is a nice integration of a glucose monitor and a pump. It looks and feels as if it's one unit.

Okay. The next topic I'm going to talk about is the artificial pancreas. Now, we don't have an artificial pancreas on the market yet, but I'm going to tell you what the artificial pancreas will look like in a broad sense when it is available.

First, it will contain a continuous sensor. It will contain an insulin delivery system, which you can think of as a pump. There will be a controlled processor which receives a glucose signal and then uses an algorithm to drive the pump. That links the glucose measurement with the insulin delivery, and then there will be a radio that will first link the sensor with the insulin delivery system so that it knows how much insulin to give and with an external monitor so that the patient will know what their blood glucose level is at all times.

This is a potential candidate to become an artificial pancreas. They still have a lot of work to do, but this is the Medtronic MiniMed long-

term implanted sensor pump or sensor and pump system. This round system is an insulin pump. It's implanted in the abdomen, and you see the different parts of it.

At the tip of it is an insulin delivery catheter, which would be way out here. It's a little bit cut off, and then it's also connected to an intervascular glucose sensor here. So this device is put in the abdomen. The tip of the sensor goes into the peritoneum, and the peritoneal delivery of insulin has some advantages because it goes right to the liver, and the other end of it is an intravascular glucose sensor that's intended to stay in the superior vena cava for a year. So that's one way, but there's other ways.

An artificial pancreas could contain an external insulin pump. The insulin could be delivered subcutaneously, and so there's different combinations, but there are some problems that have to be solved in order to have a successful artificial pancreas, and each component has problems. The continuous sensor, for example, will

have calibration drift. There has to be some way of recalibrating regularly. When you put a sensor in, you can't just leave it.

You can have a lag between dynamic changes in blood glucose and interstitial fluid glucose if the sensor tip is not in a blood vessel, but in the skin, and the majority of artificial pancreas systems that are being developed have the sensor in the skin.

There can be lag. There can be fouling of the sensor. There can be immune rejection or fibrosis of the sensor so that the body forms a capsule around it, and then it's not reading true interstitial fluid but just some kind of altered fluid that's within the cap. And there's local complications.

Insulin delivery in an artificial pancreas could have some problems, namely, nonphysiologic response to elevated blood sugar. There are some other stimuli that affect insulin beside glucose, and the current artificial pancreases are not really taking that into account.

Insulin can be denatured if it stays in the body, which is nice and warm, for three months at a time. There's systemic complications, and there's anesthesia and surgical risks of putting it in and taking it out.

And then additional problems with the artificial pancreas is that you just can't have hypoglycemia. You're the manufacturer. Your algorithm must protect against severe hypoglycemia or the patient is going to get sick and sue. There could be product recalls. A lot of bad things could happen.

So you have to run the sugar a little higher than you need it, and yet the whole idea of an artificial pancreas is to keep it normal.

Currently the artificial pancreas is being developed to treat low blood sugar because it's so important to avoid low blood sugar means that in effect you're going to have more high blood sugar than you want, and then finally there's the economic impact of improving control from current levels to better levels with the artificial pancreas

is unknown. This can be very expensive. It's not clear who's going to pay for this technology.

Another device that's being developed is a bioartificial pancreas, and this is a device that would substitute for an endocrine pancreas, but instead of being purely bioengineered, it contains synthetic materials and functional islet cells that are encapsulated within a semi-permeable membrane to protect them from immune rejection.

So within the membrane, glucose comes in. The eyelet cells see it. They figure out how much insulin to make. The insulin goes out, and this membrane protects the eyelet cells from being destroyed by antibodies or lymphocytes. The results look good in rodents, but we don't have good results in larger animals or in humans.

We need better immunoisolation to protect these cells. Every year I go one year further out. So you come back next year and it will say 2009 maybe, and it's certainly going to be expensive, about $20,000 a year. I'll show you a picture of an artificial pancreas.

This is produced by a company in San Francisco called Islet Sheets Medical. We'll look at a liver, a dog liver, and on it is this sheet, and within the sheet there's a little cuff that's dark, and then this sort of milky white square. This milky white square are islet cells, and this sheet was sutured to the liver in a pancreatectomized dog in the hope that these eyelet cells would protect it from hyperglycemia.

Unfortunately in this particular experiment the sheet fell off. The sutures broke, and they don't know why this tends to happen. So that's a problem they're working on.

The last area I want to discuss is alternate routes for administering insulin. Dr. Langer covered some alternate routes for drugs in general. Insulin has some areas that I think are, I think, interesting.

Some promising technologies include inhaled, oral, buccal, nasal, transdermal, all of these ways of getting insulin into a person other than with a needle.

Now, here's why inhaled insulin looks promising. If you give a person, say, in the hospital intravenous insulin, which is red here, what happens is it gets in very quickly. You want rapid action.

If you give the person subcutaneous insulin, which is yellow, it lasts for a long time. So that can be good in some situations.

If you give inhaled insulin, what tends to happen is you get rapid absorption of insulin so that what you're seeing is similar to IVs. So it gets in quickly the way IV insulin gets in, and it lasts for a long time the way subcutaneous insulin lasts. So in theory inhaled insulin would be very useful for people, especially at mealtime.

Now, I'm going to show you what the system looks like from what used to be called Inhaled Therapeutics, now known as Nektar. I was an investigator with three of their trials that they did with Pfizer.

This is the lady taking out the device. It looks like an asthma spray device, but it's a

little bigger. She's putting in an insulin-like little sheet. This is powdered insulin, and there's a bubble that's going to go inside the device. So she's putting that in.

Now she's sort of getting the trigger pulled back, and when she pressed the button it's going to fire. She's turning the mouthpiece. It's going to be facing her, and now she's firing the trigger, and what's happening now is that the blister of insulin is ripped. Air comes in, and suddenly disburses the insulin into a cloud, and now you see a cloud of insulin. This is correct. It's white. They call this a standing cloud. It's inhaled insulin, and she's inhaling, and in just a moment it has gone clear. I'll show you that again.

Here it is, a cloud of insulin. It's clear. Where did that go? It went into her lungs So that's inhaling dry powdered insulin.

Now she's finished. She puts the two cylinders one on top of the other and puts it away. So that's one way of delivering inhaled insulin. She's all finished.

Another way is being developed with liquid insulin. This is by a company -- I should say Inhaled Therapeutics, Inc. is in San Carlos, California. This is being developed by Aradigm, which is in Hayward, California. This is a first generation device. This is a second generation device with liquid insulin.

They're putting a blister in here. The insulin blister strip is inserted. Now you rotate this mouthpiece, and a pin punches the blister strip, and when the person inhales, they're getting an aerosol of liquid insulin.

This is a third generation device by Aradigm. They call it the AERx pulmonary drug delivery system. In that you're going to have buttons and a mouthpiece and a screen.

But an interesting feature here is this green light. This is the breath control guidance light. Here's why this is important. In order to make inhaled insulin work, to get it into the alveoli where you want it and not have it land in your mouth or in the trachea, you have to breathe at

the right speed and without turbulence. It has to be even and at the right speed. If you breathe fast and jerk, it's going to go too fast and it won't get into the alveoli.

So people are trained to breathe properly, and the idea of this device is as the manufacturer claims, that only if you're breathing the right way will it fire and deliver the insulin, and if you're the patient, you don't know whether it fired or not. You can't even taste it. So if you see a green light, you know you got your insulin. If you see a red light, you have to take another dose until it gives you a green light.

This is a method known as PDC Technospheres. This company has been known as PDC, Pharmaceutical Discovery Corporation. Recently it has been acquired by Mannkind. Now these are Mannkind technospheres. We're about to do a Phase II trial at Mills Peninsula on these spheres.

This is an interesting technology. You take fumaric acid. You polymerize it, and you form a shell around powdered insulin. You get an insulin

loaded Technosphere, and the fumaric acid was selected because at the pH of alveolar air it melts, turns into liquid, and now the insulin is in the alveoli. It gets absorbed. Fumaric acid is absorbed.

And according to what the company has told me, that the fumaric acid is not toxic, and so they found another way of delivering powdered insulin to the alveoli. This is what their inhaled device looks like.

Another method that actually Dr. Lander is associated with, I'll just say a word about it, is Alkermes' air particle. This is an interesting particle. You want an aerodynamic diameter of one to five microns if you want this powder to be absorbed. This particle has a larger geometric diameter, five to 30 microns, but it's very fluffy. It's looks like a flower, and it functions as if it has the small aerodynamic diameter, and this device uses an inhaler air dispersion chamber which delivers porous powders.

And they're working with Eli Lilly, and

one of the scientists form Lilly showed me this device at the American Diabetes Association meeting a couple of weeks ago, and he put in like an empty capsule into the cap and he started breathing, and it sounded as if there was something wrong with his hygiene.

But as it turned out it wasn't his hygiene. It's this capsule is designed to rotate around. The cup that it's in is slightly eccentric and as it rotates, it spins off the insulin. So it's designed that way, and they seem to be making good progress with this technology.

This is the last company I'm going to mention, Aerogen in Sunnydale, California. The Air Alkermes is in Massachusetts. They were in the air inhaled insulin business. We did a user study for them, but they recently announced in December that they're going out of the inhaled insulin business. They're just going to work on inhaled drugs other than insulin but use a Piazo electric effect that, in effect, shakes insulin, and it sprays out.

Okay. Now, oral insulin. Oral insulin

would be very attractive. No needles. People are used to pills. Why can't insulin be needles or why can't insulin be pills?

