controlled study with six individuals placebo from that standpoint. The primary health and variable was levels antigenic and functional Alpha-1 antitrypsin in the epithelial lining fluid, but those that are not aficionados, epithelial lining fluid is that fluid that surrounds the alveoli, which is

calculated in a number of different ways, but one of the ways is you really going to reach the dilution method from that standpoint.

Secondary outcome variables included safety and tolerability, levels of antigenic of Alpha-1 antitrypsin and the specific Alpha-1 antitrypsin in the lung. Neutrophil elastases Alpha-1 antitrypsin complex. Neutrophil elastases concentrations in the ELF and then the counts of neutrophils in the lung as well from that standpoint.

So, in 80 milligrams per dose you can see there was substantial increase. Normal level being around 2,000 nanomolar concentrations, but in 80 milligrams once a day, it gets substantially higher. Close to almost 6,000 nanomolar concentration. So, very large doses, in single dose, in fact, there are about 17 times higher than normal individuals. In the 160 dose, they were even higher, not surprisingly from that standpoint. With doses approaching up to 25,000 nanomolar from these individuals.

When you look at the anti-neutrophil elastase capacity assay, which is a surrogate for

functional amount of Alpha-1 antitrypsin and remember, when you talk about functional Alpha-1 antitrypsin, you're not just talking about Alpha-1 antitrypsin but you're also looking at the proteolytic side as well. So, the more neutrophil elastases you have, the more suppression there will be of the anti-neutrophil elastase capacity. So, in this particular case, again, the 80 milligrams dose, you can see that the anti-neutrophil elastase capacity reaches around 4,000 nanomolar, well above the normal Alpha-1 antitrypsin concentration. And the same was actually true also for the 160 milligram doses as well. Its fairly large dose is nearly 10,000 nanomolar concentration so the epithelial lining fluid for the AAT-NE as well. Now, one of the key things in also demonstrating functionality is that in an individual that has Alpha-1 deficiency it's not a replacement therapy. They have free neutrophil elastases, but the number of complexes they have is very low because the late break limiting reagent is Alpha-1 antitrypsin in that situation. So, they have low complexes. However, if you give somebody that has an inflamed

lung they have neutrophil elastases functional Alpha-1 antitrypsin they complexes go up in that case. And then this is the thing, this is proof of principal that the active neutrophil elastases were bound by Alpha-1 antitrypsin. It goes up to about 40 nanomolar concentration from that standpoint. And the same thing is true again of the 160 milligrams with the even higher amounts of complexes going in from that standpoint.

Now, does the Alpha-1 antitrypsin that's given the wrong way cross into the interstitium. Well, we can't prove the interstitium. Nobody likes to have their lymphatics cathed, so we use the M-specific assay and we can show that there's substantial amounts of Alpha-1 antitrypsin given by the lung that appears in the bloodstream from that standpoint, indicating that it did cross from that standpoint and indeed we can even see higher with the higher dose or the more frequent dose in this particular case with eight times higher. Now, the next thing is, does Alpha-1 antitrypsin inhaled decrease the neutrophil? Now, just for those people that are not aficionados, the normal

neutrophil count in normal individuals when they're running is about 1 percent to 3 percent. In Alpha-1 antitrypsin deficient individuals, it's obviously higher than that on a proportional basis from that standpoint and indeed in the baseline in 80 milligram individual it was around 17 percent in this cohort of individuals with relatively normal lung function. They were around 70 percent predicted. In after therapy for 12 weeks, these individuals basically had a substantially group reduced amount of neutrophils in their lower respiratory tract. And it wasn't significant in the 160 group, but if you look at the other outcome of the one look at, neutrophil elastases, you can see there was a substantial decrease in the amount of neutrophil elastases, antigenic neutrophil elastases in the lower respiratory tract in the 180 dose, as well as in the 160 dose from that standpoint.

So, based on these observations in this Phase II study, I think that there is highly purified Alpha-1 antitrypsin we can get good deep delivery. It appears to have an

anti-inflammatory effect, at least decreasing the proteases in the lung and gets into the Interstitial space. It gets in the lung. It activates neutrophil elastase and it seems to decrease inflammation as defined as neutrophils in the lower respiratory track. Thank you very much. (Applause)

DR. MARKS: Okay. I think we have Dr. Wagner.

DR. WAGNER: Thank you very much. All right. First of all, I want to thank the organizers, NIH and the FDA for the invitation. I'm going to present on behalf of Inhibrx and I'm going to talk about our recombinant human Alpha-1 Fc fusion protein. My financial interest is obvious as I am an employee of Inhibrx. I'm moving on quickly to the presentation.

So, I know the background on AATD was well covered by previous speakers and probably other speakers will cover briefly as well, what's really happening in Alpha-1 antitrypsin deficiency. I want to point out three key features of AATD here. There is a quality issue. So, we have mutations in Alpha-1 which lead to

misfolding and retention in the liver. That needs to be addressed. There's also a second quality issue that some of these mutations in AAT might not be fully active as neutrophil elastase inhibitors. This is critical because ultimately that's what Alpha-1 antitrypsin does. It binds mostly to neutrophil elastase and AAT mutations that are not able to do that properly would be an issue.

And then there is the quantity issue, and I think nobody has mentioned that so far. After albumin and immunoglobulins, Alpha-1 antitrypsin is one of the most highly produced proteins in hepatocytes. On average it is produced at levels of two grams a day. So, there's a huge quantity issue here, which needs to be addressed, because you need to produce and substitute large quantities of protein. It would be difficult for other therapies than augmentation, like small molecules, to correct this.

And then the third issue is distribution. As Dr. Brantly pointed out, we really need to get Alpha-1 into the lungs. Serum

levels are nice surrogates, but ultimately, we care about the amount of AAT in the lungs and the interstitial space. Now that space is impossible to sample, but at least we have the bronchoalveolar space as a surrogate for that. So, that's the third.

If you're looking at the current therapies that we have at the bottom of the slide in the gray, I'm not going to spend too much time on that, but I just want to point out the different means to treat Alpha-1 antitrypsin deficiency. So, this is one of the key slides looking at the serum profile of AATD patients when they get the current treatment, which is human plasma derived AAT. So, what you can see here is when a patients start out their AAT serum levels are clearly below 11uM which is the threshold; a somewhat arbitrary threshold. When AATD patients receive doses of 60mg/kg of plasma derived AAT they nicely go up into the normal range, but they do not stay in the normal range for long. So, for at least three to four days of the week, a lot of these patients are in the intermediate AAT serum range between 20 and 11uM and as Dr. Pierce pointed out, there's nice

data showing that that might not be the ideal range AATD patients want to be in, and that is with weekly dosing. However, we've heard very often that weekly dosing is not ideal for patients and then they are also in this lower than normal level where you might not want to be, you might want to be higher.

So, now why do you want to be in the higher AAT serum range? And again, this will be covered by Professor Chapman and has been touched upon earlier, and there's more lines of data, but this is one of the data points from the RAPID trial showing that lung density decreases measured by CT correlates to serum AAT trough levels. Plasma derived AAT treated patients at 60mg/kg have a higher serum AAT trough level and that relates to less lung density decline than placebo treated patients. However, there are patients on this study who achieved higher serum AAT trough levels in the 20uM and above range and you can see that there seems to be a linear correlation that higher serum levels are correlated with less lung density CT decline. Now, we don't know if this effect is going to plateau out, but certainly

based on this data and others, higher serum AAT trough levels might be preferable.

Now, how do you fix this and how do we achieve this? And again, I'm not going to cover the talks which were presented by other speakers today, but there's two ways, right. So, you have a mutation. So, what you could do is in vivo gene editing and correction, super nice, you edit the mutated AAT gene and you correct it. And I think this is an interesting future development and I think in the long-term it would be fantastic because you're addressing emphysema and cirrhosis. However, you have to also address potential toxicities of gene editing and correction because you are cutting DNA and have to make sure there are no off-target cuts with consequences. But it's certainly something for the future to consider.

Chaperons or correctors have been covered before and they'll be discussed in the next talk after me so I won’ talk too much about that. The only comments I want to make about chaperons is that you are treating with small molecules for a lifelong therapy, which might

have toxicity and tolerability problems. Second, the chaperones need to cover the large quantity of AAT; and they need to cover the quality issue of correcting a potentially less functional AAT. So, that's that.

In terms of gene therapy, again, that approach was very well covered before so I'm going to skip over that, except pointing out the prior failures with too low serum AAT levels.

Then there is the most obvious solution, the recombinant production of AAT. Today essentially most of the plasma derived products or other therapeutic proteins are all produced recombinant. The first advantage here, of course, is that you're not relying on human plasma donors. So, blood donations will be less of an issue. Next, the cost of goods has come down significantly over the last couple of decades for recombinant production of proteins similar to next generation sequencing.

And so, people have tried to make recombinant Alpha-1 antitrypsin. It was produced essentially in every species you can use for recombinant protein production, starting

from bacteria over yeast to eukaryotic cells and even transgenic sheep. And what happened most of time was, either the quantity of protein was not sufficient, again, the quantity is an issue, or the neutrophil elastase inhibition of the produced AAT was insufficient, which means these recombinant Alpha-1 antitrypsins were not active and lost activity during the purification process.

Now, if you would be able to make recombinant human alpha-1 antitrypsin, you would still have to deal with the short half-life. So, the half-life of plasma derived alpha-1 antitrypsin is about five to six days. That's is consistent with liver produced proteins, they have generally short half-lives. So, what we decided to do is, we wanted to make a recombinant AAT with all the advantages of recombinant products, but we wanted to make it as an Fc fusion protein. We are not the first to propose that Fc fusion proteins are very elegant to enhance the half-life of a protein to the half-life of an antibody. So, what we have created this molecular called INBRX-101 or recombinant human

antitrypsin Fc fusion protein (rhAAT-Fc). And what you see here is that we have two Alpha-1 antitrypsin molecules fused to an IGG4 immunoglobulin domain. Notably, our two alpha molecules are fully active in inhibiting neutrophil elastase. We have introduced two changes into Alpha-1 antitrypsin of rhAAT-Fc to facilitate potential resistance towards oxidation mediated inactivation of Alpha-1 antitrypsin which can occur in the oxidizing environment of the lung. We have also introduced a couple of mutations into the IGG4 domain of rhAAT-Fc. These are commonly used in antibodies. These amino acid changes either further silence the effector function of the immunoglobulin, because we don't want an active Fc domain, or extend the half-life of the antibody further. And so, what we have been able to show here is that our recombinant Alpha-1 antitrypsin Fc fusion protein (rhAAT-Fc) is fully active, which was not trivial. Actually, we've a lot of protease inhibitor biology experience at Inhibrx and theses are neutrophil elastase assays shown here. If you benchmark rhAAT-Fc to Prolastin C

you can see, essentially, they are virtually identical in blocking neutrophil elastase. Notably, this is normalized per molecule of AAT. So, each of the AAT parts in our rhAAT-Fc molecules are fully able to block neutrophil elastase and we show this for multiple species because we've chosen these species for GLP TK/toxicity studies. That is shown here for human, cynomolgus monkey and rat neutrophil elastases.

Now, moving on, as I pointed out earlier, we have an Fc fusion which means the molecular weight of our molecule rhAAT-Fc is larger, but we also now have an Fc which is actually quite exciting. And as Dr. Brantly pointed out, the lung is not a space where antibodies cannot go to. It's actually the space where antibodies like to go. And the FcRn receptors, which are mostly there to enhance the half-life of antibodies by avoiding intracellular degradation, they are also part of transmembranous transport. We know in the lung environment the lung capillaries and the lung alveolar cells express these FcRn receptors and

its part of the transcytosis of antibodies in and out of the lung. So, what we have done here is we used the human FcRn transgenic mice from Jackson Laboratory, which is transgenic for the human FcRn receptor, and then we dose these mice IV with either rhAAT-Fc (INBRX-101) or plasma derived AAT and then we looked in the bronchial-alveolar lavage fluid after 24 and 48 hours and we wanted to see how many AAT molecules actually make it into the lung compartment. And what you can see here is, that rhAAT-Fc is certainly not less able than plasma derived AAT to get into the lung. In fact, rhAAT-Fc might even be enriched in the lung, and in these experiments rhAAT-Fc is always compared to plasma derived AAT on an equal per AAT molecule basis.

Now, moving on to PK of rhAAT-Fc, so this on non-human primate data and we are dosing here rhAAT-Fc and plasma derived AAT at equal levels of AAT, adjusted for the molecule weight of the molecules, so rhAAT-Fc is dosed at 80 mg/kg and Prolastin C is given at 60 mg/kg. And what you can clearly see here is in these cynomolgus monkeys, over time we have prolonged drug

exposure and a significantly longer half-live of the alpha-1 antitrypsin Fc fusion protein compared to plasma derived AAT, which is again, not surprising due to the function of the Fc domain for half-life extension. And so, if we confirm this PK data of rhAAT-Fc in humans, this would ultimately, lead to less frequent dosing of rhAAT-Fc and at the same time we would be able to achieve a high serum AAT trough levels which we and a lot of us wanted to see.

In terms of our current human study, so, we have this Phase 1 study ongoing. It's a Phase 1 study and we want to thank the FDA for giving us the IND late last year. This slide has been stating the NCT trial number shown here on top. It's primarily a safety and tolerability study. It's the firsttime testing rhAAT-Fc in humans. So we have two parts, we have a single ascending dose part and a multiple ascending dose part. The dose levels are shown here so with ranges from 10 to 120 mg/kg for Part 1 and 40 to 120 mg/kg for Part 2. We are planning to dose rhAAT-Fc at least every three weeks, but hopefully we'll be able to dose less frequent than that. In terms of AATD

patients for this study, we accept plasma derived AAT augmentation naïve patients, but also patients on augmentation therapy, which would just have undergo a washout period of 4 weeks. And on the trial endpoints I want to spend a little bit more time on. So, obviously safety and tolerability are key. So far plasma derived Alpha-1 antitrypsin products are generally considered very safe and we didn't see any pre-clinical toxicities with rhAAT-Fc either, which is very encouraging. But then the key clinical endpoint, of course, is serum PK and I want to point out that antigenic PK is obvious, but we also care a lot about functional AAT levels. As I said, the quality of the Alpha-1 antitrypsin protein matters, so we want to make sure we have active functional Alpha-1 in the circulation and in the lung, and not just an Alpha-1 protein which cannot inhibit neutrophil elastase. So, we go look at neutrophil elastase inhibition capacity in serum, called functional PK. We will also look at immunogenicity of our product. We have several ADA assays, including neutralizing ADA assays, developed and ready to

go. And then we will also determine lung exposure of our molecule rhAAT-Fc in brochoalveolar lavage fluid with Dr. Brantly with his help. We're going to do that in his lab looking at the antigenic PK and neutrophil elastase functional PK, in addition, to other PD biomarkers. And then we have other pharmacodynamic biomarkers for alpha-1 antitrypin and I know there's going to be a talk about that, coming about later too. Sorry, I am rushing a little bit long. We are running out of time.

