ANTHONY WINDEBANK: Today is a really exciting presentation. One of the areas of drug discovery that's really moving forward fast is something that we're going to be hearing about this morning. And we're particularly fortunate at Mayo to be involved in this kind of what's really world-leading academic partnership in the area of drug discovery. I was joking with the presenters that I could spend the whole time just introducing the presenters, but I'm not going to take up the time because this is such an exciting area.

Dr. Andrew Bradley's going to begin the presentation. He's professor of medicine here at Mayo Clinic. And then Richard Bram will be talking as well. Richard is professor of immunology and pediatrics here Mayo. And then Dr. Tom Chung, who's the director of outreach strategy and alliances at the Center for Clinical Genomics at the Burnham Institute. And as you hear this, you'll understand what an important collaboration this is. So Andrew.

ANDREW BRADLEY: Well, thank you, Tony, and welcome, everyone. Today we're going to talk about some of the activities that we've been involved with drug discovery. And to begin with, I'd just like to point out what's been going on in the pharma industry. So over the past several years, the productivity of pharma has gone down, and the price to creating new drugs within the pharma industry has gone up.

And if you look at the origination of products that have eventually made it into a drug, you'll see that in blue on this slide, the number of products that are from self-originated projects-- meaning that the drug company said, let's make this a target, let's make a drug for it-- is somewhere in the 30% range. And what that means is that 70% of the products that are eventually made come up from other mechanisms. And one of those mechanisms is academia.

And so the in-house resources that are devoted to basic scientific discovery within pharma is being cut, and more and more pharma is starting to partner with other opportunities for determining those lead compounds, or those lead targets. And that prompted Francis Collins in 2011 to take this on as an initiative and really to incentivize NIH funded researchers to partner with drug discovery, and partner with pharma to get into the drug discovery world.

Now Mayo Clinic, as you all know, has been long associated with drug discovery and drug development. These are just three examples. My field is infectious disease, so of course I have to highlight a couple of the infectious disease stories.

But of course, we all know about Kendall and Hench and their discovery of cortisone. Streptomycin was first used at Mayo Clinic. It was developed at Rutgers University. But it was used first in Cannon Falls at a Mayo Clinic sponsored sanatorium for TB. And we all know that streptomycin is a very important drug for therapy of TB and other microbacterial disease. Similarly, penicillin was first used at Mayo Clinic, and this is the first case of erysipelas being treated with penicillin with rapid resolution of the symptoms. And as was pointed out to me the other day, it also makes you grow a mustache.

So what are the benefits of investigator-initiated drug discovery? Really they're multiple. And first is that, in pharma, in order to have a target as a target that they want to pursue, typically they'll wait for 30 or 40 publications in the field and a consensus to be that it's an important target. And they'll make a business decision, and they'll meet with their stockholders. And then make a decision to pursue the drug.

In the investigator-initiated world, you don't need to do that. You can have preliminary data that you think this is an important target. If that preliminary data is sufficient, that's enough to move forward. Therefore, you're not limited by the consensus of stakeholders, and you're not limited by the target market. Pharma wants to do big blockbuster drugs, statins, blood pressure medicines. Those diseases with limited populations that are affected by the disease are still amenable to the investigator-initiated process.

Moreover, and this is as important as anything else, we can be quick to kill a process. So if you set up a screen, and for whatever reason the screen doesn't work, we haven't put sufficient time and resources in it that we can't stop it. We can say, OK, it's not working. Let's move on to a new target.

Finally, success is different. Whereas success in pharma is a drug that is given to patients that treats disease, success for Mayo is many steps along the way. Licensing the product out, partnering for further development, learning the new scientific advances that come associated with the drug discovery are all different kinds of success that are involved.

So very recently, Mayo has plugged into drug discovery. And this is an article from*Discovery's Edge* within the past month or so. And just what I'd like to do very briefly is oversee the overview of the capabilities that we now have at Mayo.

Mayo Clinic, for years, has been involved in target identification with our best scientists, and we've done a great job at that. We've also done a great job when it comes to preclinical animal studies on through phase 1, 2, and 3, and eventually licensing. What we have not done a good job at because we didn't have the resources was developing a screen to screen for a drug. Once you take that screen, you develop hits, you make leads, you do p.k., in-vitro, and in-vivo optimization. We've not had that capability.

So to accomplish the first phase, we've created a partnership with Sanford Burnham. And Dr. Thomas Chung will come up and describe that in great detail in a few minutes. But this is an important collaboration that I think people will begin to take advantage of.

In addition to the screening, there's a variety of other things. So the areas that we're funding now are developing the screen and using that screen to develop hits. That's the part we fund. There's many other steps in the drug discovery pipeline, and so we're coming up with strategic partners to accomplish that and funding mechanisms to accomplish that.