Well, if you can have an oral insulin, you would need to avoid the acidic degradation of the stomach, the enzymatic degradation of the intestines, but preserve the potency of the insulin molecule. That's the challenge.

So three different solutions have been proposed. One is to conjugate a low molecular weight polymer to the insulin to preserve adequate activity and resist digestion. That's what Nobex Corporation is doing.

Or you can have a delivery agent that carries intact insulin into intestinal cells as Dr. Langer showed. That's what Amesphere is doing, or you can PEGylate -- that means conjugate with polyethylene gylcol -- the molecule and then create a micelle with Casein, and this will increase transport to the gut epithelium.

This is an example of the polymer where you've put a polymer onto insulin. This is an

example of how you have a delivery agent mixed with insulin. You've just got a plain, old pill, and this is an example of a calcium phosphate insulin that has been pegylated and you've formed a micelle, and basically because you have a casing coating around these little blue insulin balls, this means that you can pass through the stomach of the intestine, and it sort of falls apart. It stays intact in the stomach, but it falls apart in the intestine, and then because it has been pegylated, it can get into the small intestine.

Here's buccal insulin delivery. It looks like you're spraying it into the -- as if you're inhaling it, but actually you're not. You're aiming at the buccal mucosa here. It contains permeability-enhancing agent. It gets absorbed very rapidly just like we know nitroglycerine from buccal mucosa gets absorbed rapidly.

Nasal insulin requires dissolving insulin with some type of calcium carbonate, and there's different forms of calcium carbonate.

Finally, there's transdermal routes of

injection, that is, getting insulin to the skin without a needle. You could use a jet injector or a patch or an implanted chip, which you've seen, or micro needle.

This is the Med Ejector Vision. We've done a study on this one at Mills Peninsula Health Service. The ideas are injecting the insulin not as a puddle, but as a spray, and that perhaps the insulin can get absorbed more quickly than if it was injected by a needle. That's being studied.

This is using encapsulation systems with an ultrasound to break the skin cell barrier. This is similar to what Santra Medical is doing. This is a company called Encapsulation Systems, Inc., in Pennsylvania.

This is using the MicroCHIPS technology, which Dr. Langer discussed and showing how this could be applied to insulin. Each of these pyramids here, which are sort of small, here you see blown up in this case contains insulin, and when you put the right charge on it, the gold cap in the presence of a high concentration of electricity just blows off,

and now the contents, which are here, this spray, the insulin, are strayed into the body.

So a person could program how much insulin they need with a wristwatch or you could use different kinds of microneedles. This is a human hair to show that micro needles are not much different in size than a hair. This is a 25 gauge needle, which you think of as small, but it's massive compared to these microneedles.

And this is one other type of device which uses a microneedle, and it's so small you can't even touch the needle. So you program it with a wrist watch.

Okay. the last question I want to ask now that I've shown you all of the different toys that we endocrinologists have to work with is, how good is the new technology, and there are three types of questions that I think should be answered with new technology.

Is the patient receiving the desired dose? Is the innovatively delivered insulin safe? And is the innovatively delivered insulin effective?

So regarding the dose, if you have a blood glucose meter determining the insulin dose, is that really the amount that's needed by the patient? We need to be sure.

Also, is this innovatively delivered dose predictable and consistent? People want the same amount every time. Is this innovatively delivered insulin lost to the environment? And if so, how much is lost?

And is absorption of the alternately administered insulin predictable and sufficient?

These alternate routes tend to not have as good bioavailability as injection. It all gets in. If you give it by mouth or by nose or by inhaled, only a small percentage gets into the body.

Safety. Is there local toxicity of the innovative insulin delivery system? Is that system itself irritating to the body? Are there immune problems? Is the insulin itself causing local toxicity? Could it even be causing cancer because it's a growth factor?

And finally, effectiveness. Is the

bioavailability of this alternatively administered insulin, is it adequate and consistent? Is the availability affected by common environmental factors, such as perhaps inhaled insulin? Could it be affected by a person with asthma or smoking?

Do the pharmacodynamics and pharmacokinetics resemble subcutaneous insulin, and are both types of doses, bolus, which is short acting, and basal, which is continuous dosing options, available for the patient?

So I raise some questions. I'm going to show you how one man's approach to this, and this is Dilbert. This next to the last slide shows innovative technology according to Dilbert, and here Dilbert is getting a report.

The new product brochures have already won design awards. Dilbert is going, "That's great, but our product won't do any of the things you claim here." I wonder who says that all the time.

"Well, who should we believe, the award-winning designer or the guy who can't stop complaining?"

(Laughter.)

DR. KLONOFF: So in conclusion, regarding new technologies for innovative insulin delivery, improved metabolic monitoring now allows improved bolus dosing. Continuous monitoring will allow improved basal dose adjustments. Closed loop artificial and bi-artificial pancreas systems are coming, and new routes of administration will remove barriers to use of insulin.

And if we do these things and have better methods for delivering insulin, then all of our patients will have better glucose.

Thank you very much.

(Applause.)

DR. FEIGAL: Well, thank you.

Our next speaker, changing topics, is going to take a look at the emerging techniques and technologies for treatment of solid tumors. Dr. Jonathan Kruskal from Harvard University.

DR. KRUSKAL: Dr. Feigal, colleagues, I, too, would like to thank the organizers for inviting me to participate in today's seminar.

One hat I wear is that of an interventional radiologist performing minimally invasive tumor oblations in solid human organs, and I'd like to share with you this morning in the time remaining some of the exciting emerging new techniques and new technologies that we are using both in the laboratory and already in the clinical setting.

Some of the challenges that we face in a daily basis for treating solid tumors include, first of all, vector engineering. How do we optimally take drugs or genes to get these to a site in the body for optimal efficacy?

Secondly, how do we deliver these? What are the options available to us as interventional radiologists that allow us to deliver drugs or genes into solid tumors in pretty deep cavities of the body?

What you've heard so far this morning are the transdermal, the inhalational. They're pretty superficial ways of delivering drugs in genes, but in the real world setting with solid

tumors, you really need to get deeper, and image guidance provides us with opportunities to get needles pretty deep into the body and to deliver locally.

And finally, how can we inhibit efflux? It's all very well dropping the payload into a tumor. It's all very well trying to enhance uptake of that payload into a tumor, but if we just leave it, it's simply going to be washed out or metabolized, and we need to see what options are available to us now in terms of inhibiting efflux of drugs out of solid tumors.

What I teach our fellows in residence in terms of drug delivery into tumors is ways of an approach to enhancing the payload efficacy, and the way we would like to look at it is simply how do we deliver drugs. How do we deposit these into tumors? How do we get these to be detained within the tumors? And how can we ultimately destroy these tumor?

Some of the innovative techniques that we're now using for treating solid tumors can be

categorized either into the intervascular area, interstitial treatments and efflux inhibition, and I'll go through all of these in the remaining time and show you what we are already doing and how some of these can be approached.

Well, let's start off with payload with efficacy. How can we look at the new strategies available to us in terms of delivering drugs with genes into tumors?

These tumors on the left, you can see this is a typical conventional delivery of drugs into liver tumors. This is a catheter inserted by the groin all the way up the aorta into the hepatic artery supplying the liver, and you then deliver -- you can see these lines over here of the pacified arteries going into the tumor. You can deliver drug into these large round liver tumors. This is drug that we on a daily basis deliver in a poppy seed oil extract called ethiodol, which is a depo delivery system for enhancing retention of drug in these tumors.

We can image this. We can see exactly

where the drug is going. We can look at the efficacy of the drug in terms of serial CTOMR to know if a tumor is being made any smaller.

But what we don't know at this point is, in fact, is the drug getting to where we want it, and on this complementary electromicrograph, you can see this small lipid particle, this liposomal aggregate which has got into the tumor cell and is actually adjacent to the cell nucleus.

So what are the ways that we can do right now to enhance delivery both from delivering it in an endovascular route all the way into the nucleus of the cell to effectively get the treatment we want?

Well, let's look at some of these ways. Catheter design. There are some remarkable new advances in terms of catheter design for delivering drugs. We will be hearing a little bit later on today about some of the drug-eluting stents. These right now are primarily for cardiovascular or angiogenic type treatments, drug eluting stents or other deliver chemotherapeutic agents, those that

will prevent stenosis. We are putting stents into livers to, in fact, prevent portal hypertension in patients with cirrhosis.

But what's equally important is to deliver drugs into the wall of these stents that will prevent these from occluding and allow these patients to continue living good quality existence.

We are currently seeking further oncologic applications. These are minimal right now, and I'm sure there's a huge amount of opportunity for oncologic applications of these drug-eluting stents.

Intervascular circled in vivo bioengineering, which is where genes are delivered into endothelial cells via catheters. The catheters are inserted into specific vessels in the body. You can then implode. You can drive these genes into the cells lining the vessels, endothelial cells, effectively to create, for instance, a situation where these blood vessels will not be blocked off.

And, once again, we have not taken adequate advantage of the entire field of

angiogenesis. Right now in tumors a lot of the theory behind tumor treatment right now is unblocking the blood vessels, destroying the blood vessels to the tumor.

But a lot of the patients we see, again, on a daily basis, the minute the blood vessels have been knocked out supplying the tumor, it effectively takes away a lot of the options we have for treating these tumors. Since we are delivering a lot of drugs via the vessels by blocking these major vessels going to the tumors, we've effectively taken away several major options for our patients, which is not an optimal situation.