So, these are our inclusion/exclusion criteria. They're pretty straightforward. Obviously, patients have to have Alpha-1 antitrypsin deficiency. We will accept most genotypes of Alpha-1 antitrypsin deficiency that's perfectly fine as long as their serum Alpha-1 antitrypsin level is below 11uM and patients have to be able to undergo bronchoscopies. In terms of exclusion criteria, they are pretty standard as well. For somebody that has allergies to components of either immunoglobulins or Alpha-1 antitrypsin they would

be excluded, but otherwise we're very flexible about criteria. So, with that I kind of want to leave with the last slide here. So, for us, of course, it's very important to have clarity of how we're going to develop our product recombinant Alpha-1 antitrypsin Fc fusion protein in the future, and so I put up a couple of considerations for the panel and future food for thought.

So again, quantity, quality and distribution of Alpha-1 antitrypsin matter to us and to patients. So, if you get antigenic PK data, of course, it's the gold standard and has been done for decades, but I think the functional activity of Alpha-1 antitrypsin products are key. The serum Alpha-1 antitrypsin trough level goal might also be too low and that was brought up by multiple speakers. I don't think that's trivial. I personally would rather my Alpha-1 antitrypsin serum levels be above 20uM and not just above 11uM. So, that's something to consider for future development of Alpha-1 therapeutics. And then we on purpose sample bronchoalveolar lavage fluid to measure rhAAT-Fc in the lung because we also think that the

biologic compartment matters and with this molecule, rhAAT-Fc, we might even see additional enrichment through to the Fc domain of rhAAT-Fc in the lung compartment which will give an additional efficacy advantage. Surrogate endpoint, such as pharmacodynamic biomarkers are also key. Again, we're looking at elastin degradation biomarkers such as desmosine and isodesmosine in serum and bronchoalveolar lavage fluid. Many components of the clinical study designs are important as well. So, it will be very difficult (hard), I think, with the existing potential efficacy data for plasma derived Alpha-1 antitrypsin augmentation from the RAPID trial and others to get patients excited about placebo control trials. I think the efficacy has been proven enough that the patients would have a hard time to stop their plasma derived Alpha-1 antitrypsin for these studies. So, if placebo is an issue, we should allow plasma derived Alpha-1 antitrypsin at the dose of 60 mg/kg as the control arm as the standard. I know there are going to be questions on statistical designs with non-inferiority, that's something which I would

like the panel to touch upon. And then, I'm just going to point out the obvious at the end.

So, there are about 10,000 AATD individuals on augmentation therapy in the U.S. So, if we would run a study with 500 individuals, that's 5 percent of that AATD population and that is very hard to achieve, right? Because we all know these patients are geographically spread across the U.S. and even if you have 50 sites, one in each state, patients would have to be able to drive to these centers. So that's very challenging and it's just something to keep in mind. As a comparison this would be the equivalent of trials in lung cancer enrolling 10,000 or 15,000 patients per year, which is again, a difficult hurdle and not easy to achieve. So, with that, thank you for the opportunity and I'm happy to take any questions now or --(Applause)

DR. MARKS: Finally, we have Dr. Charlotte McKee from Vertex. Thank you. Thanks again to everyone for staying on time. This is great.

DR. MCKEE: I promise to stay on time. Good afternoon. Thank you for your attention

after lunch. I hope everyone is still awake. I'm Dr. Charlotte McKee and I lead the cystic fibrosis and Alpha-1 antitrypsin deficiency clinical development group at Vertex. So, I am a full-time employee of Vertex.

Thank you to the conference organizers for this invitation. I'm going to speak today about, as you already have a heads up, about small molecule protein folding correctors which represent a new mechanism of increasing Alpha-1 antitrypsin levels and potentially treating AATD. As a lung transplant pulmonologist, I have treated patients with Alpha-1 and as the head of Vertex's cystic fibrosis clinical development program, I've actually seen the impact on that disease that correctors, small molecule correctors have had. So, I'm very excited to be bringing our knowledge and that potential to this disease.

We all recognize that Alpha-1 antitrypsin deficiency is a serious multi-system disease for which new treatments, and we've heard this throughout the day, new treatments, specifically treatments that target the lung and

the liver disease are needed.

Today, I'll show you data demonstrating why we're confident that our oral AAT protein folding correctors can address the underlying cause of this disease (that's the misfolded AAT proteins present in the vast majority of patients) and have the potential to treat both the lung and the liver disease. I'll also explain why we believe that circulating Alpha-1 levels should remain the basis of regulatory approval. And finally, I'm going to speak a little bit about the critical need to address underdiagnosis of AATD, to ensure that patients receive appropriate therapy and to accelerate development of new therapies in this important disease.

So, we've heard so eloquently today from patients and others that AATD is a serious multi-system genetic disease for which new treatments are needed. We know, of course, what causes it: mutations in the SERPIN-A-1 gene. We also know that the vast majority, well over 90 percent, of patients produce the misfolded Z variant of the AAT protein. This results primarily in lung and liver disease illustrated

here in this figure. The lung disease we know is due to a loss of function: to low levels of protective AAT leading to these well-known manifestations of emphysema, bronchiectasis or more broadly, COPD. We also know that an estimated 70 percent of severely deficient patients have early onset lung disease in this category. The liver disease on the other hand, as we've also heard, is due to accumulation of the misfolded Z AAT protein, and up to 30 percent or more of patients are affected by the liver disease. However, we know that the current treatments only target the lung manifestation of this disease.

Protein folding correctors target the misfolded protein, which is the underlying cause of AATD, and this toxic variant of the protein is responsible for the liver disease. In this figure here on the left, we see the misfolded Z AAT protein and, on the right, we see the goal: the normally folded AAT protein which can be normally secreted from the liver into the circulation. Our small molecule protein folding correctors, we believe, act where AAT is

synthesized, promoting the normal folding of this AAT protein and its normal secretion from the liver. This has two important consequences. First, the circulating levels of functional AAT are restored by enabling a normally folded AAT protein to be secreted from the liver, and two, by correcting the misfolding, the accumulated Z protein, this “soft glass” that we've heard about, is decreased in the liver. We have initiated clinical development of the first oral small molecule protein folding correctors, in fact two of them, to target the underlying cause of this disease.

So, how do we envision this molecular mechanism, the correcting of this misfolded protein, translating into the potential to treat both the lung and the liver disease? The top half of the slide is a quick recap of much of what we've heard today – here we see what happens with AAT deficiency. Misfolded protein is produced here in the liver where it accumulates in its polymerized form and causes liver damage. Because of this misfolding, very little AAT is secreted into the circulation, so there's less

anti-protease to protect the lung from inflammation and infection.

So, here on the bottom half of the slide is what we believe happens or what we see happen with AAT protein folding correctors based on our preclinical data. First, corrected normally folded AAT protein is secreted from the liver, which means that this polymerized toxic form doesn't accumulate. And because the protein is folded normally, it is secreted into the circulation (as we see here in the blood vessel) and restores the anti-protease balance to the lung.

Now, how do we know that our small molecule protein folders correctors actually do these things? This comes from our preclinical data. I'm going to show you on this slide data from our PIZ mouse models where animals were treated for 12 weeks with one of the protein folding correctors that we've taken into the clinic. Here, on the left half of the slide, are functional levels of AAT. Here on the Y axis are functional levels of AAT in the plasma of these PIZ mice and then here on the X axis are treatment

days. What we can see in the blue line here, these are inactive vehicle treated control animals, and we see over the 12 weeks that there's no change in their functional AAT levels. Here in the red line you see that the animals treated with the small molecule corrector have a rapid and sustained increase in functional AAT levels well into the carrier range, which we know is protective.

Here on the right of the slide are data showing that our protein folding correctors also result in a reduction of Z polymer in the livers of these mice. The dark staining here is this "soft glass" -this is the accumulation of Z polymer and Z protein here in the livers of the inactive vehicle-treated mice over 12 weeks. Here on the right side of the panel is the evidence that 75 percent of this polymerized Z protein has been cleared or reduced compared to the vehicle treated animals in the corrector-treated mice. So, this tells us that - both of these data tell us - that these small molecule correctors have the potential to treat both the lung disease and the liver disease of AATD.

The natural history data - and you've heard this also throughout the day - the natural history data strongly support the clinical benefit of higher AAT levels across all genotypes. The risk of emphysema, for example, and the severity of disease increases with decreasing natural levels of AAT. In the ZZ patients, who have lowest levels of AAT, they have the highest risk of lung disease and, of course, more severe disease. People with the MZ genotype on the other end of the spectrum, especially people who have never smoked, have a very low risk of emphysema, and then, of course, the SZ patients or the people with the SZ genotype who are in the middle have intermediate levels of AAT and have intermediate risk of disease.

In other words, the higher the AAT levels, the lower the risk of disease. Circulating levels of AAT have, of course, been the basis of regulatory approval and we believe that they should remain an appropriate end point for registration of new therapies that increase AAT levels in the circulation.

Finally, I want to say a few words about

under-diagnosis. I think this is something that we've all recognized for a long time as a barrier to effective treatment for patients with AATD, but it's emerging as a truly critical issue now that we have the potential for more and better therapies. First, the scope of the problem is huge: 85 percent to 90 percent of patients with AATD are simply not diagnosed and we believe this speaks to the significant gap in the need for new and better treatments. Second, educating patients and prescribers about AATD and increasing adherence with established clinical practice guidelines - which in fact, recommend that, for example, any adults with symptomatic fixed airflow obstruction are tested for AATD - would have a number of important consequences: increasing diagnosis and treatment, accelerating enrollment into clinical trials and ultimately, helping to bring new AATD therapies to patients faster. The Alpha-1 Foundation has been a leader in education and advocating for testing and we can all build on that strong foundation together.

So, in conclusion, AATD is a serious multi-system disease for which new treatments,

and especially those that address both the lung and the liver disease, are sorely needed. Vertex is testing two oral small molecule AAT protein folding correctors in the clinic now. These target the underlying cause of the disease and have the potential to treat both the lung and the liver disease. We believe that restoration of circulating AAT levels remains an appropriate basis for approval, including for new therapies that increase AAT levels in the circulation. And finally, we all need to work together with the Alpha-1 Foundation, with the patients, with providers, and with the regulatory agencies to tackle this critical problem of underdiagnoses: first of all, to ensure that patients receive appropriate therapy, and also to accelerate development of new therapies for this important disease. I thank you for your attention and sincere thanks to the Alpha-1 patient and provider community for their partnership on our corrector development programs. (Applause)

DR. MARKS: So, I'm going to ask Dr. Flotte, Dr. Marciniak, Dr. Brantly, and Wagner just to come up here and just going to ask the

Senator to cede five minutes of his time and yield five minutes of his time and we'll yield five minutes of break later on. Questions for anyone who -- of these five presentations? Everyone's speechless. We solved the problem. Go ahead.

DR. MARCINIAK: Well, I'll get the ball rolling then, Mark. So, one of the reasons that we can do this is because the epithelial surface of the lung is incredibly water tight and you know that as Alpha-1 moves from blood through the lung there's a gradient of the concentration going down. And although you can get a signal finding M protein in blood, when you've given it by aerosol, do you really think you're really going to be able to protect the lung by substantially raising the level there as opposed to on the air surface?

DR. BRANTLY: Well, again, I think that it's only -- again, the claim is that it passes through. So, what the concentration is in the interest level is unclear from that standpoint. The fact that we see a biological affect with reduction in neutrophils and neutrophil elastase makes me think, I'm not -- I don't really -- I'm

not really that concerned about what's going on from that standpoint.

DR. MARCINIAK: I hope you're right.

DR. BRANTLY: I hope so too.

MR. IRVIN: Ken Irvin, I'm on the board of the Alpha-1 Foundation. Earlier today, we had two presentations by the FDA. One suggesting the sample size should be 400 patients. The other suggesting that the sample size for a rare disease, possibly should be much less. I am just wondering what the people on the stage might think is the right sample size for a clinical trial?

DR. MARCINIAK: I'm not a clinical trialist, so I can't do the calculation, but I can say that I know a country where they speak English and they don't use augmentation therapy yet and it would be a very good place to do such a study.

MR. IRVINE: You're talking about Ireland?

DR. FLOTTE: I guess in a -- my concluding slide was to say that we wanted to know what the endpoint is. I don't think you can determine the number of patients needed to reach to endpoint until you know what the endpoint is.

And that, of course, means you need to know what the difference is between the treated and untreated groups of patients and the variability of measurement of the endpoint is. So, if the endpoint is reconstitution of the plasma levels then the numbers of patients is going to be much far, far smaller. It's because if we have simple reproducible assays to determine that endpoint, so, I think the consequence of having a very clear endpoint like that, but it's the ability to license additional therapies to make those therapies available to the patients and the providers to decide which ones are appropriate to their patients based on a variety of considerations. It is conceivable that some of the therapies could end up being equivalent to augmentation therapy with regard to that endpoint, but one or another might be preferable to individuals if their equivalent safety and at that endpoint.

DR. MARCINIAK: So, this is a question for Charlie and for the other people as well. So, we have some on SZ and we have found that SZ you don't smoke. You're not at an increased risk for

COPD compared to their MS or MN symptoms, but if they do smoke, they have a significantly increased risk for COPD. So, the question is, I suppose, we come with the levels and we know that in MN individuals we get infection, the level goes up, but the process of giving intravenous infusion once a week, or even the gene therapy, we get a standard that seems to be not replicating what's happening really in reality. (In a very high level or can we modulate to that unresponse to infection from. Do you have any thoughts on that?

DR. FLOTTE: That's an excellent point. I think that just the entire pattern of the pharmacokinetics is very different in all of these scenarios, so with augmentation you have the rapid spikes and troughs that are not related to particular stimuli in the way that your therapy functions. I mean, some of those graphs they showed were a year and five years, albeit, at two low levels that were too low, but they were not fluctuating at all and what you're suggesting, which would be a more physiologic response, would be to have, perhaps something like an IL-6

promotor element, which we haven't designed yet in our own vectors, but it seems like it would be feasible to do that. The other point that you made, or point I made, that I think relates to what you said about the SZ individuals, is that at neither S nor Z has the full specific activity. Although S my understanding has better functional activity than Z, but a therapy where the gene therapy would at a same level might be -- at least in the same antigenic level, might be different if that antigenic level is being reached with, you know, pure M as opposed to the antigenic level that might be reached through some other modality when you're still getting draws or elevating some of the others. A good tight anti-neutrophil elastase assay which there are several of them measured in many of these trials could get in that.