So far, we have signed agreements with all of the companies listed here to partner in the drug discovery pathway. And as projects come to our attention-- and we have a joint steering committee with Sanford Burnham and ourselves that oversees the review of these applications-- as these partnerships are needed, we're happy to refer people towards them. And I won't go into much detail as to what they are today, but I'll talk to people individually.

So in the target identification, there's a little bit of dollars. In the screening and hit generation and lead compound optimization, there's a lot of dollars. So we're taking steps to fund each step of the way.

So the first RFA, which is the one that most people have heard of is an RFA that was piloted in 2012. We've reissued it in 2013 and 2014. And we hope to reissue it in 2015. And we expect the announcement will come out soon.

And the purpose of this RFA is if you have a lead target and you think it's important for whatever disease it is you're studying, and you have good scientific data to suggest that if you alter this target, a beneficial phenotype occurs, then take that data, propose a screen, talk to TC and his colleagues about whether that's an appropriate screen, and submit an application to the RFA. And those will be reviewed on a competitive basis.

If awarded, you and the Sanford Burnham team will work together to develop a scree. That screen will be optimized. The operating characteristics of that screen are important in the drug discovery world. They will be optimized, and then that package of information, along with the package of information you have on the scientific merit of that target will be used to submit for other sources of funding.

Thus far, we have submitted to a variety of places. And as you'll hear, we've received RO1 awards for this. We've received awards from the Michael J. Fox Foundation, Leukemia and Lymphoma Society, state of Florida money. So there's plenty of venues to apply for to get funding to move this along.

I'm pleased to announce today that there's going to be a second funding mechanism that you will hear about shortly. This is a very brief overview. And this has occurred in partnership with our CCaTS, CTSA, and the University of Minnesota CTSA. And this is in partnership with the Minnesota Partnership for Biotechnology and Medical Genomics. So we all know about this program comes out twice a year. Some of those monies are going to be carved off towards a new funding mechanism. And it's going to be called the Translational Produce Development fund.

What is unique about this new funding mechanism is it will not fund science per se, but it'll fund development of a project which is intended to create a product. That product can be a diagnostic tool. It can be an artificial hip. It can be a new IT capability for some medical purpose, or, as it pertains to this, it can fund the implementation of a screen that has already been developed through the Sanford Burnham type mechanism.

So with that as a very brief overview, I'll just point out that Dr. William Mayo in 1866 pointed out that scientific research is critical and that we need to invest our time and future into scientific research. And we think this is a pathway which will facilitate that. And I think in the two or three years this has been in operation, it's really started to change the paradigm by which Mayo researchers do research. And I think very much for the better. So with that, I'll invite Dr. Chung up here to give an overview of the Sanford Burnham collaboration.

THOMAS CHUNG: Thank you, Andrew, for the kind invitation. And thank you all for bearing with a lunch time meeting. I'm Thomas Chung. I go by TC. So just to clarify one thing. We're a department within the larger Sanford Burnham. And so what I'd like to do, and I need to do disclosures that you're familiar with that just lawyers have to do.

With regard to continuing education credits, there are three learning objectives I hope to get to you [INAUDIBLE]. I'll summarize for you what Andrew has put up some great slides of the drug discovery process as kind of practice today. So anyone in that should be able to after this seminar recite at a high level what those steps are. The second is with specifically why Mayo has partnered with Sanford Burnham and our department. I'd like to share with you our capabilities and some case histories.

And lastly, the important thing to what Andrew said is it has been almost two years. So what is our organization's goals of this joint steering group? How do we operate? What is the value that we create, and what are some of the achievements that we can point to in this steer collaboration? So I think that's all important to communicate this to your colleagues, and to also, more importantly for you as a PI, to see why it might be worth it to participate.

So just really quickly on Sanford Burnham-- I won't belabor this-- if you go to our open web page, you can download this fact sheet. But the seminal points is we're scaled to be not quite as large as Mayo, but we are complimentary. At our peak, we had a fairly high budget of $190 million, but it's been tough going with all funding all around. So we have had some [INAUDIBLE] realignments. The key thing is we're moving forward as an organization and drug discovery innovation licensing out is becoming a cornerstone of our organization within the nonprofit space. It's as important as the impact of basic research. So I'll let you look at that. And you can look that up at your leisure.

With regard to the Conrad Prebys Center, or-- it's such a long name-- it's just the Prebys Center now. Conrad Prebys was a Hungarian who came to the US at age 13 with $100 and built a multi-billion dollar real estate empire like most people do. He is very much interested in science, and that's how the name is on our facility.

The key things about our facility is we're about now 70, 80 FTEs, full time employees. We focus solely on translation enabling drug discovery for PIs. We work in partnership. All of us all have gone through pharmaceutical companies. You could say we're refugees of pharma companies. Sort of what happened to us is we've been reorganized out of existence.