So there are ways of taking advantage of angiogenesis to find a nice match between the two.

This is two examples I've taken from an article of John Thomas in radiographics in 1998, and these are types of catheters which are being developed now for drug or gene delivery. You can see over here this is simulated vessels. Two balloons are blown up in this catheter, and you can then perfuse a drug or gene mixture in the vessel to

allow it to deliver into the endothelial cells.

More exciting is this type of catheter, this patch type catheter where the wire is inserted into a vessel, it's blown up, and you can see this loop which develops, it does not block the vessel. It allows the blood to continuously pass through the vessel without causing any ischemia or occlusion, and you can then profuse your drug or gene in this helical tube, and it then leaks out. It's a very permeable membrane, and it leaks out into this little cavity over here, and it will then allow it to basically be taken up by the endothelial cells.

These are the types of systems that are now being delivered and explored for local delivery of drugs or gene product and peptides into the endothelial cells lining vessels.

What about some of the therapeutic vectors, the therapeutic ways in which we delivery payload into tumors?

And the four categories I will be talking about will be radio immunotherapy, vector

engineering and design, some of the new cell delivery techniques, and some of the new gene delivery enhancement techniques.

Selective internal radiation therapy, I'm sure many of you have heard about this. As an example I've just selected the Yttrium microspheres. These are very small, 32 approximately micron resin microspheres onto which is bound some radiation, Yttrium 90.

This is then delivered. We put a catheter all the way up, again, up the aorta. We target this catheter with guide wires into the tumor, and then you can deliver these small, little microspheres directly into the tumor. There's preferential deposition in very vascular angiogenic tissue, and we can deliver, therefore, therapeutic dose of radiation to the tumor and not to the entire organ.

The liver, as an example, is a very sensitive organ. If you expose the liver to conventional doses or radiation treatment, you're going to wipe out the liver function, and the

patient might succumb. However, if you can deliver this local radiation treatment to solid vascular tumors, it allows you to then subject this to a much higher radiation exposure than conventional radiation treatment.

However, this technology certainly needs to be optimized. There are lots of companies out there which are exploring it. We need to see some good comparative prospective studies. We need to see the technology optimized before I would certainly be happy about administering this to any of our patients.

Immunocongugates monoclonal antibody therapy also is being used right now, not with too much success in our experience, and as an example, if you take colon cancer, which expresses what's called a carcinoembryonic antigen on its cell surface, you combine radiation Iodine 131 to these monoclonal antibodies. You can deliver these intravenously, and these will then bind onto the cell surface of any tumor cell which is expressing this antigen.

The problem, of course, is that many other normal cells in the body might express it, such as the colon, and so we need to basically improve ways of targeting the immunoconjugates. It's not sensitive enough at this time. The monoclonal antibodies need to be worked on. It's not enough to simply use a rather specific monoclonal-type antibody. You need to use antibody fragments and small, little peptide fragments, cyclic peptides as well, and this might improve the localization.

The other area which is explored in many laboratories is once you've actually delivered these onto the surface of the tumor cell, how do you get these inside. How can you internalize either this radiation or, in fact, whatever you might put on it. This might be drugs. This might be other types of therapeutic agents. How do you get these in?

And the areas which are being looked at now with some, in fact, quite optimistic early results include radio frequency or heat, sonoporation using focused ultrasound, and UV light.

All of these techniques are being explored in the laboratory setting for enhancing uptake and internalization of delivered immunoconjugates.

Vector engineering is another area which is receiving a lot of interest in the laboratory setting. I'll give an example of what we refer to as immunoliposomes. Some of the very good work has come out of David Cheresh's group in La Jola, and what they've done is they've taken advantage of tumor angiogenesis. The integren off of E-beta-3 is expressed on very early angiogenic vessels.

What they've done is they've bound a monoclonal antibody to this integren, to a small, little liposome which contains gadolinium. We can see gadolinium with MRI, and therefore, if you give the small immunoliposome into an animal at this stage, it will actually localize in areas where there are integrens being expressed in very early angiogenic territories, and you can see it because of the gadolinium.

In further studies, what they've done is they've also then bound doxorubicin, the

chemotherapeutic agent doxorubicin, to this same agent, and this, again, will then target the doxorubicin to the integren which is being expressed.

Phage display technology is a very exciting, I'd like to say, new technique. In fact, it has been around for a while, which really allows us to target far more specifically than monoclonal antibodies would, and in using phased( )display technology, that group and others have certainly been able to identify small what they call cyclic peptides, and these will target not only small integrens, but as more work is done, in fact, they're finding that these probes target multiple different receptors.

They're able to target angiogenesis. They're able to target receptors on tumor cells. They're able to target other enzymes which might be expressed prior to angiogenesis, such as the so-called metalloproteinases.

So, in fact, a more and more basic science is being performed, they're identifying more

and more applications for each of these probes.

Similarly, tumor receptor is another big, exciting area. A lot of work has been done on tumor proteases. Ralph Weissleder and his lab in Boston has developed a lot of imaging probes to the cathepsins and other proteases. Metrics metalloproteinase is one of our own optical imaging probes actually showing a circular room of matrix metalloproteinases being expressed around the periphery of a colon cancer metastasis in this video micrograph of a colon metastasis in a mouse liver.

And there are also a variety of growth factor receptors which are now being targeted, and remember we can use these not only for diagnostic purposes, but also for therapeutic purposes. So we can try and look at developing probes which show us on an imaging basis where these receptors are, confirm that they're being expressed, and then block them with a lot of these very exciting, new factors which are being engineered.

VEGF, the vascular endothelial growth factor, also very exciting. VEGF is being used.

You'll hear in subsequent talks this morning about the way in which it's being used in Hans angiogenesis.

VEGF can also be targeted for gene therapy. We use VEGF; in fact, we drop it onto tumors with needles, and it enhances the permeability of the leakiness of tumors, and we can then pulse this with drugs off to its enhanced delivery of drugs into tumors.

So whereas VEGF might not be the ideal agent being expressed by tumor cells because it enhances angiogenesis in growth, we're also administering it to enhance delivery of drugs into these tumors.

Targeting tumor-associated cells, this is something that we hit on inadvertently a couple of year ago through our radio frequency ablation program. It's well know that many solid tumors, breast, for instance, will recruit systemic macrophages. Systemic macrophages are recruited into the center of solid tumors, and these then might play either a pro or an anti-tumor effect

depending on which specific population of macrophages these are.

However, we have now found, in fact, that when you ablate a tumor with radio frequency ablation, you can actually recruit specific types of macrophages that would have an anti-tumoral effect on the tumor.

And we have taken advantage of this. This is a small colon cancer metastasis. This is a video micrograph of an exteriorized mouse liver with colon cancer, and by sticking a needle in and ablating this for about 30 seconds and waiting for a few days, we've recruited these very Agard phagocytic macrophages into the cell. These black cells infect all systemic macrophages which have taken up these small carbon micro particles, and this is a different population of macrophages to which reside in the typical growing antiogenic tumor cell.

So therapeutic macrophage recruitment is interesting not only because of its anti-tumoral effects, but because these avidly phagocytic cells,

to me, seem to represent a wonderful delivery site for drugs or for genes.

Taking advantage of tumor permeability, you have already heard in the previous two talks about pegylated liposomes. We have certainly played around with these a lot. This is just an image. You can see this is a diagrammatic illustration of a liposome. These yellow bands along the periphery are the polyethylene glycol.

And what this does is they provide stearic hindrance. What this means is that if you just inject these into the blood stream, they will circulate. They will have a prolonged intravascular residence, and these thin strands of polyethylene glycol will prevent these from being taken up by macrophages throughout the body. They, therefore, would stay in the blood stream for up to two days.

The illustration on the right, again, is one of our small -- this is about a two millimeter colon cancer tumor growing in a mouse liver. You can see PV is the portal vein, is the blood vessel supplying the tumor, labeled as T, and what we have

done is we have simply given these animals an injection of a small amount of these pegylated liposomes containing doxorubicin, and these will simply leak out because of the leaky vessels within the tumor.

And more interesting, in fact, is that the doxorubicin will only fluoresce once liberated from the actual liposome, and all of this bright white area is the liberated doxorubicin which we can see in real time.

So taking advantage of tumor permeability is another broad area that to me seems quite optimistic and hopeful.

So we've looked at the vector engineering. We looked at the catheters. Now let's look at cell transplantation. Cell transplantation certainly we've heard in this previous talk. There's a lot of opportunities for diabetes.

We are injecting islet cells into patients in our institution, but what's sort of strange and bizarre to me as a radiologist is that clinicians come to us; they give us a little vial;

they provide the patient' and they say, "Please inject this into the spleen."

And we inject these eyelet cells into the spleen, and we have no idea where these cells are going, and this, of course, I think is one of the big challenges we're dealing with in liver cells as well. We're injecting hepatocytes into the spleen, and there's a lot of work that needs to be done in the laboratory to know exactly where these cells are going. They seem to be working in some patients, not working in others.

And interestingly, we're finding with our liver cells, which we're giving to patients to tide them over prior to transplantation, that they seem to reside within the spleen and do quite well and actually work.

So that opens up another whole possibility. You can have ectopic location of normal functioning cells. They don't need to be in the organ where they normally function.