DR. MARCINIAK: Thank very much. Related in some way to the above, when your studies are described to scientists and we actually get questions about it and the biggest question about the protocols are while it's wonderful that you're able to raise levels to near

normal in some of the animal studies that you've done, it's that level is of a Z protein which is considered to be not as good and lasting inhibitors the M protein. Do you think that that levels that you get of activity are going to be sufficient?

DR. MCKEE: So, I think it's a good question because what I can tell you is, again, based on the PIZ mouse models and the data in the PIZ mouse, that the levels of AAT that we see in the serum are fully functional and again, as I showed you, fully capable of moving well into the carrier range. So, we do believe that they're acting the way they need to in the serum.

DR. MARCINIAK: So, today we spoke about if Alpha-1 Antitrypsin therapy works and what the levels are correct, or the level might be more higher. Some of the data on the aerosol study that Dr. Brantly showed have been able to increase Alpha-1 to do something to that in normal individual. In Alpha-1 deficient individuals about 0.3, but interestingly enough, we continue seeing what was significant on a neutrophil burden decrease some 130 percent to

about 15 percent or 18 percent normal, which is still 18 times higher than a normal mutual fields burden in a lung of individual at 1 percent or maybe even a smoker at 5 percent. So, when done times deliver of Alpha-1 aerosol into the lung component yet have not been able to turn off the neutrophils and neutrophil states production which is by far 20, 30 times higher than normal individual. So, the question is, is level of Alpha-1 and 2 the only thing we have to talk about because by augmentation or aerosol, or perhaps it's time to start thinking about neutrophil states inhibitors as a therapy. So, I just wanted to hear your thoughts on that?

DR. BRANTLY: Well, I think that's a good question. And so, without being trite, for all the physicians in the room, when we used Digoxin a lot, how much Digoxin immuned us -- for atrial fib enough. How much anti-inflammatory molecule do you need in the lung when it's inflamed, enough. So, targeting, and particularly this concept of variable amounts of Alpha antitrypsin that our bodies so eloquently designed a liver, is probably a difficult thing

for us to do from a dosing standpoint, particularly with Alpha-1 Antitrypsin. So, it's likely that we need to go with the highest tolerable dose to cover most of the circumstances in which we have inflammation going on.

Now, addressing the issue about that, a drug doesn't reduce inflammation to zero, particularly in a three-month study, I think that chronic inflammation takes a long time, and as you mentioned, there are a lot of things that drive chronic inflammation. One of the things that drives chronic inflammation is also basically structural damage. Over time -- and that's not going to go away. So, we may be having to be satisfied with significant reductions without complete resolution because we are not going to turn a emphysematous lung or destructive lung back to normal from that standpoint. It's just not going to at least happen, at least without lung transplantation.

DR. MARKS: Thank you all. So, thank you everyone again. And I -- another round of applause for this group who really stay on time. (Applause) Okay, and with that I want to say

thanks -- a number of months ago, what helped actually get this workshop on the books and on the docket, we were at some meeting that was held with Alpha-1 that former Senator Rick Santorum helped -- was involved in and he's been very actively involved in trying to help develop and enable development of plasma therapeutics for various disorders. So, it's a pleasure to welcome him to give some remarks here. There he is. Okay. Thanks.

MR. SANTORUM: Thank you, Dr. Marks. I appreciate it. I usually get applause when I'm introduced, but I guess -- (Applause) that's okay. I have a fragile ego. I'm a politician, so it just sorts of makes me feel comfortable. First up, I just want to thank Dr. Marks who was kind enough to say what he said about me, but let me tell you, as somebody who's been a politics a long time, I was first elected way back in 1990 and served 16 years in the House and Senate, so I know a little bit about working with people in Government and I had, over the last now year and a half, I've had to opportunity to work with Dr. Marks and I can tell you, I have never worked with

anybody inside the Government who is as accessible, is as clear in what he lays out as far as what he will do and what he won't do. As is patient focused as he is and not only does, he say what he is going to do and what he's not, but he says how long it will take him to do it and he does it in that timeframe. That's great for anybody, but much less someone in Washington, D.C. which is not necessarily known for any of those things in the Government. So, I just have to say what an extraordinary experience this has been for me and I'm just thankful on behalf of the company that we're here to talk about, but also on the other reason that I'm here, as a dad.

As you saw, I am blessed that we have seven children that we are raising. Our youngest is named Bella. Bella was born 11 and a half years ago, and she was born somewhat premature, four weeks premature. She weighed a little over three pounds and four days after she was born, we were given a genetic diagnosis that she had a condition called Trisomy 18. If you look up Trisomy 18 in the medical literature, it says "incompatible with life." And so, we were taken

-- sent home from the hospital, from the ICU after 10 days on hospice care and told it was simply a matter of time before our daughter died and she could die from respiratory failure from getting a cold because the infection will overwhelm her system. She wouldn't be able to breathe, and she would die.

Well, that almost came true. In the first six months of her life she had two colds and both times that she had those colds it did overwhelm her system. She did stop breathing. Her heart stopped twice, and we had to -- my wife, who is blessed to be a neonatal intensive care nurse in her former life before she became a mom and a wife of a senator, she revived her until the EMTs came and stabilized her. But she was living on the edge for the first several years of her life dealing with very dramatic problems. You may recall, unfortunately, you all don't recall, that I actually ran for president back in 2012 and I believe it or not, came actually fairly close and during the time I won the Iowa Caucasus which is now in the news these days again because of the primaries and right after the Iowa Caucasus, a few

weeks afterwards, I had thankfully to be home one day and my daughter had another one of her bouts with a respiratory infection and we ended up having to take her to the hospital. She had very severe pneumonia, was unable to breathe and we had to suspend the campaign for a period of time. So, my life with Bella has been a private life, but it's also been a very public one and in dealing with someone, and I will tell you, the thing that transformed my daughter's life, was after the campaign we went back to try and figure out is there something we can do and thanks to the great people at Children's Hospital, they diagnosed that she had an immune deficiency and so, we started on Ig and for the last eight plus years she had been on Ig and she is not been back to the hospital since. It has changed not just the quality of life; it has saved her life.

As a dad, this issue of plasma and the availability of plasma products is very, very important to me. So, when a good friend of mine, who came to me and said, "Hey, I've got a great new technology." Gene Surlow (phonetic) came to me and said, "I've got a great new technology that

can actually provide more Ig and more Alpha-1 antitrypsin than the current process and would you be interested in helping me?" I said, "Well, sure. Tell me about it." And he described the process that he was replacing an 80-year old process. Yes. We still use 1940s technology to fractionating plasma. Eighty-year old technology instead of using alcohol, which I can't think that -- I put alcohol on my blood on many occasions. I don't think it's necessarily been a good thing for me or my blood, but we do that on a regular basis to fractionate plasma. And so, he said, "We use a salt precipitation step. In fact, we get substantially higher yields than the current system. I said, "Well, that's a good thing. We could use higher yields. That would put less pricing pressure." Because at times, I've been without insurance. I know you think well former senators; presidential candidates don't lose insurance. We do too. And I've had to pay for my Ig out of pocket on occasion and it's not a very easy thing to pay for. So, I said, "Yeah, that's interesting." And he also said something that really concerned me,

which he said, "that we have a shortage in Ig." He said that, "the industry doesn't recognize, FDA doesn't recognize it, but anecdotally, in other places I can tell you if you talk to the folks at Immune Deficiency Foundation they will tell you that there is a shortage and that usage has going up 7 percent, 8 percent a year and the fractionators can't keep up with it. Now, that really worries me because my daughter has Trisomy 18.

I told you who she is. I didn't tell much about her. My daughter can't walk. She can't talk. She can't feed herself. She gets feed by internal feedings. In the eyes of the world, my daughter doesn't look particularly useful to an insurance company or maybe to her physicians who prescribe medications. And if there's a shortage, then I see my daughter as someone with a bullseye on her back. As someone who is not seen as useful. Doc will tell you that my daughter had made the life of every person she's ever met better. I don't know if you can say that about yourself or any member of your family. I can't say that about all my children,

but I can say it about her.

And so, when I hear shortage my -- as they are right now, the hairs upon my skin go up and I want to make sure that that doesn't happen, so I'm never forced to have to fight to get the care my daughter needs. So, I am here today because I want to make sure that we have plenty of plasma products available for people like my daughter and others and like people in the Alpha-1 world.

So, I said, "Gene, I will help you." I said, "What's the problem?" and he said, "Well, the problem is the FDA. For the last 10 years we haven't been able to get a new Alpha-1 product approved because of the standards that the FDA uses is block innovation and because fractionators won't do a new technology that changes the way, as in the case of our technologists, the front end of the fractionation process is right at the beginning. So, you have to relicense the whole facility and you have to relicense all your products, including your Ig and Alpha-1 products, and since you can't get an Alpha-1 product approved, no fractionator is interested in talking to us because they're not

willing to sacrifice their Alpha-1 product for the other products. So, I said, "Well, that's something I know a little bit about. I've been involved in Government for a few years." So, I said, "I will try to help you." So, I contacted a friend of mine who used to work for me and who knows a lot about the FDA and she said, "The person to talk to is Peter Marks." So, I got in contact with Dr. Marks and Dr. Marks said, "I'll meet with you whenever you'd like. Just let me know."

Well, I wasn't ready because I still had to learn a lot, so I talked to really smart people. I talked to Miriam O'Day, whose educated me beyond the belief and helped me understand the Alpha-1 world along with Sandy Savanosta (phonetic), Charlie Strange in the Alpha-1 world, as well as a guy I know who's here in the back, at least he used to be, there he is; John Boyle from the Immune Deficiency Foundation. I worked over a period with my partners, Dennis Curtain and Gene Surlow to put together a presentation to Dr. Marks and we went in, in August of last year and met with Dr. Marks for an hour and a half over at the FDA. I have to say, but it was a remarkable meeting I've

been too with a regulatory body. It was fantastic. It was curious. He listened. He and his team that were there were really understanding of the problems that Alpha-1 and the immune community were facing -- our gene community were facing.

After that meeting, he said that he would take four months and in four months he would research and find out whether what we were saying was actually planned out -- panned out and that he would -- and if it is true, he would make changes. He did take four months and two weeks, but because the Government was shut down for two weeks, I gave him some slack and in four months and two weeks, he worked over that time with Miriam and with John. He talked to them. Patient advocates, as he should and working with the and delivered the message to them that he would in fact -- that what we said checked out in his estimation and that the FDA would hold a workshop to come up with a better understanding of how to improve Alpha-1 products going forward, but there would be changes. And there'd be changes in not just how we do it, but also in the people that are going to be looking

at it. And the final things he said, which is very important to me, which was, "we need a partner. We can't do this on our own. There are a handful of fractionators out there in the world and we need to get a manufacturer, a fractionator to at least look our -- frankly, not just ours, to any technology and there's other out there that can improve yields from," like I said, "this antiquated system." Well, that's happened. A couple of things have happened. We've been in contact with fractionators. With all of them and many of them are still in discussion with us. Some have even gone so far as to pilot test our product, our technique, our technology, I should say, and to great success and we're very excited about that. And I would say to Dr. Marks, he has stayed in touch with us. He has said, "Look, I'm," -- he went out and reached out to fractionators and has said the same. "Look, we need to do better. We have an 80-year old technology. An 80-year old technology that destroys Alpha-1 85 percent of it. Destroys 85 percent of Alpha-1. Think about that. Do you want to drive the car that survived the demolition

derby?" Because that's what we have. We have a demolition derby going on with this product. You got 15 percent of your protein surviving this process and you're saying that's the product we're going to use to actually -- how about a process that does better than that. That actually has a more natural protein. Something that can may be a better protein? I'm not a scientist. I'm not making any claims up here. I'm just saying, as a common sense to me, that if you have something that the Ig that destroys 40 percent of your protein and Alpha-1 that destroys 85 percent, that maybe if you have something you have no detectable loss, and everything comes through it might be a better protein. It might do a better job. It might get better results. I don't know. All I'm saying is, we have an opportunity because of Dr. Marks and what he has laid forward. We have an opportunity as a community of patients and advocates. We have an opportunity as people in the business that provides -- I'm very impressed by what I see and I hope all of these things work and work well, but I know what we have now, and what we have now can

be better. What we have now can be better and can be in much more supply.

Our process gets six times the amount of Alpha-1 than the current process. Six times. Now, imagine if you have something six times the yield and again, not the car that survived the demolition derby, what you might be able to do with that. So, I just share with you that the door is open. Everything I've seen is that the door is opened here at this agency and I would just encourage all of you to help and whether it's my innovation, other innovations -- Gene's innovation, not mine. I got carried away for a second with it. Whatever innovation it is, I'm saying, let's do something to help patients. Let's do something to increase the amount of plasma proteins and I would just add one thing, I mention Alpha-1 and I mentioned Ig, there are hundreds of other proteins and plasma getting destroyed by the current system. None of them as far as we can tell are destroyed by ours and there may be other that do the same. What other types of cures of therapies are available that can't be researched because there's not product to supply?

So, here is the opportunity and I would just encourage you to come out, come to the table and let's make something really great happen for patients. And one final shout out. Thank you, Dr. Marks for everything. I appreciate it.

(Applause)

DR. MARKS: Thank you and I'm a little bit blushing. You shouldn't have. It's very sweet of you and thank you for sharing the story of your daughter. I actually didn't know the whole story, so thank you for sharing that. With that, we are remarkably on time believe it or not, which means my blood pressure is now down from at least 20 systolic points because I thought by this time of the day, we're already going to be an hour over time. So, everybody, we have a 20-minute break. I'm not sure we have anything. We have left over stuff from lunch, so, if you didn't really go out for lunch, anything that's back there enjoy, and we'll see you back and we'll start promptly at 3:00 again. Thank you very much.

(Recess)

DR. MARKS: Okay. We're going to go

ahead and start to get started again. So, a hopefully people will start to wander back in as they hear a noise coming from the podium. We're trying to run like a Swiss railway. Switzerland's the only place where you can actually go where they'll have connections for railways with two minutes between two trains, and you can get off of one train, and the other train pulls up for you to get on -- it's really quite amazing. They really do run on Swiss time.

So, we're going to continue on, and we have a couple of more presentations, and then a panel discussion, and, so, I'd like to welcome Dr. James Hamilton to talk about therapeutic approaches to liver disease, and it's something that we've heard a little bit about, the background of it. So, thanks very much.