We're now at a very good space. It's not a shameful venture to come out here and not be in a pharma company. In fact, it kind of helped us rediscover why we're doing medicine development. It's fun to do really interesting things. Pharma companies are not [INAUDIBLE]. I bring that up because we actually are expertise [INAUDIBLE] and our staff. We represent biotech in a [INAUDIBLE] number of pharma companies. You can see some of the lists there. Just to say that we know how to do discovery in what pharma does. But now we're actually having the space to do it in a different way.

Our core expertise is our assay development and follow up of structure activity relationship development. And we have these kinds of sub-functions and then you can see the allocation of people. I'll go into each of these in more detail. And then if you're going to test things, you need to test compounds. And you need to have collections of compounds as way to manage that. So like if you've gone to the [INAUDIBLE] and seen a clinical lab, there's all the sample management supply chain stuff. So we take care of that as well.

When you're working to scale, you cannot look at a spreadsheet. You cannot look at your notebook. You have to look at massive, big data. And so there is software and systems to do all of that. And chemists who knows how to do IT work to kind of make that all happen, medicinal chemists will actually take the compounds that you initially find and make them better, so you can evolve them. So that's a critical piece of that, as well as structural biology.

And then once you get into potency, you need to be able to make sure it gets into a person. So that's an exploratory pharmacology part of it. And we don't do this casually. At its peak, we're running 50 concurrent projects per year. That's like a meeting a week. So you can't just be casual. So that's why I am the director of outreach, but for the road map, I really was the functional project manager for it. So product manager in the best sense of the word.

So we all integrated. We have all of this. We have this world-class assay development. We have some automation you saw a picture of, but I'll go into more detail. The compound collection in total if we add up the bits and pieces is about 850,000 compounds that exist in containers. The core collection that we test in high throughput is 300,365. So we have two batches of them. And the other ones are specialty things. That last thing is a key technology I'll talk about a little bit more.

In this more pictorial view, if you look at in green, the process of drug discovery from concept to assay to this clinical high [INAUDIBLE] screening and validating what you find, you can see that process. We've seen several diagrams. What this hopes to show is that each of those steps and transitions, we are fully aligned. There's functional units that are aligned to optimize each of those processes.

So you have a concept. The PI, you probably have some test tube-based assay, Western blot, something-- some basis of it. And if you've done something, you might even test a few compounds. So you have some calibration points. Then you talk to the Conrad Prebys Center, and it's our job to basically speed that up to make things better, faster, and cheaper.

And the way you do that is by scaling things. So rather than working in test tubes, you go and plate the microtiter plates. And what we try to drive to there is we're trying to drive from a 96 wall format into next level 384 walls. And ultimately, about 80% of our screens are done in a 1,536 test tubes per plate assay. A human being can't do that. You need robotics to do that.

So from that stage of assay development, we convert the assay into a plate reader, plate format. Look at the plate as a chamber. And we look at a small collection of compounds. And that basically validates everything to make sure the assay doesn't break down. You in fact can' find some interesting things. and You know the characteristics when you've put it in stress.

Then you move it over to the middle high throughput section, you see the plate. There's a nice picture of the robot that's in Florida. In La Jolla, we have a smaller robot. And that's a compound collection. That's how it actually looks in the lab. It's a stack of plates. And there's somehow 286 plates. And we have robots to take care of that.

Once you find the compound, you have to evolve them. So now aside from finding out whether it's hit, you're trying to see if it's potent. That means you don't have to take a big horse pill. And that is selective that you hit the target tissue that's the disease, but not the normal tissue. So that therapeutic index, as you all know, is very important.

So in the test that we drive and the way that presents is you drive it by doing dose response curves massively parallel. So a key concept just for numbers is in a 50 [INAUDIBLE] plate format, each plate I can do 128 dose response curves over 10 points. I can test 128 compounds per plate. We typically run 50 plates a day. So you're testing 1,000 to 2,000 compounds per day. So we are not the [INAUDIBLE] step for finding that data. That's what we're trying to do.

Then the chemist will make compounds. And then they have the structure, and they're trying to elucidate it. The way we make the compounds is in parallel so that we can make several compounds at once that are related to the main scaffold. And we have technologies. One is a microflora chemistry that you can accelerate and combinatorialize but with solution phase chemistry so that you don't have to re-engineer all the chemistry that you read in a paper of how a molecule is made.

And the last thing is, of course, once you do that, you want to be able to do all the exploratory pharmacology to get a very good [INAUDIBLE], the best compound with the right absorption connect properties. And then at that point, we, ourselves, pass it off, and work with collaborators for the clinical supply material. And then eventually, when it's to the clinical test, then we want to-- we do everything up to the preclinical work.

So that took a while, but our core competency is we've run many different assays in the high throughput mode. But for every primary high throughput target and assay, you need supportive-- two to three on average-- supportive assays to counter-screen away artifacts et cetera. So therefore, while we've run maybe about 150 projects, we've developed about 300 or 400 assays or formats that are used.