In our oncology patients, we're injecting the fibroblasts and the dendritic cells

into the peritoneal cavity. We do this under image or ultrasound or CT guidance, and again, these are cells which have been transduced to produce things like human growth factor, some of the clotting factors in our hemophiliac patients, and this again provides a wonderful opportunity.

However, as has been said before, we certainly await new techniques for improved targeting of these cells, and I think this is another big area that a lot of work needs to be done.

So recruitment I've mentioned here. Some cells can be recruited. Certainly image-guided MCF delivery; what I mean by MCF is the macrophage chemotactic factors. You can literally pick up the sigma biochemicals catalogue and purchase overnight a whole variety of different chemotactic peptides, and a lot of these now that we inject in an image guidance into a solid organ in the body will then recruit macrophages, which might have an anti- or pro- tumoral effect. And we need to explore this area further. There's a lot of opportunity here.

Radio frequency tumor ablation we've shown. Our own institution recruits macrophages, and this, again, was data that was sitting in front of our eyes for years and years, since every time we did this to an animal or patient we would get histology that would show a lot of macrophages, and the assumption that we made, that this was simply the RF-induced inflammatory response.

So certainly there's a lot of data out there that we just need to look at again and take advantage of.

And these cells, again, are a wonderful depo for drug and gene delivery. These are two micrographs, again, in our little mice in the lab. This is an exteriorized mouse liver. You can see the vessels draining out. This is the portal vein coming into the liver. These are the individual liver cells, and these small white dots, in fact, are the liver macrophages, also known as the Kupfer cells, and we've delivered a fluorescent peptide to these, and you can see the broad delivery of these.

Whereas once we let a tumor grow inside,

we ablate this tumor with RF. You can see a different population of macrophages which takes up a different dye, which has been localized around these tumor cells.

So depos for drug and gene delivery, I think, are another bit area that deserves some further work, and this is, again, one of our images. This is radio frequency recruited into two macrophages, and what these have now done is they've taken up liposomal doxorubicin, and it is being released in these macrophages.

So this is a one millimeter tumor. These are macrophages which are being recruited often within the center of the tumor for about two to three days after RF ablation, and these are not there before, and you can then deliver drugs to these.

And these are also a rich population for delivery of gene products.

Adoptive immunotherapy, I don't want to get into this in too much detail, but it is certainly being performed in patients in our

institution. What we mean by this is one of several things.

First of all, you can take natural killer cells from the patient or others. You could activate these with lymphokines, reinject these into the patient, and then hope that these will somehow attack the tumor for some therapeutic purpose.

The trouble is the nonspecificity of these cells, and again, to improve targeting of these natural killer cells.

And then lastly, in this category, the so-called TIL, the tumor infiltrating lymphocytes. What we have in our institution is one of the basic science researchers takes lymphocytes. He transfixed them with a cDNA of carcinary rheonic antigen, and then what they do is they actually ultimately start making an antibody for the carcinary embryonic antigen, and we then reinject these back into the patients, and they will then home in on our patients with colorectal cancer metastases in the liver.

And we are just sharing these, and this,

again, is one of our micrographs of a small mouse liver. This is looking directly into a live tumor in the liver through a microscope, and these small, little cells here are the lymphocytes which, in fact, fluoresce under the appropriate conditions, and we can target these to the tumor.

However, clinically is it successful? I'm not convinced. It seems to target other parts of the body, such as the colon, and it's an area richly in need of good research and optimizing this technology.

Gene-based therapies. We hear earlier that gene therapy has not been performed that much in humans. Certainly in our institution it appears to be. We've seen some major hurdles over the last couple of years, but with a lot of trepidation and being extremely gentle with the patients, we certainly are delivering genes to patients.

Two of the major innovations that I think we're going to hear about for treating solid tumors are the use of tissue specific promoters and the use of inducible enhancers. And what I mean by

this is the ways in which genes are being synthesized now are to allow specific factors on them to promote gene expression, and one which is being used is VEGF, the vascular endothelial growth factor.

And what this means is that in an animal model you could introduce genes into solid tumors, wait for these to become angiogenic, become invasive, and the minute VEGF starts being expressed, it turns on therapeutic anti-tumoral genes.

And then what we'll also look at is how we can actually enhance delivery of genes, and the areas which are being looked at with most interest are heat, hypoxia, and ultrasound.

The inducible enhancers of gene expression, a little gene fragment, a little cDNA fragment consists of an enhancer subunit, promoter subunit, and the actual gene.

And what you can do is, if you can basically subject this enhancer subunit to one of many ways of activation, it will, in turn, activate

the promoter subunit, will activate expression of the gene product, which will then be released and go off and have the therapeutic effect.

How can we take advantage of this? Well, certainly with hypoxia. Hypoxia inducible factors can be inserted on the enhancer unit, and then in the presence of hypoxia, these will then be activated to express genes, such as the gene for VEGF of a variety of other genes.

Believe it or not, in the year 2003, we are delivering chemotherapy to patients with solid tumors. We're then blocking the vessels in the hope that this will occlude the blood supply and kill the tumor.

But as I've just shown you, in fact, to make a tumor hypoxic, it, in fact, stimulates VEGF expression and should, in reality, induce further growth of the tumor. And this really is sort of the take-home point I'd like to leave us all with, is that a lot of things that we are doing to patients right now, they seem to have a wonderful, positive effect on a lot of patients, and in theory some of

these might not work that well.

Ultrasound is something that Bob Langer mentioned, and certainly he deserves even more credit than we can give him for what he has done in this field, but heat shock protein is another protein which has recently been identified as a protein which can be up-regulated by the presence of the heat delivered by ultrasound. If you can make a gene that has heat shock protein inserted into it, you can then target ultrasound directly to this gene and it will inactivate this and induce gene expression.

The trouble is that this has not been done with too much efficacy at this point, and we need to look at all of the entire spectrum of other available heat opportunities for this.

So we've not delivered vectors. We've delivered genes. We've delivered drugs into the tumor. How come we enhance the delivery here?

First of all, drugs, which can enhance permeability and, secondly, mechanical; there's a variety of different pre-targeting drugs that we can

look at. VEGF again, as I said, we drop it onto tumors to increase endothelial pores. We can actually deliver via catheters transient permeability enhancers. You can see all of these that I've mentioned over here on this slide: platelet activating factors, bradykinin, all of these will, in fact, enhance permeability.

Mannitol is used by neurosurgeons to a large extent to disrupt the endothelium, and then mechanical enhancement. It's well known that RF ablation as well as electrophoresis or antiphoresis, all of these will enhance permeability to allow drugs to be delivered.

This is one of our tumors we have subjected to 30 seconds of RF ablation and changed this with small fluorescent microbeads, and all that you can see the track of the needle inside the solid tumor, and you can see how the microbeads, they leak out around the tumor. So certainly RF can enhance permeability.

Something I suspect we might be hearing a little bit more about later on, these so-called

magnetic targeted carrier particles. These are small, little magnetized particles onto which different chemotherapeutic drugs can be bound. This is then delivered via catheter into a patient's blood system, and then these magnetic particles can effectively be sucked out by a magnetic field placed onto the patient's surface.

Here's an example of this, a catheter that has been delivered into an artery supply in these liver tumors. These magnetic targeted carriers are delivered into the liver tumors. Magnetic field is placed over there that would suck these out, and then these are delivered into the tumor.

And you can use MRI to actually see this small, little magnetic particles in the tumor. What needs to be looked at, in fact, not only is the system being fully optimized, but once you've got small magnetic ion particles in the liver, what effect would this have on other therapies?

For instance, if you use ion and RF ablation, what effect would ion and RF ablation?

Would this be synergistic? Would this be antagonistic?

There's a lot of additional exciting work that can be done here to further optimize this, and this sort of falls into the category of what I call cooperative therapies, something that hasn't received much attention, but for an example, RF can be used to recruit targetable macrophages.

We already are injecting the genes for P53 into solid tumors, and what these do is they then allow the tumors to, in theory, re-get into the normal way of dying, but P53 also allows us to subject these tumors to a lower level of radiation.

Radiation-inducible promoters are another entire area. Thermally-activated vectors, vectors which can be delivered in the blood system, into solid tumors and then shattered by subjecting these to different heat techniques.

In vivo electroporation, sticking a needle into a solid tumor, delivering drugs systemically, and then by subjecting this to a local electric field, allow these drugs, just as we do in

the laboratory, to be taken up into the tumor cells.

And then, of course, a nice combination that we have done and published last week, in fact, is a combination of radio frequency and liposomal doxorubicin, and our theory here was that once you have a tumor in the liver, you can give the patient liposomal doxorubicin or, in fact, any liposomal agent. It will then surround the periphery of the tumor.

We then, using image guidance, stick a needle into this tumor. We turn on the RF ablation. You can see the red heat, and then what this does is it actually extends all the way out to ablate the entire tumor.

And I was also actually very excited. We've done this in quite a few patients. The regulatory issues in and of themselves are very interesting because RF ablation is approved. Liposomal doxorubicin is approved. So we've taken two approved technologies, and what we're getting over here, this is one of our patients, and it's showing us some very surprising results.

This is a tumor which has been ablated. This is the liver. This is a CAT scan through the patient's upper abdomen. This big, black area is the dead tumor, but you can still see a few blood vessels within it.

And about two weeks later these blood vessels have disappeared completely, and the types of results we're seeing, in fact, is that whereas a couple of months ago we could only ablate tumors up to four centimeters in size, we're now getting up to eight centimeters in size. So a 100 percent increase in tumor size.