DR. HAMILTON: Thank you for that introduction and thank you to the conference organizers for the invitation to present. Today, I'll be discussing RNA interference as an approach to the liver disease in Alpha-1, and, so, Arrowhead's been working in this space for about eight years now. We're working on a compound

called ARO-AAT, that is an siRNA compound, and this is our second compound in development. That earlier compound was called ARC-AAT, and, before I get into the development process to these two drug candidates and the data, I just wanted to highlight some of the things that we've learned over the last eight years.

As it has been described earlier today, actually multiple times, that drug development in this space, in the Alpha-1 space, for lung disease and also for liver disease, it is quite challenging, and, so, I've listed some of these challenges that we've encountered, and how we are attempting to address these as well, and, so, of course, Alpha-1 is a rare disease. The liver disease in Alpha-1 represents a subset of patients. So, that's even more of a rare condition, and this is important to us because it impacts how the one designs from the trials, how you can power a study, how you can enroll a study where there's sort of a posse of patients.

One of the ways that we've addressed this, it is by working with U.S. regulators to adopt an adaptive clinical trial design that is

facilitating the use of patients in phase two, that seamlessly phase and feed into a phase three component of the study, and I'll talk about that design at the end of the presentation. One of the other issues with drug development in Alpha-1 liver disease, and maybe more so in the liver disease than in the -- with the lung condition, is really the lack of natural history data. There's -- this has changed over the last decade or so. There are several publications, even a recent publication, about progression of liver disease and how long it takes to progress, the liver disease takes to progress in Alpha-1, but it's not entirely clear what some of the other contributing factors are, what the time till progression is, in Alpha-1 liver disease, in terms of how rapidly liver fibrosis may progress, and once they become cirrhotic, is there a flat rate any different than, say, in NASH cirrhotics or Hep C cirrhotics, and, so, one of the things that we've been working on, and we're in the final stages of a retrospective natural history study, in 101 PiZZ patients with liver disease, and intend, at some point, to publish that data.

The last point, here, and maybe one of the most important, at least from a drug developer's standpoint, is that there's no well accepted efficacy surrogate for liver disease in Alpha-1. There are other histologic grading scales for things, like NASH, or Hep C, or Hep B, that have been used as, sort of, measures of liver health and liver inflammation, and, so, this is something that we're working on, is the development of a novel histologic grading scale that's really specific to Alpha-1, but at least has features that are specific to the Alpha-1 condition.

So, just a refresher on RNA interference, or siRNA. This is an emerging therapeutic modality. For many years, it was thwarted by the challenge of delivery. The technology has the capacity to silence any gene expression, and the problem has always been delivery, how do you get these short RNAs into the cytoplasm, where they can sort of do their thing, and, least, then, this is still a challenge. Delivery is still an issue for extra-hepatic tissues, but, for the hepatocyte and for the

liver, it appears that this challenge has, for the most part, been overcome at -- there are companies that have used lipid nanoparticles or liposomes to transfect hepatocytes, and there's actually an approved drug, called Patisiran, that uses a liposomal formulation to deliver an siRNA.

However, what's becoming more and more commonplace and sort of the standard of delivering siRNA to -- at a site are the so-called single molecule GalNac conjugates, and, so, these target the hepatocyte through the asialoglycoprotein receptor on the surface of the hepatocyte and gain entry into the cell through a clathrin coated pit pathway. These siRNA triggers are modified. The backbone is modified, such that they are somewhat resistant nucleuses inside the endosome. So, they're able, to some degree, survive the degradation pathway of the endosome. This sufficient amount is able to escape, where, once inside the cytoplasm, they can engage with the RISC Complex, or the RNA Induced Silencing Complex. These oligonucleotides become loaded into RISC, and, then, can silence, in a sequence specific manner,

the complementary mRNA, and, so, this siRNA is protected inside of risk, to some degree. This allows deep gene target knockdown. So, measuring proteins, like an Alpha-1, in the serum, and I'll share some of our data later. One is able to achieve reductions of 90-plus percent, and, with a single dose, the duration of effect can be very long. The single dosed can generate knockdown for about -- for months, six months, or even longer. Now, this is a transient effect, as these patients are followed. The levels do return back to baseline over time. So, it's important to distinguish that from something, like gene therapy, where the effect may be more permanent, this is transient.

Just briefly, there are a few situations where siRNA may be ideal. In a setting where a protein is intercellular, so, it's difficult to target with an antibody, this might be an ideal circumstance to use a RNA interference approach, or when there is a risk of off target toxicity with a small molecule that, when given orally or intravenously, may not target to a specific tissue, and siRNA single

molecule conjugate can be targeted, tissue targeted, none of them tissue targeted, but also specific for only a gene that may be expressed in this specific tissue may be advantageous.

As I mentioned, there is one FDA approved siRNA therapeutic. This is Patisiran for the treatment of hereditary transthyretin mediated amyloidosis, and this uses a liposomal delivery system, and, then, most of the later stage siRNA compounds in development use this, the GalNac technology, to target the asialoglycoprotein receptor.

One of these compounds is ARO-AAT. This is Arrowhead's investigational product, in development to address the liver disease in Alpha-1. Again, this is a hepatocyte targeted RNAi molecule. It specifically targets for silencing the Alpha-1 mRNA. It's designed to minimize off target effects, and, again, silencing is hepatocyte specific, and, so, I think we've covered the general pathophysiology of Alpha-1 several times today, as it relates to our mechanism of action in the disease state. There is this accumulation of the abnormal

polymers that polymerize protein in the liver. Because of this, there is a deficiency in the serum because this protein is poorly secreted into the serum. The deficiency in the serum, of course, can be addressed with augmentation therapy to raise the levels. However, the accumulated protein in the liver, over time, leads to endoplasmic reticular stress, cell injury, inflammation, cell death, and sort of a repeated cycle of inflammation
that -- inflammation and healing that leads to fibrosis and, eventually, cirrhosis, and there is no specific available treatment for liver disease in Alpha-1, as of yet, other than, of course, transplant, in the late stages.

So, how common is liver disease in Alpha-1, and there are various figures in the literature I've seen, that ranges from 10 percent to greater than 30 percent. There was a recent study done by Drs. Brantly and Clark, where they enrolled 94 of the patients from their cohort, all ZZ patients, that underwent liver biopsy, and, out of this cohort, about 35 percent had what was described as clinically significant liver

fibrosis, so, F-2 or greater liver fibrosis, and, as this slide shows, as the severity of liver fibrosis increases, there is also an increase the number of globules and the density of -- has the staining sustaining specific for the Alpha-1 protein. As we go from F-0 to, you know, bridging fibrosis near the cirrhosis, an increase in globules and in PAS-D stain.

So, what's the therapeutic rationale for an siRNA approach in this condition, and, again, this is -- our approach is to essentially turn off the production of this abnormal protein that is being produced by the liver, and is accumulating in the hepatocytes, allow the endogenous clearance systems to clear accumulated protein that is already present and repeat this cycle of cellular injury and inflammation that leads to fibrosis and cirrhosis, and the goal would be to prevent worsening of fibrosis that is already present, but also could potentially allow for a remodeling of fibrosis, and, as has been shown, other liver conditions, things like Hep C and NASH and others. If the insult can be removed, the liver can heal

itself, to some degree. The fibrosis can regress, and cirrhosis can improve, to a degree.

So, the biggest question we get asked about this approach, about the gene silencing mechanism, is that -- really relates to the lung disease. Of course, Alpha-1 is a storage disease in the liver, and it's a deficiency state in the lung, and, while emphysema takes apparently decades to develop in Alpha-1 deficient patients, there is evidence to suggest, at least in the null/null patients, that a severe deficiency, so those making no Alpha-1 protein, may develop emphysema faster. So, the question is, while through a gene knockdown approach and only targeting the hepatocyte production, so you're not targeting local production or production by neutrophils, but the question is if you take someone's hepatocyte production from 20 percent of normal, and knock that down to a five percent of normal, does that potentiate lung disease, and, so, pulmonary risk, for us, is the key disease specific toxicity to be addressed in clinical programs. One of the things that we're thinking of, if feasible, is finite durational

treatment. So, could you treat someone with near maximum gene target silencing for two to three years, essentially attempting to clear out the liver of accumulated azine protein, and then, maybe, either observe them, or use a maintenance dose going forward. Something like that may be preferred, if feasible, and the last point, here, gets to the question of off target effects.

There's a lot of effort that goes into screening siRNA triggers to ensure that the gene silencing that's achieved is only the silencing of the gene of interest. So, they could only knock it down, the protein that you want to be knocking down, and not silencing other genes, and while we go through extensive bioinformatics work, and animal work, to exclude off target effects and, of course, the usual GLP tox in two animal species. Of course, unexpected toxicity in humans is always a possibility.

So, briefly, I thought I'd just share some of the animal data with a siRNA mechanism of action. We've done a lot of work in the PiZ transgenic mouse model, and some of this has been published already, but what you can see, here, is,

with the RNA group versus the -- this is the control of an active control scrambled RNA and a saline control, that we're able to prevent the accumulation of globules. Similarly, in the same mouse model, able to prevent inflammation versus a saline control, and this was over a 33-week study, and while these animals don't get a lot of actual liver fibrosis, so they don't develop real -- a real clear fibrosis on histology. They do show increased expression of fibrosis associated genes, so genes associated with collagen production and what we've shown with this is with our older compound, but still the same siRNA mechanism, that we're able to prevent the expression of these genes, so that the treatment group is looking more like the baseline versus the saline control.

ARO-AAT has progressed through a phase one study of healthy volunteers. This was an open label study, single dose escalation, in healthy volunteers, and then a double blind multi-dose escalation, all, also, in healthy volunteers, and this is the serum knockdowns, the reductions in serum levels, after a single dose,

presumably due to a gene target silencing in the hepatocyte, and this was originally presented at ASLD last year, and, so, that was in November of last year. I think the key takeaways, here, are that the, even at 35 milligram, single dose level was quite active, showing mean nadir reductions about 60 to 60 percent, compared to baseline, and there's a degree of dose response going from 35 to 100 milligrams, 200 and 300 really look about the same, and then these individuals start to rebound back towards baseline, starting at Route 12. This was through end of study. So, week 16 was end of study, and then we continued to follow, actually, all of these subjects until their levels returned back towards baseline, and all of them will be followed until at least they're above the protective thresholds, roughly 57 milligrams per decimal, and many of them are followed into levels that were higher than that.

Summary safety data from this study, 45 healthy volunteers received one -- at least one dose, 28 active, 17 placebo, no severeor serious AE's were reported. There were reports of mild injection site reactions in about four percent,

about four percent of injections, and there were no AE's secondary to platelet count declines, adverse changes in renal function, or adverse changes in markings of liver injury, or markers of liver function. There were three treatment emerging in grade I ALT elevations all returned to baseline by the end of the study, and the highest ALT elevation was less than two X, upper limits of normal.

So, in this phase one study, again, the pulmonary considerations -- the pulmonary effects were taken into consideration. Again, this was a relatively short study, and, while emphysema takes a long time to develop, in patients with Alpha-1 Antitrypsin Deficiency, we took steps to exclude patients at risk for developing lung disease and monitored lung function throughout the study. We required that all of these participants had a normal FEV1 at baseline, normal Alpha-1 levels at baseline, and then we conducted spirometry at multiple time-points throughout the study.

At the beginning of the study, the per protocol, and minimally important difference in

FEV1 decline was set at 200 cc's, and one of the things that we learned over a relatively short study was that FEV can bounce around quite a lot. It can be an effort ended test, and intrasubject variability can be initiated over the short term.

So, here's some of the data. This is through day 113, so through end of study. There were no AEs of dypnea or other symptoms consistent with lung parenchymal damage, nobody complaining of shortness of breath with exertion. There were three AE's of FEV1 decline. So, that's -- those were based on that 200-cc cutoff. That's what the investigators were using, one in an active subject, two on placebo, and then none of these reported symptoms. The one on active, their FEV1 bounced around quite a bit throughout the study, and then it rebounded to above their baseline value, even while their Alpha-1 levels were still close to danger. Declines in FEV1 from these 200 cc's, on day 113, there were 8.6 percent of active with this finding, and about 15 percent of placebo subjects.

In terms of declines in FEV1 of 200 cc's, at any visit through day 113, we saw this

in 21 percent of -- in active, and 11.7 percent of placebo. However, when we looked at changes of FEV1 at every timepoint, regardless of magnitude, so regardless of this 200 cc cutoff, there was no statistically significant difference at any of those time-points where FEV1 was measured between active and placebo, and, so, this data, here, show -- these are just -- these are the means of change from baseline at day 113 with, in terms of change, FEV1 from baseline, or about the same, than if you look at maximal adverse excursion in the same range for active and placebo.

So, we've initiated a phase two-three clinical study. This is the ARO-AAT 2001 study, and I thought I'd go through the design. This is the adaptive trial design that I spoke to at the beginning of the talk. So, the way the study works is everyone who is -- it is randomized. It's a pre-dose liver biopsy, and then there are randomized to one of three dose levels, 25, 100, or 200 milligrams versus placebo. They receive their assigned dose level on days one, two, and 113. This is all part A of the study, and the

first 36 patients enrolled in part A. Once they get their last dose in part A, they undergo a second liver biopsy, and, based on changes in serum Alpha-1 levels, as well as liver Alpha-1 levels, so levels measured in a biopsy, and, of course, also, based on safety data, including changes in pulmonary function over this period of time, the DSMB, in consultation with FDA, selects the dose level that's carried forward in part B of the study. The sponsor stays blinded, and there is no histologic evaluation on this biopsy. It's only to look at protein knockdown.

All of these patients roll into part B at their assigned dose, and all new patients that are enrolled into the study go directly into part B and receive nine doses at the selected part B dose level, and then all of the patients, at the end of the study, and this is enrolling PiZZ patients, all of the patients, at the end of the study, did a liver biopsy, and then there's this other -- a two-year study. The comparison for the primary endpoint is a histologic one, using the histologic grading scale, and comparing pre-dose to end of study. All placebo patients

have the opportunity, at the end of the study, to roll over into an active extension study.

So, the key questions for us to answer, from this trial design, phase two is really all about dose selection, and evaluating dose response; in the liver, Z protein levels in PiZZ patients. Of course, safety intolerability will be key, particularly pulmonary safety. Although, this is, again, very part A's short duration study, and, so, the selected dose will be carried forward in the part B. This is the phase three component of the study, and the key endpoint is improvement in an Alpa-1 specific histologic grading scale, based on liver biopsy, without any worsening of liver fibrosis, and then there are several secondary endpoints that look specifically at certain features of the grading scale, and liver fibrosis in isolation.