So this top one shows you the kinds of signal formats in technology we use. We've hit pretty much all target classes. The figure on the right here is important because if you break up assays, they either are test tube cell free or have cells. And within the cellular assays, they can partition to the cells that you do not do any automated microscopy on, and high content screens where you do microscopy in an automated fashion.

And these numbers just show you what we've done for the NIH roadmap. But add on top of this our current portfolio of collaboration. So you could add another 50. But we have been doing this. And the good thing is we've been doing this for seven years now, so we know how to do it. And we have a pretty good idea of how to do things.

Andrew showed pictures of this. But the key concept of this-- the reason why I belabor this-- this particular automation system allows us to take a peripheral device, and swap it in and off the main robotic arm. So it's highly flexible rather than very rigid.

I have been in pharma. I've been in an organization where I hated robots, and then I joined an organization where we're forced to buy robots. I was forced to buy one at a time when this vendor just showed up literally one month before I had to make a decision. No one else has this flexibly. It's very important because in academics we have to really change the assay formats, and we have to reconfigure things. And nothing is ever as simple as you would predict.

In pharma, it's different. They force the rest of the organization to fit everything to that format. And they say, if you're not ready, we just don't do that. And they have 50 assays queued up time and time again. So it's true production. In Florida, we have the same thing, but in three pods. The key thing [INAUDIBLE] plug and play is every device, if it ages out, I just buy a new device and reintegrate it very quickly. Every device can be turned away from the main robot arm, so I can use it offline. I'm maximizing the use and speeding up the obsolescence cycle and not being captive to that.

The other critical piece of equipment is something called acoustic dispenser. You take a source plate that has your compounds. And you basically have a little sonic cone that shoots a sound wave coupled with water droplets. And it encourages a drop of 2.5 nanoliters to pop off the surface of that meniscus. And then it's captured on a target plate.

What this allows you to do is highly precise contact less transfer. You don't put a pipette in. You don't put a pin in. You don't contaminate your precious core sample. It's very precise in what it. gives. There's a precision over two nanoliters to 25 nanoliters. Essentially less than 1% invariant. That claim is made by high stroboscopic pictures as a perfect sphere.

This is what happens for error if you look at manual pipetting or contact pipetting. You cannot get down to those low levels. Why do we do that? Because for the seven past years, we have had a library that comprises 50 microliters. For the last seven years, 50 microliters is what we've used. And then we have a copy. And from the copy, we do our cherry picks and dose response curves. And we then have backups of the backups. But it's critical because it allows you to get maximum use.

The other critical thing about it is you never have a chance of contaminating. And the last thing is we can do dose response curves. You can just pipette or transfer a longer line and get higher volumes. So when I tell you I have a dose response curve, this is the machine that created that, in triplicate, and each transfer is not a serial dilution transfer. It's an independent experiment. So they're truly separate independent replicates.

OK, so it eliminates all that. The other key thing is image-based stuff. Every one of you have played with confocal microscope. So what this does, it takes everything that's great about confocal microscopes, automates it so that you don't have to sit there scanning it. And so you can go from a plate to find in a well of a plate. You can find the field of image, and you can find the exact cell. And you can actually determine the cell, image it, pretty pictures. But more importantly, there's machine language so that it can convert all this automatically to reams of data.

And you can do very complex things. You can multiplex because, of course, you've done this and confocal labs. You can stain nuclei. You can stain organs, acting filaments and some other things. So you can actually do layer upon layer multiplexing all at once, and that's important. So you get high resolution. You get spatial resolution. And you get [INAUDIBLE].

You can actually use this technology to do actual pharmacology-- your dose response curves. This is a GPCR. We're using condensation of beta [INAUDIBLE] couple with the GPCR receptors. One thing interesting that happened if while you have a normal type of pharmacological curve at the high end, you have this apparent diminution signal. But because you now have the image as you understand, the diminution of signal comes from the condensed puncta re-diffusing out and becoming broader. So that apparently lowers your signal.

What's happening is as we start with image-based assays, we start with one thing. As you do the experiment, you start learning. You actually create new science. It's been pretty exciting that way.

You can't do this casually. You have to have the microscope, so our core system is this. That's about $2 million. We have 200 copies. And then all the data has got to be done automatic-- you don't want to be doing this manually. It has to be archived somewhere. It has to have ways of looking at it. So every single image of that plate within that [INAUDIBLE], if I take 5,000 tiles and each tile has 40 objects, and each 40 objects has 25 or 100 parameters, every single bit of the information I capture on a large terabyte file.

And it goes and it's stored and archived in our supercomputing center. And you or anyone once you log in can say, I'm not sure about that assay. I want to see that plate, see a picture of the plate. Click on the plate, open up the field, click on the field, open that up, and actually see the pictures. Click on that and see what the data looks like. So it's like automatic image processing. I know that's revolutionized medicine too for [INAUDIBLE] So we have that.