We've even showing in our animal studies that the survival of the animals has increased. We're also getting slowed growth not only when the entire tumor is ablated, but when parts of the tumor are ablated, and we're also knocking out blood vessels which may be residual.

So the combination of interstitial treatment, such a microwave or radio frequency ablation and drug therapy, certainly is being used at this point in patients and deserves further

investigation.

In such activation of expression of drugs or genes, you can certainly induce local liberation of contents of drugs with photoactivation, radiation of sound radio frequency, heat sensitive liposomes, a lot of great work being done by Needham's group down in the Duke hypothermia project, and here they are using special liposomes which are activated or shattered apart by heat.

And of course, sonoporation of using ultrasound to shatter liposomes, and this is an example. Some of the ultrasound contrast agents are being designed to have a biomaterial on the outside, which are antibodies which can target these to specific surfaces of tumor cells.

They have a polymer inside which is specifically designed to be shattered by using conventional ultrasound waves, and then inside they could have a drug or a gene.

And then what you do is you subject this to ultrasound waves. This will then break it apart, release the small, little peptides, and allow local

release of gene or drug inside a tumor.

And we, in fact, are doing this in the laboratory. This is the liver ultrasound delivered doxorubicin. This is a small liver in a rat, and there's no ultrasounds being given when you subject this to conventional ultrasound, and by conventional, exactly the same ultrasound that many in this room may have gone to have your fetus, your embryo imaged. It's not using any higher frequency ultrasound whatsoever, and you can show the marked increase in the fluorescence of this doxorubicin when this is subjected to approximately 30 seconds of conventional ultrasound.

What we have shown that's even more interesting, in fact, is that in the presence of a tumor, you can get even further delivery. So this really opens up a whole new ball game where we can use conventional ultrasound, and already we're exploring this.

The patient comes in. We can image the tumor in the liver. We can then give a drug and actually use that exact same ultrasound while we're

imaging it, target the beam, and try to deliver this, get local delivery and implosion of the ultrasound contrast agent.

Detention of the payload. We're almost done. There's certainly a lot of pharmacologic inhibitors. These are efflux inhibitors. Once you've got the drugs into the set tumor cells, we could take advantage of the ATP dependent pumps, P-glycoprotein multi-drug resistance pump is something that a lot of drugs being used for other purposes will block, and there are a variety of these multi-drug resistance-associated proteins.

Any of these infective, once the drug is inside the tumor by giving these to the patient or to the animal, it will inhibit efflux of these drugs out, and of course, the mechanical inhibitors.

And there's some very good work that has come out of the laboratories of Genzyme in Boston showing that gene delivery intravenously in animals by inhibiting flow out of the liver, by occluding the hepatic veins, will cause significant increase in the uptake of genes into these cells.

So, of course, using catheters and other engineering techniques to cause local increase in interstitial pressures certainly may have a positive effect on gene and drug delivery, and this is, again, one of our small colon cancer cells, and what we've done is we've given verapamil and Cyclosporin A, and this has inhibited efflux of doxorubicin out of this tumor cell.

So these are types f therapies, types of approaches that need to be looked at once you have delivered the payload, once you've deposited in the cell. You need to prevent it from being released.

So in summary, this was a very brief overview. For the treatment of solid tumors there really are a variety of emerging techniques and new technologies. There are a huge amount of opportunities for optimization of these techniques, especially these combination therapies. However, someone who is doing these on a daily basis -- and I think this is where the challenge really is -- we still do await some good quality, peer reviewed, published science showing which techniques are the

best. We need to compare the techniques, and we would really as clinicians love to get involved in some good, prospective, randomized studies to see which are really going to be best for our patients.

Thank you very much.

(Applause.)

DR. FEIGAL: Thank you.

Our final speaker before the break is Richard Kuntz, who will be talking about the novel technologies for the treatment of cardiovascular disease.

DR. KUNTZ: Good morning. I'd like to thank Dr. Feigal and Dr. Provost for inviting me to this wonderful session.

And I'd like to talk in the next few minutes about the clinical impact of some of the technologies that you heard about this morning, mainly focusing on the drug eluting stent experience.

We all know that coronary stents use funny, little metal cages that have been around for about 15 years, made of about three different types

of materials, mainly stainless steel 316L or Nitinol or recently cobalt chromium. These materials are now referred to as bare metal stents because of the drug-eluting stent environment, have basically revolutionized the treatment of coronary disease throughout the world.

That is, these cages basically open lumens that are blocked in the coronary arteries and maintain, because of their physical properties and mechanical properties of plastic deformation, can maintain an opening in the artery despite injury sustained by the stent, and overcoming the reaction of vascular injury.

Now, one of the problems is that when you start to expand any new therapy, you start to see a problem associated with expansion of the clinical outcomes. We initially evaluated stents in basically simple patients, and they could be defined by patients with large vessels and generally non-diabetics. They had rates of failure that were very, very good and basically were associated with pretty much a breakthrough therapy in coronary

disease. That is, only about ten to 20 percent of the patients who were treated with coronary stents in the simplest lesions would ever fail over the course of the restenosis period, which is about six months.

But as expansion included diabetics and longer lesions and vessels that are smaller, we started seeing that these parameters are actually quite influential on the geometry of renarrowing. So that when you have patients who are diabetics with long vessels and small lesions, failure rates approach 50 percent.

So this is, I think, a pretty typical cycle of any new technology, that when it is initially introduced it is with really fantastic results. Clinicians figure out a way to expand it to patient populations where it fails again.

(Laughter.)

DR. KUNTZ: And then it's time for us to now engender a new need for a new breakthrough therapy.

So the drug-eluting stent process

started out, and it wasn't necessarily that it was a drug-eluting approach. Early on we know the biology of thrombus and neoplasia, which is the renarrowing process of restenosis, is guided by four different types of pathological processes.

One is that when you put a stent or injure any artery, you get initially thrombus that forms on the artery. This engenders an inflammatory process at the site with recriminative white cells and macrophages. This leads to stimulation of the deeper tissue in the vasculature of proliferation, both of in situ perivascular cells and also media which transform to macrophages in the fibroblast and recruit more cells and they basically heap up the scar that if you're in a vascular bed, generally it causes a reduction in the lumen size.

And then finally, arteries that don't get stented actually can contract around the inflammation itself so that there are these four process that we have known for years cause a problem.

The problem has been that almost every

drug available in the last 15 to 20 years has been tested in over 40 or 50 multi-center randomized trials, and all have failed. So the notion in the mid-'90s was that maybe we should reevaluate some of these drugs with the emerging technology of local drug delivery.

That was always in the back of the mind of many of the scientists that not enough drug was getting to the tissue site because it had to be given systemically. So the notion of local delivery really has been manifested as a success and the poster child for drug delivery at this point is the drug-eluting stent.

Now, in conjunction with this concept that local delivery was important was even more science that was added by Nurse, Hartwell, and Hunt, who ultimately ended up winning the Nobel Prize in 1991 for their similar work on understanding the importance of specific key proteins orchestrating cell division. These include Cyclin CDK, CDK1, and a variety of P proteins.

Simplistically one can look at a variety

of compounds that have been around for a while and look at their impact using this model on the cell cycle and, in general, knowing that the implementation of a stent would cause activation of inflammation followed by cell division, and trying to process some of the data from those Nobel Prize winning science, we could see that potentially these drugs that have been used in other areas, including immunosuppression and chemotherapy, might be valuable loading a stent to stop a cell from getting into mitosis.

Now, early on we know the radiation therapy is extremely effective in that, and there was a heads-up with respect to that working because radiation therapy is extremely effective in the prevention of in stent restenosis, that is restenosis happening a second time.

So we do know that we can inhibit mitosis, and radiation therapy is kind of a no brainer approach, but we can reduce this problem of repeat failure after stenting.

A variety of different drugs that are

mentioned here include Sirolimus, which is the brand name for rapamycin; paclitaxel and actinomycin D.

Now, if we look specifically at the first compound extensively studied, which is rapamycin, Sirolimus, we know that processing some of this data that a variety of cell receptors, both stimulated by white cells and by platelets lead to activation of some of these key proteins that are synthesized at some unknown protein enzyme, and this has been referred to as the target of rapamycin because it is felt that rapamycin works after combining with a KPB12 to inhibit the function of TOR in leading to the synthesis of these key proteins, which lead to cell division.

So one had to utilize this science with the emerging technology, as was pointed out by previous speakers, of polymers that can hold and deliver the drug.

So the concept of drug-eluting stent was started, pioneered throughout several centers throughout the world, including MIT, with some of Dr. Langer's students, including Elazar Edelman at

the Biotechnology Center.

And these agents were felt to be part of a three-part process of combination, including the initial stent itself, which was generally just a stainless steel stent on the market; a pharmacologic agent which was going to work and have some theoretical advantage to prevent mitosis, and, of course, the most critical thing was the drug vehicle.

And if you follow the coronary field in polymer science in the last 15 years, we actually didn't get off to a good start initially. Polymers were probably the harder nut to crack rather than the drug itself because the initial polymers were so toxic that they in themselves would cause dramatic vascular responses.

Well, after a lot of work, and this is almost ten years of work at Cordis in conjunction with Wyeth-AIRS, there had been multiple efforts to try to develop the ultimate polymer-holding drug with a top coat that would allow for delivery to stent without rubbing off the drug, and ultimately

release of drug over the course of 30 days that would, in fact, interfere with the process of thrombus and inflammation, which was the kind of ring leader of the restenosis process that occurred subsequently for six months.