So, in summary, RNA interference can consistently induce deep and prolonged reductions in serum Alpha-1 levels, presumably due to hepatocyte gene silencing. In healthy volunteers, there's been no clear association between Alpha-1 decline and adverse FEV1 changes

or pulmonary AE's, granted this was over a short study linked in a several month period. While FEV1 declines as a measure of pulmonary toxicity, we're not expecting it in this phase one study. Results from this study are encouraging, particularly if ARO-AAT can be used as a finite duration therapy, something that's used for a couple of years to ameliorate the Z protein accumulation in the liver. Of course, more data is needed in an Alpa-1 patient population with longer treatment periods, and the ARO-AAT 2001 study is the result of constructive collaboration between U.S. Regulators and sponsored the development of novel clinical trial approach to Alpha-1 liver disease. This study is open for enrollment, and it's the first study to evaluate the impact of gene silencing on Alpha-1 liver histology, and also on pulmonary function, as it relates to gene silencing in this condition. Thank you.

DR. MARKS: Any questions? Going once. Okay. Thank you very much, and, now, we're going to kind of complete out, today, and this should lead nicely into our panel discussion

because I think the, kind of, the 800 pound gorilla sitting in the room are endpoints, maybe two 800 pound gorillas, and, so, we'll start with, right here, Dr. Jeanine D'Armiento.

DR. D'ARMIENTO: Okay. Thank you for asking me to speak. I was asked to talk about clinical biomarkers in COPD, which, will obviously relate to Alpha-1. I have no conflicts, except that I'm the Chair of the Alpha-1 Foundation Board of Directors.

A biomarker, as we're going to talk about today, is characterized as something that objectively measures and evaluates an indicator of either normal processes, pathological processes, or a pharmacological response to a therapeutic intervention. We can have, obviously, diagnostic, prognostic, or predictive biomarkers. We, in Alpha-1, are not really concerned about diagnostic markers because we have a clear genetic way to make the diagnosis. So, I really want to refer to what could be a prognostic or predictive biomarker.

Over the years, the COPD field has performed a large amount of work towards

obtaining biomarkers. That's very helpful to us in the Alpha field -- there have been a lot of studies looking at transcriptomes, and panels of transcriptomes, and large numbers of patients. Unfortunately, what has come from these studies is that these transcriptomes often don't correlate with protein changes. Also, changes in transcription is often difficult to assess since you require tissue for analysis. In addition, they are extremely sensitive, and levels can be variable. Therefore, they are not typically ideal biomarkers.

Investigators have been leaning more towards proteomics and proteins, both with immunoassay methods and protein separation with mass spect technology. There are several sources to obtain material to measure a biomarker, obviously, blood, sputum, and urine. We will go through these. Then I will briefly talk about imaging. Our next speaker will talk about imaging. Although we tend to think of biomarkers as analysis in the blood and sputum imaging is also a biomarker.

So, what are the potential blood

biomarkers that we've obtained from the COPD field? The first one, here, is listed as CRP. CRP was shown initially to be increased in patients with COPD, compared to non-COPD smokers and non-smokers, and was then replicated again in the ECLIPSE study. Finally, CRP was shown to be an independent predictor of hospitalization and death in COPD.

However, the problem with CRP has turned out to be the fact that many patients with COPD have low grade systemic inflammatory response, which could change CRP during an acute event, and the CRP has not really been responsive to pharmacological interventions, except for steroids. So, there's a limitation in its ability to be utilized as a marker. Fibrinogen is a prognostic marker for patients with COPD and has been approved by the FDA, mainly because of the second study, listed here, demonstrating that COPD patients with baseline fibrinogen levels greater than 403 had a greater mortality over 18 years, a study published by Mannino, I'd like you to take a note of the long time frame to find the change in mortality.

Surfactant D protein, again, somewhat linked with inflammation, but has been shown in COPD patients, compared with non-smokers, to be increased and an indicator for an increase in the risk of exacerbation. I list, here, a few others. CC16 is quite promising, but, again, may be linked slightly to inflammation. There was some excitement over sRAGE as a biomarker. However, the problem with sRAGE is it goes down, and anything that goes down is a little bit harder to develop as a biomarker. It has been consistent in all of the studies that have been published, showing that it goes down in patients with COPD, and it's also associated with the severity of emphysema and airflow obstruction. Finally, one note is that patients serum immunoglobins lower than seven actually exhibit an increased likelihood of developing an exacerbation. So, that is, I believe, something that could be a biomarker for risk of an exacerbation.

So, the status of the serum biomarkers is like this. There are promising markers, but they have not panned out to be perfect. Also, these biomarkers seem to be further away from the

pathology that is going on. Now looking at sputum biomarkers, investigators are trying to link the biomarker to the pathogenesis of the disease, and this way you have better success with the biomarker. So, as we all know, in Alpha-1 and in COPD, neutrophil elastase plays a significant role in the disease process, and neutrophil elastics has actually been shown to be increased in the sputum of patients with COPD during an exacerbation.

I'm going to talk about this a little bit more, but this is one of our most promising biomarkers, desmosine/isodesmosine. Desmosine levels are shown to be elevated in the sputum of COPD patients with Alpha-1 Antitrypsin, and, as you heard earlier, Alpha-1 Antitrypsin augmentation therapy decreased levels of desmosine. Desmosine being tightly linked to the pathogenesis of disease. Some studies on MMPs have shown that MMPs are elevated in COPD patients, but these are all over the place, mainly because MMPs are not as stable enzymes as may be for other enzymes, and, so instead of looking at the MMPs themselves, investigators have begun to

look at the breakdown fragments produced after degradation by MMPs, such as PCG, PGP -- and Alpha-PGP.

Dr. Wells' group has shown that PGP is detected only in COPD sputum samples, and also is a developing biomarker, and he's been kind to provide us with some unpublished data that I'm going to share with you.

So, now getting back to the desmosines being a promising marker for us in Alpha-1, I'm not going to go through this in great detail. This has been talked about, and I think, maybe, the next talk will go more in depth, but this is -- I want to introduce -- the desmosine I'm going to talk about data from the RAPID trial, and from the fact that this trial did show a change in CT densitometry between patients on augmentation therapy in patients with Alpha-1 Antitrypsin Emphysema, who received Alpha-1 replacement, than those who did not, and what was shown in this study, using desmosine and isodesmosine, was that the biomarker associated with change in densitometry, as I told you, this is linked closely to the pathogenesis of the disease, those

who were identified to have reduced levels of isodesmosine and desmosine were in patients receiving Alpha-1 Antitrypsin, at all time frames, relative to baseline, and there was a significant increase in this marker, from baseline, 24 months, in the placebo group. The correlations with isodemosine and desmosine remained with FEV1 and DLCO at baseline and at 24 months.

This is a very nice piece of data from the study I just described, where the investigators were able to show that the changes in isdesmosine, desmosine were linked to the level of decline, as measured by CT densitometry, which we are going to talk about at the next lecture. So, clearly, this is a very promising biomarker, and having linked it to some of the clinical correlates of FEV1, and DLCO, and CT densitometry, it seems that one could use this in short term studies to determine effect, efficacy, of pharmacological therapy.

Another marker, PGP, is extremely exciting in our field, and, as I said, not a bunch has been published, but Dr. Wells has provided us

with some unpublished data today, to see the promise of this biomarker. This first study showing that PGP is elevated in COPD and can be pharmacologically augmented. This is very important. We can actually see -- shown here, PGP levels, in control, and smokers, and then, over here, when you treat with Roflumilast, you can see that the sputum levels of PGP are responsive to the treatment.

Now, this is a study, that is going to hopefully be published soon, that demonstrates a correlation of PGP with clinical parameters of COPD, FEV1, that it correlated with FEV1, FEV1, as we see, GOLD stage, and CT scan, as you see here, and I'm very excited for some of us who are looking at trials in exacerbation. We can see that the PGP levels, are lower in the population without severe COPD exacerbations. Patients who had a low level of PGP really did not have exacerbations, and patients that had above the mean had a significant number of exacerbations.

So, I believe that both the PGP and isodesmosine present very promising biomarkers for us in both COPD and in Alpha-1. So, another

method which we can use as a biomarker is imaging technology. Presently there are limited modalities for the imaging of pulmonary disease since most of these imaging modalities reflect the damage that has already occurred. This is not very beneficial to predict the course of the disease since we are only detecting damage after it has occurred. The CT as a biomarker will be discussed in the next talk. So, I'm not going to go into too much detail.

One other method that's been developed is something called the parametric response, and that's, again developed in COPD. This is a method by which you match inspiratory and expiratory CT films, and then you can get the measurements of the parametric response mapping, SAD, and then EMP, which have been shown to correlate, shown here, in this published study, with lung function. Here if you measure the PRMFSAD -- you can see there's an association with FEV1 decline in GOLD 0 stage, and both measures, over a five-year period of time, correlate with decline in disease.

So, what's important about this

measurement, though, is that the decline is only measured in mild to moderate disease, and the difference was only detected after a five- year period of time. Therefore, this measurement is not easy to use if you are trying to use to study efficacy of a drug. There are other CT scan measurements that have been in the literature recently. Mean segmental wall thickness and total emphysema percentages were shown in the COPD population. Just take note of the number of patients that it took to see an effect. Airway fractal dimension is a measure of the complexity of the airway we modeled. This, again, took five years, 8,000 patients, but it did correlate with FEV1, FEC, and CT in the emphysema patients.

So, I think these imaging modalities are important, but, again, I want to reiterate they reflect irreversible damage, and they also require thousands of patients in all the studies that I just listed. I'd be remiss if I didn't mention this, an MRI shown in Alpha-1 Antitrypsin patients was able to detect the difference between Alpha-1 Antitrypsin, as you see here, and COPD patients. These are the normal COPD and

Alpha-Antitrypsin patients, but this is really not feasible, performing Helium MRI in patients is not feasible and it's not feasible, certainly, financially to do a study with an MRI, for patients on a large-scale.

So, our laboratory actually, and many others, have turned to something called functional imaging in lung disease, again, with the idea that you're targeting the pathogenesis of the disease. So, most of the imaging agents I just told you about target end stage destruction. What we wanted to do is develop a method to image cellular process that are related to the disease pathogenesis. Thereby, you actually can get prognostic information over the time course of the disease, and potentially would be useful to examine the efficacy of a therapeutic intervention.

Functional imaging is not new. It's been used in multiple other diseases. Cushing's disease, we're all probably familiar with studies tracing red blood cell and white blood cell studies. So, this is not something that was new in the field of imaging, but it is actually new

to the field of lung disease. So, the approach we utilized was to think about what is the pathological process going on in the lung and can we target this pathway. As you heard earlier from the speakers, Alpha-1 patients exhibit increased apoptosis in the lung. Emphysema patients also have increased apoptosis. We therefore targeted apoptosis with a functional imaging approach to determine if we could detect ongoing disease and potentially, in the future, use this imaging modality as a biomarker.

So, as I said, Alpha-1 Antitrypsin has increased apoptosis in the lung, as demonstrated by multiple investigators, and can we image this process? We've used something called Annexin V as a target, and we've utilized SpecCT imaging to try to identify the tagged Annexin V in the lung. I won't go through all the details of this because the hour's late, and I don't want to put everybody to sleep, but what we did is we labeled Annexin V-128, injected the agent and scanned. Shown here is the data, here are the normal patients, and we're able to show this Annexin V agent clears the lung by three hours, and, so, we can utilize

this timeframe for, eventually, our patients. There are unpublished studies, and we found that the Annexin V signaling increased in COPD patients and was elevated as obstruction worsened in patients with COPD, compared to the controls and the smokers, shown here.

Dr. Goldklang my colleague has taken on these same studies in Alpha-1 patients, and had been able to show -- this is just an image of a healthy subject and an Alpha-1 patient with Annexin V, and you can see the increased uptake in the base of the lung in this patient, and in the Alpha-1 patients that she has studies thus far, she's been able to show that the Annexin signaling increases with the development of COPD in the Alpha-1 patient, and not only that, the Annexin V correlated with the FEV1. The higher the uptake, the lower the FEV1 in the patients.

So, the use for, well, our hope for this, as we continue to develop it, is that this reflects the pathogenic process that's going on and that we can actually visually see what we're attempting to block, and hopefully we can develop shorter studies with this imaging agent and

decrease the expense of intervention, and, also, it is very critical in a rare disease that we can perform studies with less numbers of patients. We think that this imaging agent potentially can, in correlation with the biomarkers that I talked about, can allow us to use lowers numbers of patients in studies. In the study that Dr. Goldklang has completed, we are actually measuring some of the other biomarkers, that I told you about, in the sputum to see if we can correlate with the imaging.

So, this my sort of summary slide, and my chance to say what I think, and not just what's in the literature. We unequivocally need a very specific marker in the disease that will not require four years of a study and a very large number of patients. We're a rare disease. We can't afford 400 patients in a trial and follow the FEV1, which we all know in lung disease, now, is really not a good moniker of a progression of disease. We know, in COPD, we have patients with the same FEV1 and totally different clinical characteristics. So, that's not an endpoint for us anymore. I think that isodesmosine and

desmosine are really, really promising, and that everyone who is performing Alpha studies now will be looking at this and confirming its use as a biomarker for the disease. PGP has not been tested in Alpha, but very rapidly, I think, the group will find some evidence as they are continuing to work on PGP as a biomarker. What's important is that PGP is already shown to correlate with the clinical picture of COPD so it has great promise. I believe, when you have a biomarker that correlates with a clinical parameter you can use that biomarker in subsequent studies without having to perform long term studies in an attempt to get a clinical signal.

CT densitometry, I'm going to speak about next, and then airway fractal dimension. I think it's promising in COPD, but, again, in Alpha-1, it's not been established, and it also requires a large number of patients. We don't have the numbers, nor do we have the time to wait for what a COPD study would take, and we need to use the information obtained from the COPD studies to put into our trials.

So, finally, I should mention all the people in my group. Well, I just won't mention them all. I'll show you the names. I want to acknowledge my collaborator Monica Goldklang for the imaging studies in Alpha1 and also our collaborators Lynne Johnson and Gebhard Wagener for their support of the project. Also, we are grateful for the funding of this project. Our functional imaging would not be possible without NIH having put out an RFA to make us think about functional imaging in the long, and we really are grateful for that, and we're grateful to the Alpha-1 Foundation for funding the Alpha-1 imaging project, and, most importantly, of course, it wouldn't be possible if we didn't thank our patients who participated in our trial. Thank you.

DR. MARKS: You know what? Just stay up there just in case there are questions for you.

DR. D'ARMIENTO: Oh, sure.

DR. MARKS: Any questions?

MR. ZARNIACK: Hi. Michael Zarniack - about functional imaging with, kind of, regards to hyperinflation and air trapping with emphysema

and whether you think there could be a clinical correlation and used as a biomarker, along with CT.