Now here's just an example list of some of the targets, the cellular functions and what we use and size of the screens that we did. And you can see how the images work between controls and treated [INAUDIBLE]. And so rather than [INAUDIBLE] each and every one, you get the picture that you can use these to find very interesting biology and that we have industrialized it. So that was our main core thing.

This is important because-- I don't have the paper-- there is a paper from Anthony [INAUDIBLE] that was published about three years ago. And it looked at all first in class drugs by assay type. So it turns out if you have a targeted assay-- you know the target and biochemical-- you can always do very good optimization for follow on drugs. But it turns out that they plotted out the first in class drugs, they actually came from phenotypic assays where the true targeted mechanism wasn't known. But they had nested sets of cell lines and good phenotype that led them to drive it that way.

In the pharma industry, the pendulum swings several times. I've been in six pharmaceutical and biotech companies now. So I've seen that swing design everything. Screen randomly, design everything, make it optimal. So the truth is always somewhere in between. The key thing is how robust is the biology, and how well you can sort out the bad actors from the noise. And that means you need nested assays. The controls are more important than the phenotype per se. But you all know that's good design.

This is just to show you that I personally have run this. So I worked with 131 PIs over six years, 42 institutions, 31 locales, four countries, conference calls a lot. So I learned to live virtually. Andrew e-mails me a lot of times, and he always responds to it in the morning, so he didn't sleep. We are very effective at that. And all this shows that is this is a disciplined process. We have software that's [INAUDIBLE] boom, boom. Or this budget's lagging. How long has it been lagging? What stage I am at. And we can monetize our operations that way. So this is why we're working together. Mayo's terrific at the basic biology.

And I will share my personal opinion and experience has been a delight because it's not the science part that it's cool on the paper and cool in the idea, concept. It's that I know the clinician has a vision of what they need this drug for. So it's very powerful. It's a very different motivation than just I want to find a little paper. So that's very important. And even if you might not personally do the trial, just articulating that to a potential partner or licensing someone who is trying to get funding with is very important.

So together, I'll show you what we can do, and we've done it already. With Mayo, we're building it out, but at Sanford Burnham, we have done it, and we've actually transacted our first license from something we worked on as a group. And so it's a big event. It'll be probably talked about in the next couple of months. So a combined capability between Sanford Burnham and Mayo, we're going to be stronger of course. And so that will help everyone. It will help your internal programs. I think it's going to help this product development mandate that's coming up.

Here's the RFA. I'm sure you've seen it. And back again, what Andrew pointed out, so what we focus on-- the RFA's funding the Prebys Center to help your PIs go through this process. A little box called Assay Event requires all these steps. And what we have done in certain select cases, we've been able to by going through this steps, have a lot of confidence in all the robustness of it. And we can actually apply a little de-risking, and we can screen with our funds-- some of the Mayo funds and some of our own funds-- to actually getting smaller, but larger pilot libraries. So maybe 50,000, 10,000 helps us give a little more ideas of what kinds of compounds we'd find and might actually see something.

But the [INAUDIBLE] library is expensive to screen, so we screen that after we get a grant. So we're putting in grants together. There was a specific NIH FOA that the Mayo RFA addressed. And we have done that. And Richard Bram was the first one to be put in, and he got it in the first try. And we thought this would be very easy, and it's going to work great. But everyone knows our NIH funding is getting tough. I'll address that in a second.

So this is kind of the organization of the committee as it were. It's joint between Mayo and Sanford Burnham. And Mayo has more people on the committee because we don't want a tie. There's always a tiebreaker, and it's usually that Mayo will make that tie-breaking decision. So we want to make sure that we address your needs. And so the committee will look at the targets, evaluate them. There are working groups. The teams at Sanford Burnham will work on the assay, and then we report back.

So right now we're focused on this part and this part, and then have we done a grant. And then once the grant comes, we go ahead and screen. Later on, we're going to be looking at the transition to hit the lead in actually making new molecules. But that's the heart of how the organization committee works and the cycles of things.

It's also a portfolio. So I know if an individual PI's project doesn't advance as quickly, or there's a reason it stalls, or for whatever reason it's not continued, the portfolio as a whole will advance. Because there will be things that will feed it.

So where are we in terms of success and complements? I look at the target space, these are the programs that are active right now or in the course of being done. You can see we've taken a project. For confidentiality, we've taken it away, but you can figure out who it is. These all got grants. Some are on the way to filing for grants roughly. And a couple of these we've actually got the grants, so we've conducted the large screen.

This particular example is interesting. We got the grant, and then in the discussion with the science of it, I was like, we had the format [INAUDIBLE], but protein production was a little of a bottleneck, but we had enough protein to do a screen. So we said, let's go to the screen, and we did it. And now where we are, we have the protein. And Sanford Burnham made the protein. So that's the other thing. We have capabilities in protein production as well as Mayo does, so we were able to apply that.