The notion was, in fact, if you could stop the upstream processes of cell division, you wouldn't get the manifestation of heaped up neomyplasia after six months. So the notion was to develop a rapidly releasing polymer that would get drug into the vasculature within the first seven days and possibly as late as 30 days.

Now, I'll jump right to the clinical trials because we could spend a lot of time on the polymer science here, and there are better speakers than me to talk about that, but with respect to how this has manifested itself out, early on there were some studies done in South America, as are a lot of kind of under the radar screen studies that are done outside the United States, and one of the initial studies with this drug showed up as a winner.

The first in-man analysis demonstrated

that after treatment of 40 patients there was absolutely no latent loss that would be expected to be seen at six months, and this triggered initially Cordis to start two prospective studies.

Now, the prospective studies were first a study called RAVEL done in Europe, and then the FDA regulated study in America called SIRIUS, which was more of a pivotal trial study.

The RAVEL study was actually designed to demonstrate reduction in a surrogate of restenosis, which is angiographic narrowing. A 200-patient study generally wouldn't show reductions in clinical outcomes, and it was substantially and markedly positive. That is, if we look at the classical measures of narrowing, which is the crossing of the 50 percent narrowing diameter stenosis at angiograph at follow-up, it rate was 26 and 27 percent, as we would expect, in the control arm, and in the active arm it was zero.

Now, there are a variety of ways of measuring narrowing within the stent and outside the stent, but regardless of how we measured it, it was

quite fantastic, and this study was performed by Dr. Serois in Rotterdam using his European colleagues, and it was probably the most substantial breakthrough in the field of interventional cardiology in the last 30 years.

Now, this was in tandem and slightly frame shifted behind, performed with a study called SIRIUS, which was the American study. Again, this study is a lot larger because it's powered to demonstrate reductions in the clinical restenosis rates, which are lower and less powerful endpoints than that established from angiographic measures, and we see that the restenosis rates angiographically were also substantially reduced. You can see the reductions here, almost 90 percent, depending on how we measure restenosis.

This, again, is unprecedented not only in coronary cardiology, but in medicine in general.

If we look at other measures of what the target was, which is this amount of neomyplasia best measured by three dimensional intervascular ultrasound reconstruction, you can see that when the

patients were exposed to normal stenting, they had 34 cubic millimeters on average of neomyplasia compared to 2.6 from the other group, again showing substantial reductions.

And then if we go to the robust clinical measures, that is, does the patient have to be revascularized, what about if they had a heart attack and other kinds of very robust measures?

This is the major clinical outcome called target lesion revectorization, and that was reduced almost fourfold, from 16 to four. And if we look at that event plus anything else that can happen to the patient, including small heart attacks, it was still substantially reduced.

Now, it was interesting because we have a paper pending in the New England Journal of Medicine that should be out next month, and in the initial review the editors asked us to remove the words "marked" and "substantial" that we were using in the manuscript because they said it sounded like a marketing brochure rather than a scientific paper.

And we tried to figure out a way to

describe the 91 percent treatment effect without using the word "substantial" or "marked." It was pretty hard.

(Laughter.)

DR. KUNTZ: So you'll see sentences like, "A treatment one effect was found, 91 percent."

What's interesting is that this is almost a dream come true from an initial perspective, and that is the field of DES, I think, is more so than just SIRIUS itself, Sirolimus. These drugs in their initial incarnation so far appear to work without any increase in adverse events, and stent thrombosis was something of great concern because we were putting a polymer on top of the surface of the stent, and that might be a problem.

And in a variety of different studies from Europe and Canada, America, and others, the pooled analysis shows the same thrombosis rate or even lower from what we would expect at least on the patients we've studied so far.

So in general, the inclusion criteria for this trial, which included relatively sick patients, had fantastic results from a stent thrombosis perspective.

What also is interesting was that if we looked back at those predictors clinically of increased restenosis, which is the length of the lesion, the size of the vessel of the person with diabetes, there was a really uniform treatment effect -- this is looking at clinical restenosis -- across the board.

That is, if we looked at linear, nonlinear modeling, if we looked at actual results and we tried to smooth them in a variety of statistical ways, we would find this consistent effect.

So this, again, is a little bit unusual to see in medicine where almost all subgroups benefit to some degree.

Another way to look at that is just to break them down by the observed outcomes, and this is the classical odds ratios analysis, and, again,

this is a familiar graph that one takes a positive study like this with its odds ratio reduction from the active arm and its confidence intervals, and then measures it against the unity line, and then looks at a variety of subsets.

And it's very hard to come up with any other study in medicine I know of that has all of these subsets located so far to the left. So it was very hard for us to find any subsets that didn't have substantial advantage in this group overall.

What's more interesting mechanically is that we've always known that with the advent of stenting and its ability to prevent abrupt closure and other acute complications, many interventional cardiologists use a lot of stents because they could really get themselves out of problems.

But there's a price that you pay, that is, the increase in stent length was associated with substantial increase in restenosis, and this is mainly a probabilistic reason statistically.

Well, this was almost negated by our experience so far with the Sirolimus stent,

suggesting that now the interventional cardiologists can have their cake and eat it, too, that they can put the long stents in, the so-called full metal jackets, and not pay the price they have before with substantial increases in restenosis per se.

Now, we don't want these interventional cardiologists to go hog wild and start putting a lot of stents in. Surgeons certainly don't want that, but at least when one is concerning themselves about an acute complication, like an edge dissection, and you're always debating as to whether you should put that extra stent in, we feel that the patient can actually benefit from having a safe approach by putting the extra stent length in because the price we see so far of restenosis is very minimal for extra stent length.

We followed this for now a year, and what we see is that even from the initial nine month outcomes which were reported to the Food and Drug Administration and led to approval of the one-year data, still is maintained, and if anything, we still see a slight reduction in freedom from restenosis in

the control arm by the main endpoints, and it is still maintained, I assume, more robustly in the active arm.

So our treatment effects actually have lightened, interestingly enough, even from nine months to 12 months, to suggest that there is no evident catch-up phenomenon.

If we look at the RAVEL study, the one that was started slightly before, the two-year data suggests that we have still maintenance of good clinical outcomes, and there's clearly in all of the angiographic analyses no evidence that this process of delay or narrowing that occurs in six months is delayed any more than what we normally see in six months.

Now, European studies have just been reported a few months ago. Again, a new data set; again, phenomenal results overall, and I think overall the results of rapamycin with three randomized trials now suggest that this is a good drug.

Well, what about other drugs? Does it

work? Is the answer local drug delivery or is the answer Sirolimus?

Well, paclitaxel is another important therapy, and its first study was a 500-patient study done in Europe, and it also showed marked reductions in restenosis. The FDA study called TAXUS-4 in America, which has, again, over 1,000 patients will be presented relatively soon, whose results, I think, are being filed if not now, to the Food and Drug Administration, and I think they'll be presented some time in August or September.

But if it does follow this initial European experience overall, we're looking at probably another 50 to 60 percent reduction in restenosis. We're the second drug now attached by polymer to a stent.

Does that mean that every drug-stent combination now works? The answer is no. Actually it doesn't. The same drug, paclitaxel, was shown not to have substantial reduction in restenosis, 13 versus ten, when directly applied to the stent surface. Okay? Paclitaxel is a sticky molecule,

and if you spray it on and then put it in the body, it actually doesn't seem to prevent restenosis to the same degree that we certain saw with rapamycin or the other formulation of Boston Scientific TAXUS stent.

So I think the polymer technology is critical, at least from my limited perspective, so far. It looks like that is an important component rather than just drug and stent alone.

There are lots of other polymers out there. I just want to give you a little sampling now of what they look like. Abbott, in collaboration with Biocompatibles in the U.K., has access to phosphatidylcholine, which this agent is like a sponge. It essentially is easy to apply. It holds molecules up to 2,000 Daltons. It is a natural reservoir and can be easily manipulated to change its kinetics of release.

Abbott, in conjunction with Medtronic, are looking at a variety of different compounds, including a rapamycin analogue called Rapalog, or ABT 578, and both of them have licensed this

compound, and there are two studies that are ongoing right now in Europe.

Interestingly enough, there is some interesting data from basic old drugs that are off patent and have been studied before and were negative, and when combined with a polymer looks initially like it might have good results as well, and they include dexamethasone estradiol.

And of course, Guidat has another rapamycin analogue in a polymer called everolamus, and this in a study called FUTURE in Germany has demonstrated fantastic results so far.

If we look at the overall experience so far, we can start to classify them, and this is from Peter Fitzgerald, who is virtually the intervascular ultrasound core laboratory in Stanford for almost all of these studies, and what he's seeing is that he's got a marked reduction in neomyplasia using either paclitaxel or the limus family.

Now, I don't know that there's a difference between these two. These are very small sample sizes overall. I'm a little skeptical about

that. I think when we find the actual results from the TAXUS-4 study we'll be able to tell whether, in fact, they're all in the same class or not. My guess is they probably are.

In any event, they're substantially lower than that seen in the bare metal stent. Again, polymer is the key for a variety of these drugs that work.

Now, I just want to point out one other stent just to show how the technology can go further. This is just an interesting company that has a stent in which the struts now have little holes in them, and what these holes are are little wells that can contain drug.