DR. D'ARMIENTO: So, that's what we're -- so, our software allows us to do some of those calculations, and we are doing that. We're finding, though, that, despite the loss of lung tissue, we still detect an increase in uptake in the lung with this agent. Some of our other agents coming down the pike, we think, will be even better at doing this.

MR. ZARNIACK: And as far as, let's say, a six- minute walk test and PO-2 max as a clinical correlation or a biomarker for emphysema, do you have any feeling on whether that's an endpoint to measure?

DR. D'ARMIENTO: Six-minute walk?

MR. ZARNIACK: Yeah.

DR. D'ARMIENTO: No. Okay, so.

MR. ZARNIACK: Okay. Thank you.

DR. MARKS: Thanks very much. Okay, our final talk before panel discussion is from Dr. Kenneth Chapman, and he's going to talk about CT densitometry. So, thanks.

DR. CHAPMAN: Well, thank you. I'm delighted to pick up all tasks that have been assigned to me throughout the day. It's been a bit troubling to me that, as each presenter presents and shares difficult issues, he or she has stated that Ken Chapman will speak about this. As a result, I've been busily adding and subtracting slides throughout the day, and I'm not sure what's left of my original presentation. I have a number of potential conflicts listed.

I want to begin by talking a bit about phenotyping. Sandy challenged us to use slides that were new or that we all hadn't used many times over. So, I'll try and do that. I think phenotyping is an important concept to bring to this discussion of future endpoints for Alpha-1 Antitrypsin Deficiency. I'll briefly remind you of the rationale for CT densitometry, a brief history simply to point out the consistency of the results that we've been seeing across a couple of decades. I want to talk about the relationship of CT densitometry to other endpoints. Then, I want to talk about the suggestions that we move back to the general COPD research field and adopt

some of the endpoints that have emerged in that field over the last several years.

Phenotyping, this I guarantee, Sandy, is something that nobody else has used today, but the pulmonologists in the crowd may recognize Sally Wenzel's famous diagram that attempts to phenotype asthma. That's a little dated now. The graph talks about Th2 asthma. We now refer to a Type 2 asthma, but if you're a non-pulmonologist, you can read some of the labels, and you'll get a sense that each of those pastel colored shapes represents some cluster of asthma. I's, perhaps, easy to understand that the young child who wheezes during soccer practice twice a month may have a different type of asthma from the late onset adult who has life threatening attacks of severe asthma accompanied by nasal polyposis. The other part of this concept was that underlying these clusters, these clinical phenotypes would be endotypes. Asthma was driven by, perhaps, one or two cytokines that would be targets foR intervention.

This is a tremendously important concept, and I'll just remind you that

mepolizumab, brand name Nucala, the injectable intervention for severe eosinophilic asthma, now marketed around the world by one of the large companies, is a game changer in severe eosinophilic asthma, but was a complete failure when it was given to routine patients with, if you will, generic, one size fits all asthma. It wasn't until the phenotyping was applied that the endpoints began to make sense. All of this to say, in COPD, we're way behind the curve. We still are wedded to those old-fashioned concepts of undifferentiated chronic obstruction or the crude division of COPD into chronic bronchitic or emphysematous patterns. There is, however, one very specific phenotype of COPD that was described a half century ago. We're talking about it today. We learned from the observations of Laurell and Eriksson in 1963, lung that dissolves because of an imbalance of elastase and anti- elastase, and that warrants its own unique biomarker. So, I'll argue that CT scan densitometry both makes then biologic and clinical trial sense, instead of, for example, the maneuver that we would recognize as blowing

out candles on a birthday cake. Spirometry has been with us 200 years, which, as far as I can see, is its only real merit. We know how to do it. We know how to do it reproducibly, and we have a vague sense that we know what it means, but there's nothing physiological about measuring an FEV1.

So, the sort of thing that this leads to is a statement that changed the course of Alpha-1 therapy in Canada a couple of decades ago. My colleagues, looking at pharmacokinetic profiles of augmentation therapy, said, well, that's all well in good for those blood therapy wonks, but we don't understand those pharmacokinetics and what they have to do with the management of COPD clinically. We want to see spirometry, and that makes great sense, of course, until you map it out. When you map it out, you discover that, of course, emphysema develops slowly. So, you'll probably want to follow people for four or five years, randomize them into augmentation therapy and placebo therapy. The original power calculations suggested that you would need about 1,000 patients to do such a trial, based the FEV1.

I'm just going to remind you that the RAPID trial that people alluded to was filed at clinicaltrials.gov. So, you can look this up yourself. Of course, the study's completed. It was originally filed and opened for recruitment in 2005. It was seven years later, in 2012, that the last patient was recruited, studied, and the trial was closed. That was not 1,000 patients. That was 180 patients. It took 24 sites collaborating internationally, and it took seven years for that two-year intervention. I think we need to remember that as we start talking about larger and longer studies.

By the way, the Canadian Thoracic Society has caught up to the latest of news. They said yes, we believe augmentation therapy warrants consideration in patients with airflow limitation across a broad range of FEV1s because of benefits in lung density and mortality. With respect to lung density, they were, of course, reflecting what we've all seen before. I think this diagram, or these photos have been used, on the left, nice, dense, spongey lung parenchyma; on the right, the moth-eaten appearance of

emphysema, and I think this diagram has been used as well. The yellow frequency histogram is meant to be the histogram of Hounsfield units in a healthy lung. That shift to the left, marked by the red line, is a shift to lower density Hounsfield units, and if we quantify that shift, we can quantify the change in lung density. Smarter people than I have figured that out, but it's a way of calculating, in very precise terms, that darkness on CT scans that we associate with emphysema.

One of the first times that was done, of course, was Asger Dirksen's study. His was part of a study looking at repeat spirometry as a way of improving on the spirometry signal, and that part of the study failed, due to a phenomenon called autocorrelation. But there was this tantalizing result from CT scan lung density. As I'm sure everybody knows, the rapidly declining CT density, in yellow, over three years, are the placebo treated patients. The less rapidly declining CT scan density are the active treated patients, a difference associated with a p value of 0.07. Close, but no cigar, a very tantalizing

result.

In the audience is not Asger Dirksen, but Rob Stockley who worked with Asger, years later, to study this endpoint a little more closely. Their study, EXACTLE was not meant to be the definitive trial of lung density studies, but it was meant to help us to understand how to apply this technology. We see very similar curves that don't need labels. I think you can tell which one is active and which one is placebo. In fact, I can also give you the p value, which, according to the selected analysis of several possible statistical analyses, associated with a p value of 0.07.

By the way, of course, the raw data have been pooled. So, this is not a metanalysis, but using the raw data, investigators have pooled those two studies. There's a highly significant preservation of lung density by augmentation therapy in those two studies, and, remember, those are many years apart, which brings us, of course, to the RAPID trial that people have referenced many times. It was published at Lancet in 2015. The study design has been shown,

two years of double-blind intravenous treatment with either the standard dose of augmentation therapy or a matching placebo, and an open label extension, opened to the non-U.S. participants in the RAPID trial group, chose to continue. Across the bottom, you'll see the schedule of CT scan lung density measurements.

Now, something that did come up earlier, and I pointed out in a question from the audience, is there was a differential withdrawal in the RAPID trial, and I think that means it becomes a bit of a challenge to look at some of the non-significant endpoints in numerical trends. It's easy to imagine that placebo treated patients having difficulty might drop out ahead of the active treated patients. I don't know if I can convince you of that, but I'll point out, if you look at the top line on that table, there were twice as many withdrawals in the placebo treated group as in the actively treated group. For what it's worth, the numbers are small. There were three deaths in the placebo arm, one death in the active treatment arm, lung transplants are one each, and then this disparity

for the vague withdrawn consent, roughly two to one ratio. Something was going on there, and that something could have influenced some of those other outcomes.

We're back to blood, and this is the low serum levels for Alpha-1 Antitrypsin. In blue, we have the people randomized to placebo for the first two years, and then crossing over to a delayed start in the second two years of open label extension. The early start group, a rise in serum levels, promptly, and, of course, continuing through the two years of the double-blind and two years further in open-label extension. This graph has been shown before. We see the more rapid rate of lung density decline, in black, in what we can call the late start group. These are people who are given intravenous placebo, and we see the slower rate of decline in patients getting intravenous augmentation therapy at the standard dosage of just 60 milligrams per kilogram per week.

In the open label extension, we see, perhaps, indirect evidence of a disease modifying the effect. That is the late start patients now

have a slower rate of CT scan density decline, but they don't catch up. They don't recover any lost density. So, disease modification has been lost for that group in those first two years with delayed start.

What I wanted to point out, because people keep referring to RAPID, RAPID, RAPID, it was not the only CT scan lung density trial, and I'm showing you the graphs from the two earlier large-scale randomized trials. They produced very similar data, and we're looking at very similar differences between active and placebo across those decades of study, about a gram per liter per year of lung density preserved by augmentation therapy at the standard dosage. I don't think that's a questionable or fluky sort of endpoint. I think that's a highly reproduceable endpoint, and, once again, I think it measures the major abnormality of this particular phenotype of COPD. But there's a problem, and I've labeled it the double jeopardy problem, and I'm going to hesitate over this next part because I think I was invited to talk about this as a very polite Canadian, but as, a very

polite Canadian, I'm going to tell you that, damn it, all, RAPID was a positive, definitive trial. CT scan lung density measured at TLC declined more slowly in the augmented than the placebo treated patients, P values there, 0.017.

If I don't move on to the next arrow, somebody's going to question that from the audience. So, yes, you're right. In the paper, TLC and FRC densities combined was the endpoint, and people have talked about this as the predetermined endpoint. In 2005, adding together CT density and TLC in FRC was never the intended endpoint. Throughout the trial, the radiology technicians were taught to get people to take a really good deep breath in, not hard to do. It's what you do before blowing out candles on a birthday cake. It's what you do, reflexively, before you sneeze. As to the FRC, that's kind of tough.

If you're a non-pulmonologist, actually, even if you're a pulmonologist, describing to a patient that they let the air out, well, not all of it, but to where their muscles aren't doing any pulling in or pushing out, well,

that's FRC. The radiology technicians were told to aim for this and if they didn’t hit what we define physiologically as FRC, they just had to make sure it was a volume different from the TLC volume.

So, I will tell you that the decision to add the two together, I'm sure, was made with the best of intentions, but people in the field who designed the study were never asked what they intended. The Asger Dirksen study, looked at CT scan lung density at TLC and nobody in this field ever wanted to add in the volume correction signal. But to people who came to the trial seven years after it had been started, it was decided that adding these two measurements together made sense. I guess the way we define it, in our quirky, academic world, that was the a priori endpoint, adding the two together, but it was never the intended endpoint. By the way, it is not specified in the statistical analysis plan filed under clinicaltrials.gov, and elsewhere, in 2005.

Why do I go on about this? Our Chair just pointed out that he's a

hematologist/oncologist, who doesn't read CT scans all the time. I got to tell you, people in our radiology department sit in the dark and they confuse me endlessly, but I've figured out that my area of interest, on the left, full inflation CT scan, TLC, my interest, is all black. That's emphysematous lung. That's where we want to measure lung density in a trial in Alpha-1 Antitrypsin Deficiency. All that white stuff is bone, fat. Somewhere in the middle, I think that's heart, unimportant stuff, in other words.

This is not from the RAPID trial, by the way, but taken from the internet. What you have on the right is that same individual at full exhalation, and this would be representative of what we see. In other words, instead of about 60 or 65 percent of the picture reflecting lung, we see something under half of the picture reflecting lung, and, now, what we're measuring is a whole lot more of that unimportant white stuff, heart, bone, skin, fat, and I would suggest to you that choosing not to measure the key area of interest on the left, but to mix it together with the noisy signal on the right was a mistake.

I think it's time we owned up to that mistake. RAPID, as designed, was a definitive trial.

I want to share with you what these differences in lung density mean clinically. I'll show some data that you've seen already, and some that you may not have seen. If we look at the end of the open label extension, Gerry McElvaney's paper describes this. There is a correlation between FEV1 and changes in lung density. This is FEV1 as percent predicted. It's also true in absolute numbers. You can actually put some absolute numbers to that. For every three grams per liter of lung tissue lost, as measured by the CT densitometry, there's a 10 percent change in FEV1 percent predicted. By the way, that would be a valid way to calculate an MCID for CT scan lung density – anchoring it to a known MCID for FEV1.

MCID is a very quirky beast. Don't get me started, but it is reasonable to calculate it against an anchor, and if we want to call FEV1 an anchor, then we can certainly back calculate to an MCID for CT scan lung density. There's also a correlation with forced vital capacity. This

has been shown in the last presentation.

If we look for other parameters that track with change in lung density, we can look a elastin breakdown products as measured by Gerry Turino and his colleagues. We saw the RAPID trial a decrease in elastin breakdown products amongst treated patients but not in the placebo group, in the first two years of the trial. Those last two pairs of columns represent, of course, the open label extension, where we see a reduction in elastin breakdown products for all.

This has been either shown or alluded to, and I simply wanted to put it up again because the serum level story is an interesting one out of the RAPID trial. We, of course, gave a single, standard dosage of intravenous therapy 60 milligrams per kilogram. So, coming up with the dose response curve is a bit cheeky. This is a model of highly processed data, and those yellow bands have some wide confidence levels, but it's fair to say that because of different volumes of distribution, different breakdown rates of Alpha-1 Antitrypsin amongst individuals we do have a range of serum levels even using a single

dosage. As best as we can determine across the measured range, there was no plateau effect to the lung density protection effect relative to serum levels. The thought that higher dosages may offer more protection is, of course, being looked at in other studies. Higher doses may provide greater efficacy.

I'm not sure that I've seen anybody present this today, but we know from longitudinal or natural history or observational studies, there is a reduction in mortality amongst those individuals do receive augmentation therapy. because every robust finding in the RAPID trial, In our Lancet paper, we explored the relationship between lung density and mortaliy. On the Y axis, of this graph we have plotted lung density and on the X axis, years of age.The horizontal line points out what we call terminal value, an important lung density value. There were six or seven individuals who had transplants or who died during the trial. They did so very close to a value of 20 grams per liter lung tissue, and, if we look at the starting point, the average lung density in RAPID and the average untreated rate

of decline, we can see how long those individuals would survive with or without a transplant. If we look at the augmented individuals, we see a difference, and the difference matches up well with some of the data that Sandy showed us, at the very outset, this morning. Augmented individuals who live longer and the numbers that Sandy showed from the real world don't look very different from the calculations in RAPID.

There was a mention made earlier that a speculation CT scan lung density changes in Alpha-1 would not be linear. What I can show you is this graph, which has, on the Y axis, the CT scan lung density decline, and ages of the individuals in RAPID. The dashed line, of course, are the augmented individuals. Their rate of decline is less than the solid line of the individuals who received placebo, but the point is there are two separated lines. They are not alinear, as FEV1 would be. They are linear. To the best of my knowledge, CT scan lung density measures the gradual and relatively constant melting away of lung parenchyma over decades.