And so this particular project is going to have two orthogonal technologies, and it's going to be very interesting scientifically to see the spectrum of compounds each [INAUDIBLE]. In one, it's targeted to a certain interaction. The other one to protein-protein interaction, or protein-small interaction. And the other one we're looking at a similar [INAUDIBLE] just directly [INAUDIBLE] target to see if it it could probe allosteric sites. So that's going to be very interesting.

This second one is from one of your former colleagues. We tried twice. We did not get an NIH grant, but in the meantime, it turned out that all that work and investment was picked up by the Michael J. Fox Foundation. They loved it, so they funded it. And it was also funded by Sanford Burnham in Florida. Convinced the state of Florida to give 2% of its tobacco tax money to fund a mini NIH screening center. So we have to work with Florida PIs, and Mayo has the Jacksonville site. Or you have to collaborate with one of the Jacksonville site collaborators. So that was also picked up by that. And so that particular project has now been run against 700,000 total compounds. That's a lot of money and value. And we're now at the point of following up those compounds.

We have another one with one of your PIs here in Minnesota. Again, it didn't score real well, but in the meantime, we thought the target was valuable so we did a small pilot. And that pilot incentivized the whole group. And while we still haven't got a government grant, what happened was GlaxoSmithKline has this program where they are very interested in some targets. And you simply make a proposal, and because we had such strong data, they accepted the proposal. So this target will now be screened against a million compounds of the GSK library, which means if they fund them, they will be highly motivated to continue collaboration. And maybe take a license action, and work on it. And therefore, the PI will probably be involved in supporting that, and we may be too.

So Andrew also recently got a another variation of that. And that's also based on our work together, and some of the things that he's decided to do. While this is set up very narrowly to go for the RFA, we're actually finding that we're getting very creative finding other funding sources which actually have a higher success rate. So we're not going to stop the NIH stuff. Either way it's good, but we have a good value add. So just to let you know that there is a good value add that if you add up all the work that we done together and the grants and equivalent value, it's about 4 million. So it's sizable, and I think it's been very good.

We have done a couple of programs where we've done additional internal screening at risk. One in preparation to address refiling. In another one, we strongly believe the target will find us tractable materials no matter what. So that's a decision that [INAUDIBLE] company made together. So the point of this is it is successful. We are building the pipeline, and we're getting very creative at not being limited by funding challenges per se. We're getting very good at finding funding.

So once we get through all of that screening, finding hit person stage, we're going to go to a transition. So just as a preamble, I'm not going to belabor it. We were looking at LEAD generation, this RFA, and all the things I described. Later on, there will be another transition, which is more like a product portfolio where we're going to have more of the LEAD expansion stuff. And our group is also very good at doing that.

And so if you look at the whole process, it's takes quite a while. And there's a lot of different steps and involvements of different pieces. And we can do that. And just as an example of the thing we actually did is you can take a very poor compound and make a better compound for licensing. And when you do this, you have to be very clear what the ideal profile of the compound is you want with these kinds of things.

You can see this particular molecule, which came out of the NIH roadmap, would be an early lead. But to get here, there were roughly about 400 compounds interrogated. So that's the profile. So there are a couple of good things about it, but everything else in red was not meeting up to specs. And after about making 800 more compounds, we got to this molecule, which basically, every key thing was crossed off and made good. So this is what it involves. It's a [INAUDIBLE] process and takes a while, takes a lot of chemistry.

So the long term, what we're trying to build together is we're trying to build a sustainable model so that when we work with a partner like Mayo-- and this is centric to us as an engine. So we're doing it and the output, hopefully, after all this will be leads that can be licensed to someone. Pharma is part of the target. They give you a payment, and the royalty terms will be-- we're not looking for a company to grow hugely-- enough to be self-sustaining.

So the idea would be that this would go into a buffer fund that's translational and evergreen. And it would basically allow us to catalyze and continue to catalyze research. And that's the general model that Sanford Burnham is trying to build with all our collaborators.

This is a bit dated. Michael Jackson had come before. That's him. It's Michael R. Jackson, not the other Michael Jackson. It's always fun. And our new CEO, Perry Nisen, who came from GSK. He's not on this. Our group's changed a little bit, but you can see the message here is La Jolla's small. One small robot, lots of people. And in Florida, not too many people, but huge robots and lots of space.

RICHARD BRAM: OK, great. So I'll give you just a quick run through of the vignette of one of the projects. And as you've heard, there have been many dozens of these that have been handled by this collaboration. It's been just really a wonderful experience on the basic science side because I really didn't have to know anything about drug screening. You can rely on our collaborators.