And there is a manufacturing process that can precisely place in these tiny holes levels of drugs with different levels of polymer and different elution characteristics so that one could stack a variety of different drugs with different release kinetics so that if you want to have a drug for the first three days, it would be released, a drug for the next week would be released below that,

and so on and differential release both to abluminal and vessel size.

This is a very interesting type of new technology, and I think we'll see more and more of this. Trying to design a trial, I think, to deal with all of these permutations may be difficult, but in general if one comes up with a theoretical nice combination of drugs, such approach might be something interesting and may stimulate other people to think about likewise approaches.

Now, one of the important things is how does drug-eluting stents, even as in its infancy right now, how does that impact on how we take care of patients with coronary disease per se. Well, as an interventional cardiologist, we're constantly measuring ourselves against the surgeons, and early on we felt that we owned a single vessel disease problem. That is, the heart usually has three vessels, and if one is blocked, you generally don't want to send someone to surgery for that.

Well, there have been a variety of studies done on patients with multi-vessel disease

and comparisons with surgery, and in general, there's not much of a difference except for maybe a subset of diabetics with severe vessel disease. There's not much difference between mortality or other major adverse events between the two therapies.

That is, angioplasty or bypass surgery tend to be extremely effective with respect to the ability to revascularize and also has about the same major adverse event outcomes.

But the main problem with angioplasty has been that the restenosis process requires that it be reintervened on, and that gap was 32 percent when balloon angioplasty was initially out there.

This slide, by the way, I borrowed from Dr. Serois in Rotterdam who made this up. Now, Dr. Serois is also the PI of the ARTS study, which is the first stent study versus bypass surgery, and that gap for revascularization repeat in intervention has narrowed to 14 percent.

Even with conservative predictions of what the drug with the stent world can look like, it

now appears that even under multi-vessel angioplasty and stenting we not only will be as safe as surgery for many multi-vessel diseases, but possibly even have fewer revascularization failures than surgery alone, and this is going to have a tremendous impact, I think, in how patients with multi-vessel disease are going to be treated, and slowly we'll have to do clinical trials to prove that one can shift into the coronary surgical arena.

And, in general, I think that this is very good for patients because the noninvasive approaches or less invasive approaches, I think, are going to take over in a big way from the more invasive surgical procedures.

Now, if you're a stent company with a new drug-eluting stent, the question is how are you going to do your study, and if you are around a year or two ago, you could do this study, which is like TAXUS or SIRIUS, and do a 1,200 patient study compared to bare metal stent.

But now that the first drug-eluting stent is out of the bag and CMS is paying for it,

it's hard to do a study against bare metal stent because everybody is going to get a drug-eluting stent in America. It seems that way, at least.

So we have to consider looking at equivalency studies overall, but if you look at trying to be equivalent to something that only has a five or six percent rate of failure clinically, you need to do a big study, four or 5,000 patients, or if you try to beat the five percent, you know, failure rate, which would be very hard to do, that still requires four to 5,000 patients overall.

Well, I think what you're also going to see if you're interested in the clinical field here is that I think in collaboration with the FDA there are going to be several clinical investigators and others working with a large group at the FDA interested in surrogate outcomes, and we'll try to make a case for angiography and also intervascular ultrasound as very powerful measures of looking at how these stents work and prevent people from having failures, and they include measures of narrowing of the artery.

And we do have a long history of well designed studies with good follow-up that demonstrates angiographic outcomes actually very good, and when we employ these kinds of outcomes, we can reduce the sample size substantially and I think still do something there, but we have to go through the classical analysis that will support surrogacy for these endpoints overall.

Right now, what some companies are doing is, they are trying to either go through a U.S. dominant approach, which would be to try to do a large scale equivalency trial at the FDA or go to Europe where the bare metal stent is not being paid for by any third party payers, and you can still do a bare metal stent study.

So the drug eluting stent still can be randomized against a bare metal stent, and there's a lot of kinks in these approaches, and they're all trying to work out both in collaboration with notified bodies in Europe as well as the FDA, but I think that this is kind of the current status right now, and I think we'll work ourselves out a little

bit better.

I just want to spend the last few minutes on potentially other applications overall, and this is very speculative. So I don't want to say that this is proven at all, but I think that with the advent of drug-eluting stents we can actually get into completely new uses of these little vehicles.

To me, and I think to others, now that we've essentially solved restenosis to some degree, and I think we have largely, maybe we can start to do things that make sense. As interventional cardiologists, we have never really helped extend anybody's lives. We basically make them feel better when they play the 18th hole, or maybe they can walk, you know, 18 without using a cart. We make their quality of life better, and that's really what angioplasty does.

But still, almost a million people a year die of heart attacks, and heart attacks occur because of plaque ruptures, not at the sites where blockages occur. Usually they don't rupture, but at

sites that we don't treat, the ones that don't cause obstructions.

Well, we analyzed a variety of different locations for these MIs, and this is my fellow John Wang who had done this, and we found that the distribution of MIs is mainly in the LAD and RCA if we look at a consecutive series of a couple of hundred patients at the Brigham, for example.

And interestingly enough, there seems to be some clustering. That is, we can see if you look at the LAD most of the MIs occur in the first couple ten, 20, 30 millimeters of the artery itself, and that's been kind of observed by a lot of people for a while.

If we apply a continuous frequency distribution curve to the location in the LAD, for example, of where these occur, we can see that about 80 percent of the MIs occur in the first 30 millimeters of the vessel itself.

So the notion might be that we actually have vulnerable hot spots in the artery. Not actually vulnerable hot lesions, and that we don't

have to really try to search out to find the plaque that's going to rupture tonight. Just use some basic shoe leather epidemiology and say that this is where the heart attacks occur.

And if you are to look at the other notion that once you put a stent in the artery, the neomyplasia that occurs there or the scar that happens makes it impossible for atherosclerosis to grow anymore. I mean, you have basically ruined the fertile ground of atherosclerosis, and we have good evidence for this.

We can actually take arteries and remove their ability to have plaque rupture by just putting a stent there, and hopefully if we have a stent that reduces restenosis, we can have a nice, thin layer of neomyplasia and basically prevent that segment from ever having an MI.

So you know where I'm going on this one. If we are to actually look at the instantaneous probabilities of restenosis overall and apply a variety of different simulated models, this is a model for an eight millimeter single stent. We have

them up to three or four stents now.

We can see that the placement of a stent, and it's eight millimeters subsequent, can actually reduce -- we can actually optimize and find where to place the stent.

Well, to make a long story short, our initial analysis has suggested that with the use of two stents, a 28 and 23 millimeter stent, we can reduce someone's MI risk by almost 50 percent, just placing them in the proximal LAD and in the proximal right coronary artery.

Now, if you're a diabetic with three vessels, it's easier. MI risk is something like -- it was in the Berry study -- which was 70 percent of five years or your fatality risk is close to 30 percent of five years if you're diabetic. A 50 percent reduction in MI could be a substantial thing.

So I think that what you're going to see is a wide expansion of these new stents with anti-restenosis therapies to potentially prevent heart attacks in the future, and how we get to those

patients I think will be the $64,000 question, and how we utilize other diagnostic approaches such as imaging techniques I think will be quite interesting.

So let me just conclude with our experience so far with drug-eluting stents. Drug-eluting stents can definitely reduce restenosis, and right now the Level I evidence is for the CYPHER stent or rapamycin, and there's Level II evidence and hopefully Level I pretty soon for paclitaxel.

The long-term effects at this point appear not to be problematic, that is, we do have data out to three years for the first in man, two years for this RAVEL study done in Europe, and one year for the SIRIUS study, and we see no catch-up phenomenon. We see no later aneurism formation, and we see no late thrombosis problems. So far it is almost a dream come true.

Other drugs are certainly going to work. There's no question that with the wide formulation of the polymer, which I think is the key component here, drugs that we always thought should have

worked that didn't in the past are now going to be given a second chance, and they include paclitaxel, rapamycin, and possibly even other basic and inexpensive therapies, such as steroids.

Finally, cost effectiveness, which I didn't review here, actually looks quite good, and that's because restenosis is a costly event, and even at the prices that are being charged now for the Cypher stent, they're still cost effective, and hopefully with more approvals of proven therapies the prices will come down, which is what's important for most patients overall.

And I think ultimately drug-eluting stents will be used for other functions and indications in the future, including potentially to take a bite out of MIs in the future.

And I'll stop there. Thank you.

(Applause.)

DR. FEIGAL: Well, I think you'll agree with me this morning has really been a tour de force. I think almost every type of therapeutic product has been mentioned in one respect or

another.

We've run a little bit over time. So if you have questions, seek out the speakers during the break. We will reconvene at 11:30.

(Whereupon, the foregoing matter went off the record at 11:15 a.m. and went back on the record at 11:33 a.m.)

DR. HUSSAIN: Good morning. We are ready to start the second session on preclinical challenges. Please take your seats.

We had planned for four presentations on different issues with respect to preclinical challenges, and these presentations are roughly about 20 minutes. So if we get started on time, we'll have lunch on time. And I was told that if we don't start on time, lunch is on yourself.

(Laughter.)

DR. HUSSAIN: My name is Ajaz Hussain I'm with the Office of Pharmaceutical Science at Center for Drugs, and I'd like to welcome our first speaker, Dr. Leach. He will be speaking on preclinical development and considerations for

preliminary delivery of drugs approved for other routes of administration.

Dr. Leach.

DR. LEACH: Thank you very much. And thanks to Dr. Provost and the other organizers for inviting me to speak.