I want to offer a word of caution about

using COPD endpoints. In my day job, I see patients with a variety of lung diseases, and even sometimes do research in those other lung diseases. So, I wanted to point out that, in the COPD world, folks are really trying to wrap themselves around how best to approach the disease, how to phenotype the disease, and you'll forgive me if I share with you a very familiar diagram. This is the four-quadrant diagram developed by the GOLD strategy group in 2011. It stayed much unchanged until 2016, and for those of you who haven't seen it before, or haven't seen it recently, what I hear or tell you is that the task, as assigned by the GOLD strategy folks, was to place our patients into one of those four quadrants, labeled A, B, C, and D. Those four quadrants are meant to tell us something about the patient's COPD, its possible evolution, and the pharmacotherapy that we might best use to manage that patient's COPD. But it was a tricky four-quadrant diagram.

On the left, what we have is the degree of airflow limitation, mild, moderate, severe, very severe, and tricky because there's also a

right-hand Y axis, which is not having exacerbations down near the bottom, or having two or more exacerbations at the top. The X axis is a little easier. It's a low symptom burden or high symptom burden. The way to address the problem of having two Y axes was to choose the higher of the two Y axes value to categorize your patient.

In 2017, the GOLD folks said, you know, FEV1, it's blowing out candles on a birthday cake, and it actually doesn't correlate very well with much of anything in COPD (although, it does reassure us when it's abnormal that we have an obstructive process). So, they said the diagnosis, and that's what's diagramed here, is still with spirometry, and it would be good to know if the degree of FEV1 abnormality is mild, moderate, severe, or very severe, but they then said, when it comes to actually managing the patient, we're throwing out FEV1. So, a word of caution, it's disappearing from day to day clinical management of general COPD. I'd hate to see it creep back into pivotal trials for Alpha-1. Now, the four-quadrant diagram is determined by

a Y axis, which is functional, no exacerbations, frequent exacerbations, and symptom burden, low, high.

What's that done to our COPD populations? Here's ECLIPSE. It's been referred to earlier. In 2016, more than half of the patients were in group C and D. In 2017, when we set FEV1 aside as being not very valuable, we suddenly have 20 percent of the patients in group C and D. COPD gene, just under half of the patients are in group C and D, and we change. We remove the FEV1. We, now, have about 15 percent of the patients in C and D. Each time I lecture in a new country, I'll look to see if there is a COPD cohort and I'll examine this relationship, I have always seen the same phenomenon. This happens to be Taiwan. Again, old criteria using FEV1 place the percentage of C and D patients at just under 50 percent. Now, with criteria that do not use FEV1, just under 15 percent. The trials in COPD that are based on exacerbation are looking at the tail that wags the dog. The minority of non-Alpha COPD patients exacerbates. I'm not sure we want to go there in Alpha-1 trials

of the future.

A little bit more about this. You may recognize I've reproduced and blown up what is suggested initial pharmacotherapy from the GOLD strategy for category D patients. It could be a long acting anti-muscarinic. It could be a long acting anti-muscarinic and a LAMA. It could be an ICS and LAMA, but I've put up this slide in pretty big -- expanded from one because there are footnotes, and the footnote is a very crude attempt -- that wasn't a criticism, by the way, but a crude attempt to try and phenotype these minority of patients who have frequent exacerbations. If they have a very high eosinophil count, one should consider the ICS containing treatment. If they're very symptomatic, one should focus on the bronchodilators There is a follow on algorithm, which says, but if that didn't work, your initial therapy for category D, well, now, maybe you should look again, at the markers, and the suggestion is, well, if the eosinophils are above 100, you should probably move to triple therapy, and if below 100, you should start to think about

very different things, like chronic antibiotics or phosphodiesterase-4 inhibitors, such as roflumilast.

This is where exacerbation management is going in non-Alpha COPD. This brings me to a recent example of trials in exacerbation endpoints in COPD. The is the very large impact trial. This is Glaxo-Smith-Kline's trial of triple therapy in COPD. You may recall that their triple therapy was associated with the lowest rate of exacerbations and the dual therapies with higher rates of exacerbation. By the way, the results in terms and time to first exacerbation were different, and we'll have to figure that out for Alpha, if we go there. This was a 10,000-patient study. The positive studies that I've done in COPD that are not Alpha have been 2,000 patients and above. The one failed trial in exacerbation I did in Canada, the Optimal trial was about 500 patients. So, we were talking about it in order of magnitude in larger trials when endpoint is exacerbations. The only thing that makes it feasible is to enrich your population with patients who are

exacerbating frequently. We're down to the 15 or 20 percent tail, at least when it comes to non-alpha-COPD, and there's a risk if you focus on that subgroup. Here is an editorial accompanying the IMPACT trial that was said to be a definitively positive trial. The editorialists and reviewers said that the investigators were focusing so much on patients with an exacerbation history, they included patients who had a history of asthma.

This aspect of trial design exaggerated the results. The IMPACT trial, in fact, doesn't tell us anything about COPD. If we focus on our frequently exacerbating population of patients with Alpha-1, some editorialist down the road is going to say, but you focused on bronchiectatic patients with Alpha-1. These studies don't mean anything for general Alpha- 1, and we will be right back at square one. For three studies, across two decades, we have had reproducible CT scan lung density results. I'd argue that we should stick with this reliable endpoint.

My summary, CT scan estimates of lung density are reproducible, reliable. It's an

endpoint specific to this phenotype of COPD. It tracks patients' clinical courses better than FEV1. It's more predictive of outcome than FEV1, and I really do worry about exacerbation trials. They are fraught with peril in COPD. They study a minority of patients. Thank you. There can't be any questions. Everybody's been using my slides all day.

SPEAKER: Thank you, Dr. Chapman. Would you be able to share with us any difference between men and women in the apparent findings on lung density decline rate in the RAPID trial that may have been seen?

DR. CHAPMAN: I don't want to dodge what's a very interesting question. I will tell you that I've been looking at some data recently, that is not in the public domain, that makes me want to ask exactly the same question, but my quick answer to the question is no, I don't have data to share, but, boy, am I interested in that question. I'd like to know where that comes from. We'll chat afterwards.

DR. PIERCE: Understood, and I would encourage anybody, who has a data set element to

that, to take a look.

DR. CHAPMAN: Yes. Oh, look, they --

DR. PIERCE: Thank you.

DR. CHAPMAN: Don't go away, right away, after the meeting. I want to hear more.

DR. MARKS: Other questions? Great. Well, I'm going to do them as -- why don't you stay up here, and we'll invite our other panelists who are going to come up.

DR. MARKS: -- that's how you know you're supposed to come up here, but I'll call out some names. I'm going to start off with with a general question, and then we'll open it up to the audience, and then if the audience finds there's nothing to ask, then we'll come around.

DR. MARKS: So, first of all, thanks everyone for the wonderful presentations. I think it's very thought provoking. I think just to get us kicked off, we're clearly in an area where things are evolving, and this is not the first rare disorder, or orphan disorder, where we have had to rethink endpoints or tests that have been done in the past in light of emerging evidence.

So, this is, I think, a -- it's just so that people understand. This happens. Science progresses, and I think the important thing to understand is that we, as an agency, FDA, also progress, and, so, we're very happy to have this dialogue, today, about trying to think about how we can accommodate progression in science.

So, with that, I'm going to ask a general question -- we can start with Miriam and go right down the line. What would you think that the best thing would be that could come of out of today's workshop, in terms of an outcome?

MS. O'DAY: I was very struck by the amount of time that it took to recruit the patients, for a very small study, under 200, and I think the best thing to come out of the meeting, today, is an understanding that this disease is not only an orphan disease, but that it's rare, an ultra-rare, and, so, when we want to get new therapies, or next generation, or new therapies to patients, we need to really consider what the clinical trial design looks like. I think CT densitometry should really be back on the table as a clinically meaningful endpoint.

DR. MARKS: Okay. Thanks.

DR. CHAPMAN: I want to congratulate the FDA for being so open and discussing this. This is a tough area, and it's wonderful to engage in this science. So, I hope we continue to meet around this disease and others. My hope would be to continue using, as an anchor, CT scan lung density because I think we do have a sense of what that means. It is logical. It is plausible, based on what we understand about the Alpha-1 abnormality, and we, now, have quite a history with it. So, we do have some benchmarks, but that's not to say we shouldn't explore some other endpoints, some other biomarkers, and I think the most challenging question about CT scan lung densitometry is, with an intervention, how close do we get to normal or no loss of lung function, a route that I think, unequivocally, told us that we could slow the loss of lung parenchyma, but it didn't tell us whether we returned that loss to normal, and that's one of the challenging questions to answer.

DR. D'ARMIENTO: So, I agree with the assessment of the CT densitometry, but I want to

say that, in this field, we've had 50 years of research, understanding the pathogenesis of the disease, and we can utilize that knowledge to develop or maybe we have already developed biomarkers that correlate with reliable clinical characteristics, and we should use those moving forward to develop therapies.

DR. FLOTTE: Great. Well, thanks very much to FDA and the Alpha-1 Foundation for doing this and for including me. You know, I -- subjects like this, I think I'm always most struck by the voice of the patient, and, so, I wanted to thank Peggy for her comments because I think this entire activity that we do in clinical research is really intended to generate enough science around what we're doing to say that it is reasonably safe and it has some effect, that it will not -- it's just not completely futile, and I think that there was ever a reason to think that the, you know, the perfect can be the enemy of the good. It's hearing the voice of the patient, that we don't have optimal therapy right now. We have some therapy, but I think we've got to commit to finding ways to get additional therapies forward

for the -- because that's really why we're here, in order to fulfill that public trust, all of us academics, government, and in industry, and I also say that the commitment that the -- I was also struck that -- how much research has been done post-licensure with all of these products. The idea that, you know, that the licensure is the end of the line is certainly -- this is fields done as, you know, I'm just amazed how much research has been done and paid for post-licensure.

So, I think having -- understanding that whatever provisional endpoints are used that might bring forward new treatments. It can still be modified afterward, if there are others that turn out, ultimately, to be more appropriate after we get some new therapies through the pipeline.

SPEAKER: First of all, I thought that it's remarkable to have this kind of discussion, and to also have regulatory authorities here, to hear the discussion, and to feel like we're having a direct impact on the way that these many novel therapies that are moving their way forward might be approached from, and eventually improved.

I also come away feeling that we have no really well-established evaluations for the earliest stages of disease. I think by the time we find that someone has emphysema, or by the time we find someone has cirrhosis, that we've -- we're already five steps behind. I thought that the imaging data that you presented was one of the first really exciting ways to look at it, early injury of lung tissue in Alpha-1, but it's especially brought home by things, like gene silencing, when we're trying to decide whether, you know, how long can someone go with no Alpha-1 Antitrypsin before you have to worry about their lung disease. We don't really have a way of knowing that, and I think that that's -- those kind of technologies are going to need to be brought forward in order to talk about what you'd really like to do, which is to prevent lung and liver disease in Alpha-1 patients, and, so, with that, I'll stop my summary.

MS. IVERSON: I would say, as a patient, the most exciting thing, to me, today, is to bring this group together, all of these dedicated people that you -- many of you spent your

entire careers working on this. It's incredibly humbling as I listen to all of you, and the hard work and the efforts that you're making, and the conversations that have occurred, that appear to me to be really open, and especially on the part of the FDA, to help us remove barriers to what needs to propel research and studies forward to help get us to that cure, whatever that may be, or to continue to improve the lives of people impacted with Alpha-1 Antitrypsin Deficiency.

DR. MARKS: Great. Thank you. Thank you very much. You know, one of the things just is that development is a challenge here, is that -- and it's come out. It's became apparent from some of the great presentations, here, is that, for many of the novel therapies, one is probably going to think about using as a surrogate getting to levels in the normal range, right, which leads to this strange situation. Now, I have to go back and think about this more. I mean, it may be just my sleep deprivation from having been in four cities in four days, but you could end up in a situation where older products are held to a different standard, if so, that is

not -- it is completely different than standard for newer products that are coming online, despite the fact that we -- they are really, in some ways, the same equipoise about whether they work or not. Do you see what I'm -- do you know what I'm saying here?

So, I think that's something that we'll have to grapple with, back at FDA, and think about how we try to essentially set a level playing field, if we get to this, you know, this concept of could -- if you could normalize levels, what could you expect to see? I think this is, in some ways, again, my analogy for this is, again, being from the blood world, is if you -- since you can, now, normalize levels in patients with bleeding disorders, for instance with hemophilia, you can start to see what it looks like, in terms of having a essentially normalized level.

Now, those -- you don't have to be totally normal because it turns out that you can be subnormal, but we know where the level is, and as long as you stay above that level, we now have kids who don't have any manifestations of hemophilia, and I guess here the same thing was

there must be some level where you won't see any development of emphysema in this change, but -- go ahead.

DR. D'ARMIENTO: So, I think it's important to think about levels, but some of the therapies that are coming up are actually allowing the protein to do what it's supposed to do and not damage something in the process, right? So, the other thing in all the therapies are equipment, and I don't think you should always think about those because you're, for example, getting a therapy that is, you know, unfolding the protein, right? You're not damaging the liver, right? So, maybe it'll happen. It's a level less than a level, and whether they're -- so, I don't think you can -- I think we should be thoughtful about the biology of the disease -- the pathology of the disease.

DR. MARKS: Right, and point extremely well taken. I was talking about for therapies that actually were intended to change levels because I think, at the end of the day, what we're talking about is -- and I think this is something that, I think, our center particularly

has done a fairly good job with, the regenerative medicine advanced therapies. It has really taken it on itself to look at each product as its own product, and that may mean that, you know, it's true. If something -- that may be the wrong endpoint, completely wrong endpoint for a given product. So, we were -- the goal is to figure out the right development pathway, the most expeditious development pathway for each particular product.

DR. SANDHAUS: And that's the -- in, also, the example you used, that it's really easy to make those distinctions when what you're looking for is whether someone bleeds or not, in the next six months or something like that, as opposed to making -- doing those subtle adjustments and seeing if their emphysema progresses, or their liver disease progresses. It's really difficult, but I -- and I --

DR. MARKS: There's a reason why I became a hematologist. It's much easier.

DR. SANDHAUS: The other exciting thing, of course, that must be mentioned, is just since I've lived through times when the only

question was whether there was going to be another augmentation therapy, is to see the number and variety of approaches that are currently in development right now, and it's probably the most exciting drug development time in Alpha-1 lung and liver disease that I've seen in my long experience.