For disclosures, I'm going to briefly discuss what would be an unsuccessful use of cyclosporine, which would be to kill glioblastoma multiforme cells. And the goal is to learn the role of cyclophilin B in normal physiology, and then to define its potential role as a therapeutic target in brain tumors.

So we have come from just a general thought about how can we come up with better treatments for childhood cancers by thinking about some of the stresses that happen in cancer cells. And of course, they're in very uncomfortable places in the body with low numbers of nutrients and low oxygen. And so to handle that, one of the ways they deal with this is cancer cells rely on the chaperones in the cells, which are proteins that help other proteins fold and function properly.

And of course, the HSP90 machinery is well-known for serving this purpose. It's actually made up of a number of different subunits, and they stabilize different ankA proteins that are important for the malignancy of the tumors. And here you can see just a list of some of the different components. And one of them listed at the bottom is immunophilin. And that actually represents a class of chaperones which can perform this conversion shown here at the bottom which is to interconvert the cis-trans forms of prolines in a polypeptide chain.

There's three different classes of immunophilins. The cyclophilins, FKBPs. and the Parvulins. And we have been interested for some time in a member of the cyclophilin family called cyclophilin B. And if one simply looks at sort of a meta analysis of the expression of these sorts of genes in cancer cells, one fins something very interesting, which in this case, we've used the ankA mine summary of different people's studies of gene expression in tumors. Here you can see that if you look at MYC expression-- a well-known ankA gene-- that 41 studies out of 470 showed elevation of MYC expression compared to 22 studies that showed down regulation. That's consistent with it being up regulated and driving tumor regenesis.

Conversely, if you look at PTEN, which is a tumor suppressor, you can see that the majority of studies that showed a differential expression had it down regulated in blue. So if you do this analysis with cyclophilin B, you see only up regulation, and especially in brain cancers versus normal tissues.

So we got very intrigued with this and wondered if it could potentially be useful for treating brain tumors. And here you can see some of the individual tumor samples. Some in medial blastoma on the right. Glioblastoma here in the middle. Cyclophilin B is, as I said, one of these chaperones is present in the endoplasmic reticulum. And sorry, that's a typo. It's a receptor for cyclosporine not cyclophilin.

So first we had to find out what's the normal role of this protein because it really wasn't clear what its critical role was. Since it's so highly conserved throughout evolution, we decided to make a conditional knockout and created this fluxed construct shown here. We actually found that when it was completely knocked out in the mice, they were viable.

And at first, we really didn't see much difference at all. They're very slightly smaller than normal mice. And here you can see the body weights as the total knockouts get older. But after awhile, we began to notice that they had this little funny humpy back here. And that became more evident if you do plain films of the mice. Here's a wild type of heterozygous mouse. And here, a knockout you can see is a little bit smaller and has more of a kyphosis than the wild type mice,

The other thing we noted was that their skin is very flexible and lacks and tears easily. And in fact, it's very difficult to pick up these mice without them biting you because they can easily turn around and just take a big grab of your hand. So it turns out by doing some biochemical studies, we found out that the collagen is actually different in these mice. So they have collagen, obviously, or they wouldn't be born. But it's different enough that it doesn't have the same integrity. And that explains the skin defect and also the bone defect. Abnormal bone, such as osteogenesis imperfecta is a disease of collagen.

And so we proposed that cyclophilin B was a disease gene that can cause weak bones or osteogenesis imperfecta in mice, and also potentially in humans. And subsequently, that's been verified by several human genetics groups.

So to us, and at the risk of [INAUDIBLE] sort of glaring at me as I propose that brain tumors might be worth getting rid of at the risk of just having some weak bones. It could potentially be a way to attack glioblastoma and have a temporary weakness of bone strength.

So to begin to look at this, we examined some of the Rembrandt data that looks at outcomes in glioblastoma and found that patients whose tumors had higher levels of cyclophilin B had a reduced lifespan shown in red. Next, we looked at some tumors from the core facility of brain tumors run by Dr. Sarkaria. And here you can see that glioblastoma tumors from patients have much higher levels of cyclophilin B than normal astrocytes or compared to some other cell lines.

So we went on to do knockdown studies and showed that several different glioblastoma cell lines die when you knockdown cyclophilin B by lentiviral-mediated silencing RNA. We also did colony counts. And we went on to do a number of studies that I won't take the time to go into detail.

But we found that knocking down cyclophilin B activates a series of pathways that lead to the senescence of cells as shown by senescence associated beta-glucosidase standing here. The [INAUDIBLE] pathway. And also, we lose survival because these cells lose the ability to sustain MYC protein expressions. And we came up with a somewhat complex signaling diagram shown here. Again, the critical point is that cyclophilin B seems to be a central regulator of stress pathways that we think initiate from the endoplasmic reticulum, but then don't allow the cell to respond to when it's missing.

This shows that this also appears to be true in vivo. If we would knock down cyclophilin B in tumors, and then implant them in mice, we also could show that we get much less tumor growth. So obviously, we're not going to be knocking down genes in tumors to cure them, but we need a drug.