It's been an interesting morning.

I'll go pretty quickly here because I doubt that a lot of people are interested in the nitty-gritty details of preclinical sciences. So I'll try and give you an overview of some programs that have been successfully done, as well as some ones that are in the development process, as well as some that are in the early research stage, and you get to choose which is which.

Okay. So to begin with the obvious, maybe it's a good time to always state the obvious. A lot of thought really needs to go into any of these program a priori.

The first thing you need to know is has the drug been to the site before. Particularly with the lung, a lot of people have nebulized things

before and have gotten some amount of drug to some areas of the lung, and that information may be very valuable.

Is the local concentration at the new site higher than before? Well, almost always yes. We're trying to get more drug into the lung for targeted lung disease, as well as new systemic applications of drugs, existing drugs delivered by the lung.

The next thing is are the metabolic pathways present in the new site. There are usually less metabolic pathways present, for example, in the lung than there are in other tissues, like the liver or the kidney or serum enzymes, that sort of thing. But you have to make sure. Maybe your drug is a PRO drug by the IV route. You have to make sure you have the enzymes to metabolize it to the active form.

Are there new susceptible cell types? We heard before that insulin is a growth factor, and is a growth factor given in concentration of the lung which has never been there before an issue?

Will new or existing excipients cause problems? This is a huge area. For example, some excipients which are normally benign cause bronchospasm in asthmatics or even normal individuals.

And, of course, our favorite, membrane disruptors. Those are usually a no-no in lungs. You can get away with them in other areas, but membrane disruptors in a lung, which may be part of a normal formulation is a major issue.

And of course, my personal favorite, which is antibodies to proteins and peptides. Will antibodies form? Will they be neutralizing or anaphylactic? If they're anaphylactic, of course, you're out of business, and if they're neutralizing, to a large extent, then with repeated exposure your dose must go up and, therefore, it might be impractical.

Okay. So let's start out with a couple of simple examples and work our way towards the more complex. First would be approve drug, Proventil HFA. It's called Air Amair (phonetic) in Europe,

versus the existing albuterol CFC products. It was the same drug. It was in a different propellant. It was the same amount of drug delivered, same particle size distribution, but it did have some improved dosing characteristics. Okay?

So if you look at Ventolin on the bottom versus Proventil HFA, you can see there's a clear difference there in what we call the plume, and in fact, there's only about half the propellant in the Proventil HFA as there is in Ventolin, and this resulted in a warmer spray and with less force behind it.

The thought here was that there's a cold freon effect that causes some asthmatics to have a cough or mild bronchospasm, and then if you reduce that, then you could get more drug in more consistently. Pretty simple.

So to support that, we embarked -- this is a 3M pharmaceuticals product, and we embarked on a program and again went to the regulatory authorities, and this is the first time there had been a switch from CFCs to HFAs, and essentially

they said, "Gee, we have no idea what to do. Go and do something and come back to us and we'll tell you if it's okay or not."

Hopefully from the talk we heard this morning we won't be doing that anymore and we'll have a lot better communication on new things in the future.

So we designed our own program, and it basically was this. It entailed, fundamentally, what you would do with an NCE at the very beginning stages, say, through Phase I, maybe early Phase II.

And the studies we designed were actually fairly complicated in the sense that we included safety pharmacology in them, as well as recovery periods, and tried to design very well targeted studies to answer specific questions that we thought of beforehand.

There was an inhalation teratology study in rats done, which of course was negative for albuterol, and by and large unnecessary in our minds. But at that time reproductive studies were in vogue in the '90s, and everybody wanted a

reproductive study on everything regardless of whether there was an indication or not.

Okay. Just to pick out one clinical study to prove the point that this was, indeed, the same product as the old product, this clinical study was a 12-week clinical study where half of the patients were exposed to the HFA product and the other half the old CFC product, and this is a durational effect in terms of FEV, and I've actually shown you the back half of this.

The first half was when the yellow ones are the HFA. They had no difference in duration of effect through the 12 weeks, but then we did a split-off study where we took those patients who were the CFC patients at the end of this 12 weeks and then split them in half, continued one half on the CFC and put the other half on HFA, and again, we see no difference here in duration of effect.

And of course, there were many parameters involved in the study. This is just one of them.

So for this particular study compound,

then we had no preclinical surprises. We knew exactly what the old CFC version produced in animals, and we had no surprises in the animal studies that we did conduct.

We had no PK/ADME clinical surprises, and we had no efficacy surprises. So no further preclinical studies were necessary, as deemed by the developers, us and the regulatory authorities around the world.

Pretty simple, right? Well, three and a half years after we started this, we made a submission, and about one and a half years later it was approved. So this was a five-year program, and I think one of the simplest that's ever been done.

If we go on to the next most complicated one, this is QVAR. It's also approved in about 40 countries now, versus the old CFC product. Here we have the same drug, different propellant, a different amount of drug, different particle size distribution. Therefore, it went to different places in the lung, as I'll show you in a minute, as well as some improved dosing characteristics, which

I won't go into.

Okay. So here we're going to see a very large difference then. If you look at the old CFC products, they were about three and a half microns, which is actually fairly large for pulmonary delivery. Greater than 90 percent of it actually went into the mouth, and less than ten percent went into the lungs.

Not only that, but you can see a big difference here. That doesn't even cover the large airways which actually extend to the periphery in two dimensions of the lungs as opposed to the QVAR product, which is 1.1 microns, a very small amount relatively speaking, only 30 percent in the mouth and 60 percent in the lungs.

And you can see that the lungs were covered very well. Well, this was terrific, except it did raise some preclinical safety issues. This drug is going to all the airways, as well as the alveoli, and what are the safety consequences of that?

It should be great efficacy-wise, but

this did raise a lot of questions. So we performed the following preclinical program, which was, again, sort of a modification of what you would do for any NCE, range finding studies, 14-day studies, and then a 12-month inhalation study.

And the rationale behind the 12-month study was that this could cause some endocrine disruption in young animals, and there needed to be some long-term exposure. There was no scientific rationale to speak of behind this, but nonetheless, there were people who thought this was important.

The other maybe more applicable explanation for requiring such a long, hard study was that it might have an effect on the developing one on branching. Again, there wasn't any real precedence for this, but some people felt like it was important, and of course, again, in the middle '90s, being we conducted an inhalation teratology study in rats, again, reproductive studies being in vogue then.

In fact, it was negative in that teratology study, but because the class of steroids

is labeled as having reproductive effects, this ended up with a label anyway. So I'm not sure why we did the study.

Okay. Well, let's take that product then. We did a preclinical program. We showed that it really wasn't any different once you understood the dosing between the CFC and the HFA product. What happens when you go to Phase I?

And I don't really separate preclinical from clinical very well. They should fuse right into each other and sometimes feed back. So, in other words, if you set up your preclinical program and you find clinical results in your early phases, they should go back to the preclinical, explore those differences and then come back to clinical, and so forth, and have an exchange that way.

So this is a prediction then of what would happen. If you give the beclomethasone to the lungs, it's 100 percent bioavailable. It is about 20 percent bioavailable by the oral route. So if you come up with these, you can do a projection here and say if you believe the dosing, if you believe

the deposition studies, then when you do your Phase I TK study, if you give the same amount of Beclovent 100, which is the old CFC product, versus the HFA product, you should get about 2.6 times as much in the serum with the QVAR product.

So we tested this hypothesis, and we actually gave 400 microgram of the BDP, old BDP against 200 and looked at the pharmacokinetics, and you can see that, indeed, when you adjust for double the dose here, it was about two and a half to one ratio with the BDP-HFA being the yellow line here.

Now, there's a couple of things you might notice. First of all, the Tmax happens quicker with this than it does with the CFC, and that's because of the oral contribution. So this actually did confirm not only by the AUC two and a half difference, but also by looking at the Cmax/Tmax values and showing that our hypothesis did appear to be correct.

Okay. So then we're ready to go into the clinic, and so we did a dose response relationship between the QVAR and the old product,

got these lines, drew the equivalence there and saw that it was as efficacious at about 2.6 times less dose.

So, again, this is fitting with our preclinical, with our Phase I, and so forth. And in fact, when you go on to long term clinical studies, you can see breakthrough of asthma here, and you can see the yellow line being the QVAR. You can see that at a two to one switch here there was actually less breakthrough of asthma than there was with the old product.

So the safety parameter. We looked at many, but of course, urinary free cortisol is one of the major ones, and so you worry about that kind of dose being given, and is it different?

When we looked at the urinary free cortisone, this is the placebo, and these are the different doses, and in fact, we found that the -- boy, I switched colors here, yellow and red, just to see if you're awake.

In this case the yellow -- oh, the yellow is the HFA. Sorry. The CFC is the red, and

you can see that there was no additional safety concern matching doses of 800 versus 800, even though clinically 400 was equivalent to 800.

Okay. So once again, we had no preclinical surprises in the two species. We were able to predict the PK and the ADME clinical results, and there were no efficacy surprises. So there was no further preclinical studies required.

Now, this program, again, took about five and a half years to complete and another almost two years to get registered once it was submitted.

So even these simple cases have not turned out to be so simple or cheap.

So now let's move into some of the things that are being worked on. You've heard a lot about proteins and peptides and insulin. Everybody is very, very excited, as are we because there are just so many proteins and peptides that are being explored now with so many exciting results, but they have very serious delivery problems. They need to inject. No one wants that.

The time of action is too short. Native