DR. FLOTTE: I want to second that, but I also do -- I do want to talk just for a moment about the -- my experience with the environment at OTAC and CBER, I think, for people who don't all know the example, I mentioned it to a few people in conversations before, but the example of the first licensed AAV gene therapy product, LUXTURNA, for inherited retinal dystrophy, due to RPE65 deficiency. The mechanism by which RPE65 works is that it helps to recycle retinoids, and, so, basically, it helps the most with dim light vision.

So, the FDA allowed the research team, the sponsor, to develop an endpoint that would fit around that mechanism of treatment, whereas every other eye treatment had been looked at with things, like a visual acuity, or a visual field,

and none of those existing endpoints for garden variety eye disease, I shouldn't say, that for non-rare genetic eye disease, and none of those fit the mechanism. So, the ability to use a more, if you will, molecularly targeted endpoint, I mean, that was really -- I found that to be quite remarkable, as an example of work, that great work by the agency with the sponsors and the academics to make that work.

DR. MARKS: You know, thanks. That was a really great example, and, actually, that's also an example of patient focused drug development because the company, in conjunction with FDA, worked on that, figuring out -- and figured what really meant the most to patients, and it was really for kids. It was really interesting. I'm -- having watched a couple of them be interviewed. It was, can I ride my bike as it gets late in the day, as -- and eventually they wouldn't be able to ride their bike at all, but -- as this progressed. So, that was actually -- it turned out to be a very nice example that -- I think the other piece that Dr. Scott was going to mention. He was originally going to stay for

this panel, but he had to leave, unfortunately.

I think some of the things that we can try to think about, which have a -- are really evolved, over the past 10 years, with these use of complex trial designs. We are much more comfortable, now, with adaptive designs, and I think, in some ways, that may be helpful for some products. It may not be for all of them, but for certain products, I think that may be able to help, particularly where there are multiple doses that may need to be looked at, to see if there is a dose effect and selecting a dose, or particular patient populations that may benefit. So, I just wanted to remind us of those, and, there, taking advantage of the discussions with the -- both the product office, as well as the -- our Office of Biostatistics and Epidemiology, I think, can really be helpful. While we have the last couple minutes here before we start to close, do people have unanswered questions, want to ask questions on that?

DR. PIERCE: I'm Dr. Pierce, FDA. So, we've heard today that Alpha-1 protease inhibitor, Alpha-1 Antitrypsin, is a

multifunctional protein, that there are functions beyond, or activities beyond neutrophil elastase inhibition, including an anti-apoptotic mechanism, anti-inflammatory properties. I don't think we know, at this point in time, whether the dose response curves for each of those various functions are super imposable, whether the dose response curves for each of those various functions are superimposable, whether the doses at which those different mechanisms come into play are the same. Maybe they're very different.

So, I don't think we know that, for example, the RAPID trial results, when the results, looking at all of three of them, the RCTs that have been done with A1-PI against placebo. I don't think that we know the extent to which those results can be totally ascribed to neutrophil elastase inhibition, as opposed to some of these other mechanisms. So, I would kind of ask, in the thinking about using levels in the blood, for example, of Alpha-1 Antitrypsin, as a surrogate endpoint to predict clinical benefit, how would you go about picking a [target] level

in 2019?

I think we understand more than we did back at the beginning of this, and it was also mentioned, you know, there's a certain attractiveness to normalizing levels, but we heard from Dr. Brantly that neutrophil concentration in epithelial lining fluid in AATD-deficient patients had a number of 17, compared to one for normals, five for smokers, and, so, if you have this, you know, very important increase in neutrophil elastase burden in the patients with the severe deficiency, normalizing the levels still may not get you to where you want to be. So, how do we go about, in 2019, picking a target level?

DR. SANDHAUS: Well, I think that what you suggested, that we try to normalize levels, is the minimum that we should try to do. I have -- I think that we -- there are a couple of reasons. Number one, we'd like to see the largest benefit, the largest signal that we could, and I think that the decision to go to what we all agreed would be subnormal levels in our -- in augmentation therapy development was simply a matter of what we thought

patients could tolerate, in terms of infusion volume, infusion duration, and infusion frequency, and, actually, Gerry [McElvaney] is moving to the microphone, might even have a better answer to that because he was at the NIH – as was Mark Brantly – during the time of the development of these, and I think that we now have technologies, for instance the recombinant drug that was presented to us where reaching normal levels is achievable in a manner that would be acceptable to patients. What it doesn't answer is the question of why does the body make super normal levels in response to inflammation, infection, and tissue injury, and is there a way that we could match those with augmentation better, but I think we have to take that one step at a time.

DR. D'ARMIENTO: I just want to add, I think that looking at levels is somewhat simplistic because we have to look at its function. You outlined, actually, the perfect reason why levels -- looking at levels aren't ideal. In -- during a viral infection, some of the proteases go up 20-fold. So, maybe you don't want

to look at levels, you want to look at those end products, those biomarkers, as a result of treatment instead of just targeting a level in the blood.

DR. MCELVANEY: So, I just regarding the point by Ross Pierce, and this is a very important point, actually, that the -- it's not just the neutrophil elastase inhibitor that's important. It's a very potent anti-inflammatory, and not only did the Alpha-1 increase in infections, but it changes its form slightly as well. It becomes a more potent anti-inflammatory but getting up to that level is a good start because people with the Z form of Alpha-1 can't get up to the level to -- nowhere near the level, for either anti-NE capacity, or indeed anti-inflammatory capacity. So, getting levels up is obviously the good start. That's number one. The second thing is I think Mark Brantly eluded to it today is that if you have established lung disease, you're always going to be playing catchup with the cards, anti-inflammatory therapies. So, to put this in context, we've been doing some work with ivicaftor which is a

very, very good ion channel potentiator, which normalizes your sweat chloride, etc. etc. And yet if you give that to people with CF, and it does all these good things, improves lung functions, you've still got residual inflammation in the lung, and that transmits itself into certain inflammatory signals that we get when we look at patients' neutrophils etc. So, the same will happened in Alpha-1. We could be very successful and get normal levels, and, in fact, we make get levels that respond to inflammation et cetera, et cetera, but we still may have the other side of things. We may have an increased neutrophil burden, be causing structural damage as well. So, it points to another thing, we have to get people early before you had structural lung disease, in addition to all these other things.

SPEAKER: Yeah. I just wanted to echo that, and what you said, Jeanine. I mean, as we think of new therapies coming on like protein replacement, you know, you can think of gene therapies that are going to use proteins that have hyperactive Alpha-1. So, I think an antigenic level, here, is, again, I think, a bit dated, and

we should be thinking of activity in serum, as opposed to a simple level of Alpha-1 Antitrypsin.

DR. BRANTLY: So, I just want to make a comment in here, with all subject. So, we have a number of drugs that are available, and, interesting enough, they are attacking different components, but we know the pathogenesis of a disease is -- it's unlikely that this misfolded protein disease is cured by just replacing normal protein. Now, it may make it so that our patients are better. As I was talking to Dr. Reed, my patients are normally living close to normal lifespans right now, but those last 20 years are bad, and, so, I think as we chip away at the therapies for this disease, we need to not just have mortality as our endpoint, but better patient outcomes for the patients, as far as better quality of life.

Again, as -- and, so, in the end, we may be augmenting the amount of Alpha Antitrypsin. We may be correcting the folding defect. We may -- doing a number of different things because, better or worse, Alpha Antitrypsin is a very multifunctional protein. It does an enormous

number of things, and every time we look again, we find out that it modifies neutrophil defense in toxicity, and many, many other things as we go on, and that's emerging as that it seems to play a role in tolerance and transplant rejection as well. So, I think each of the tools we have, particularly our diagnostic tools, and then also our treatment tools, we may have a set to help us guide one dose. We may have it to prove clinical outcome, and we need to use all of those tools, basically, to provide the patients with the best variety of drugs that will help improve their lives.

DR. MARKS: Thanks for that comment. Okay, and this is -- it's an interesting area, where I think you get -- using a variety of different approaches is important. I think it's important, and I think what we heard today was there are obviously plasma derived approaches. There are small molecule approaches, gene therapies, RNA interference, and here's just an interesting question, really, here, has been providing the gene therapy approach.

Gene therapies have been great for

diseases where you don't need tremendous quantities of protein. The problem here is that you're needing very large quantities, and I think that's, even with the gene ride approach, you're going to end up having to have, unlike hemophilia, where you can get away with something like, you know, a hundredth of a percent or a tenth of a percent of albumin loci transfected, here, you might have to get up to higher levels to actually get the kind of levels that you'd like to see, and I got -- it's levels and activity. That was a really good -- whoever mentioned activity, that was -- thank you for that. That's an important point, here, but I think that's an -- do you want to -- do you care to comment on that, Dr. Flotte?

DR. FLOTTE: Yeah. I think a couple points, like I said, number one is, yes, it is a high-water mark, in terms of a level of a secreted protein that one will have to achieve, compared to other replacement targets. That's certainly been our challenge from the beginning. The gene ride, I didn't really have a chance to go through what we've published on that actually, if you do that early enough, early enough in life, the --

there's a selective advantage for corrected cells that not in -- includes a synthetic microRNA. So, it's actually a dual function, this being expressed from the albumin locus, along with normal albumin.

So, that is one thing about genome edited approaches, whether they be like the gene ride or CRISPR-Cas- mediated, that if there is a selective advantage for corrected hepatocytes, that's where you would see it, is in that edited approach, and that is -- would be an argument to treat early, where there is going to be additional expansion of the liver. These are earlier phase concepts in some cases, but we would think that our best, just speaking from the aggregate of the gene therapies to the four types of products I talked about, that UMASS is connected with.

We would think that the most promising one to achieve a really high level is more analogous to the current hemophilia trial, hemophilia trials with the direct IV injection to get the vector to the liver, but delivering this allele-specific augmentation and the knockdown, but we, you know, the trouble that I have with

these things is that even though the bar is very high, you know, in order to understand the feasibility of these things, we do -- it is very helpful to know where the bar is, you know, because if we don't know where the bar is, and particularly in gene therapy, estimates of the manufacturing cost per patient and the various other aspects of that, the time that it's going to take to get to a -- to BLA, et cetera. Even though I'm not in a company, but we know this drive -- this drives the investment in developing products for a given disease in gene therapy.

We have never wanted it to see -- we don't want -- I'm speaking for the standpoint of an academic, who, in fact, even though some of my technologies are in companies, I don't get any financial interest from those, but we don't want the investors to decide that other genetic diseases are the ones they should invest in. We want them to invest in therapies for Alpha-1 because we care about the patients. I mean, it is the -- this patient community deserves the -- to have their disease targeted, be, you know, have these technologies brought to bear for them, and,

quite honestly, if you scare off all the investors because of making it look like these are un-approvable therapies, that will drive the money away from solving this problem. I mean I hate to be blunt or crass about that, but that's the way things work in this country.

DR. MARKS: Go ahead.

SPEAKER: In 1978, Genentech announced that they had successfully introduced the human gene for insulin into a safe strand of E.coli bacteria, which, then, produced the protein. It took five years for that to come to market, as Humulin, which is still considered safe and in use. Where is our recombinant Alpha-1 Antitrypsin? Thirty-seven years ago, there are, I believe, from what I've been studying, that there are candidates, and there have been.

DR. SANDHAUS: So, there actually have been recombinant products tested in Alpha-1, that they've had a storied history. There have been recombinant products, made in E. coli, there have been recombinant products made in yeast, there have been transgenic products made in sheep, and most of those have failed, either because of

pharmacokinetics: They aren't processed the same way that human Alpha-1 is processed, in terms of the sugar molecules that are stuck on it.

SPEAKER: How different is insulin (overtalking)?

DR. SANDHAUS: Insulin is a very small molecule that doesn't have sugar molecules on it and works hormonally. In other words, a very tiny amount makes a big difference in someone with Diabetes, whereas this is a bulk protein that has to get there in large amounts. And there have been safety issues with some of the products that were made early on that led them to develop allergic reactions in the lung, and things like that. They certainly didn't last in the circulation anywhere near as long as naturally occurring Alpha-1 did because of the kinds of sugar molecules that were stuck on those proteins. Now, that looks like it may very well have changed. I don't know if you were here for the presentation on the recombinant product that's moving forward, but it looks like there's promising work that's been done, and, so, I guess I'd say we learned our lessons from the past, and

that maybe what you're asking for is actually on the horizon for us.

SPEAKER: I hope so. Well, I am very happy and excited about many of the things that I've heard today. It makes me feel so much more hopeful. Thank you.

DR. MARKS: Thanks very much. What I'm going to do, so we kind of end on time, here, is sum up. We heard a variety of presentations today, which I covered a lot, between the nature of the disease and the current science. We heard some about novel trial designs. Then, we heard about a variety of novel therapeutic approaches, and there's quite a lot of excitement there, and then we had some discussions about manufacturing technologies, and dealing with the approaches to the liver disease. We talked about endpoints: both biomarkers and CT densitometry. I think there's some real food for thought here. There will be a transcript of this meeting made available.

I also believe that the Alpha-1 Foundation is going to try to have a publication come from this, and I think that, when we go back

to FDA, we'll be thinking about how we can best foster further product development, because I don't want people to leave here thinking that there's just some set number of patients needed in a clinical trial to get a product approved. That's not how it works. The way you should be thinking about this is that FDA is here to work with you for each individual product, be it a plasma-based product, recombinant product, a novel molecule, or a gene therapy. I think that we have to adapt using science in 2019, soon to be 2020, using our best approaches; not just biomarkers or CT, but also what we have in our armamentarium for trials with novel designs, and applying a thoughtful regulatory approach.

So, we'll look forward to working with everyone. What we want people to leave here with is that we're committed to helping get products to patients in a timely manner, as quickly as we can, using approaches that make sure that the products that get there are safe and effective, and safe and effective doesn't mean that it's safe beyond any reasonable doubt. It means that they're safe and effective in light of the

benefit-risk considerations, as appropriate.

So, with that, I want to just finish by thanking our co-sponsors. I know why they probably had to leave but thank you to NHLBI. Thank you so much to Miriam and the Alpha-1 Foundation, who contributed tremendously to making this happen. I want to thank our staff at FDA, Dr. Pierce, and the folks from the Office of Tissues and Advanced Therapy, and particularly, Debra Ellison. Without her efforts, this would not have been pulled off. She did an incredible job making this happen, and I thank her so much. This has been a thought-provoking day, and I really appreciate everyone hanging in there. It's just 5:00. So, the Swiss train is on time. Thank you so much.

(Whereupon, at 5:00 p.m., the PROCEEDINGS were adjourned.)

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NOTE: Due to issues with transcription, his written transcript has been edited for content and clarity by several of the speakers.