And we were at first excited to do this because, as I said, there are already drugs that bind to the cyclophilins as cyclosporine. And you guys probably know all about cyclosporine because we use it clinically to induce immunosuppression. The thing is that the immunosuppressive target of cyclosporine is calcineurin, a phosphatase downstream of cyclophilin. Not normally downstream of cyclophilin, but is downstream in the presence of drug.

So when you inhibit calcineurin, you're giving much less of the drug than you would need to inhibit all of the cyclophilin in the cell. But we found that if we have high levels of cyclosporine, we can kill glioblastoma cells. And then to make sure that it wasn't an effect of calcineurin, we showed that if we use a dimer of cyclophilin which is not immunosuppressive and also not nephrotoxic, we can get actually even better cell death. And we showed that not only can we kill glioblastoma cells, but also some medulloblastoma cell lines.

So this suggests the possibility that we can, in fact, then target cyclophilin B to treat tumors, especially if they have high levels of this protein. The bad news though is that when we tried this in mice with the drug, we didn't actually get any cures of these mice. And the thinking was that not only are we probably inhibiting cyclophilin B with some of these drugs, but we need to inhibit all of the different cyclophilins shown here that bind to cyclosporine. And the majority of them do bind.

Together, these make up an appreciable percentage of total mouse protein as well as total human. The other concept going into this thinking was that there may be some of these cyclophilins which have the opposite role. Instead of preventing death, caused death. And a good candidate for that is cyclophilin D which is present at the mitochondria and is thought to initiate death-- the necrotic death that happens after ischemia reperfusion.

We went to submit an article, and said, oh, well, someday, someone should find cyclophilin B-specific compounds, and then maybe this will work. And then I said, OK, on to the next project. And then this RFA came out from the CTSA, and we decided to apply and see if it wouldn't be possible to get a compound which could selectively bind to cyclophilin B.

So this shows the 3D structures of many of the different cyclophilins. And you can see they're very similar in structure, but there are differences in charges. And so together with TC and my collaborator at Sanford Burnham, Dr. Segienko, we came up with an approach to try to find compounds that would bind selectively to cyclophilin B and actually several assays. One of the assays is TR FRET.

And this just shows that with a labeled cyclosporine, you can detect whether it's bound by a protein like cyclophilin versus free because of the difference at which the small molecule rotates. So Edward does an assay where he binds this to cyclophilin, and then displaces it by adding a competitor. And he shows that you can detect that in this multi-well format that you heard TC talk about.

We also wanted to detect more than just compounds that would specifically display cyclosporine. We wanted to detect drugs that might interact with other parts of the protein, especially from some of our own functional data which showed the [INAUDIBLE] termini of this protein are important, but also from genetic data based on a very interesting disease in American quarter horses.

There was a very fast quarter horse named Poco Bueno, who also apparently developed a missense mutation in cyclophilin B at the sixth position. And although he was fine, any of his offspring-- of which there are many-- who got a homozygous dose of this mutation have a disease called HERDA in which their skin is very, very delicate. And if you put a saddle on their back, the skin just sloughs off and they scar. So this showed that the complete opposite side of the molecule from the cyclosporine binding site was critical for the function of this protein in vivo.

So for a way to detect drugs that might bind to a different part of the protein, Edward uses differential scanning fluorimetry. Probably many of you are familiar with this technique. The protein will denature as you raise temperature. And that's detected by binding of a fluorescent dye shown here. But the interesting thing is that if you add a compound which binds to almost any part of this protein, you shift that temperature denaturation response to a higher temperature. And so in this way we hope to be able to detect compounds that can bind anywhere.

So we came up with a screen. We would do a high throughput screen using probably DSF to begin with. Then you hit conformation, and then compare it to an unrelated protein. And next, look for binding to cyclophilin D. And we would segregate those that bind to one and not the other, and then test those for our ability to kill glioblastoma cells.

This is some of the data from Dr. Sergienko. And you can see he screened-- this is just in a matter of a few weeks I think-- 385,000 compounds. There was a hit rate of 0.08%. And of these 314 hits, I think 2/3 of them were tossed out because they were pains or other compounds that didn't look interesting. And so now we have about 99 compounds that we're just sorting through to see their behavior.

And this shows some of the IC50s that Edward found. And some have higher affinity for cyclophilin D and some higher for B. As I said, we're now in the process of testing these.

So I think we haven't completely forgotten other potential uses for these compounds. In addition to potentially treating cancer, we think they may also be worth looking at for a potential role in treatment of viral infections because cyclophilins have been implicated in the pathogenicity of hepatitis and HIV, and also, as I said, in ischemia reperfusion injury. So I want to acknowledge that some of the people who have been involved here, and also thank our collaborators at Stanford Burnham quite a bit.