The Role of Sandostatin Analogue in the Reduction of Tumor Growth

Presented by Eugene Woltering, M.D.
Louisiana State University Medical Center, New Orleans, Louisiana

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It's all in the packaging, guys. Can we sort of move the projector, Bill, so that slides get on there. It probably got jostled during the meeting.

I'd like to sort of start at the beginning. You know, an expert is somebody who's at least one time zone away from home and has a of carousel slides. That means, Dr. Kvols, even though you had two carousels of slides, you're not an expert cause you're still in this time zone. And, Dr. Warner, I don't know how many slides you got. But I'm one time zone away and I got one carousel of slides.

Somatostatin and analogues are, as Dr. Kvols pointed out so eloquently, and this slide is a blatant rip-off of Larry's original slide with the little circles. This is native Somatostatin; this is what you and I make in our body every minute of every day of every hour. Somatostatin is, as Dr. Kvols pointed out, the universal off switch. I always say to my residents and interns, if you are in an exam and they ask you what turns something off and it has to do with the gut, answer Somatostatin and you'll be right 99 percent of the time. But as Dr. Kvols pointed out, this is the perfect control substance if you were the big guy upstairs designing a control mechanism, you'd design it this way. I'll give you an example. You eat a hot fudge sundae, your blood sugar goes up, you make insulin to bring your blood sugar back down. Everybody knows that. Ever ask yourself the question—what turns off the insulin so your blood sugar doesn't keep going down. The answer is Somatostatin. It's released as a peak. It goes up very rapidly and two minutes later it's gone. The off switch turns off the insulin and then it's gone. Why? Because you may decide, if you're my size, to eat a second hot fudge sundae ten minutes later. So you want to be able to turn off the second bout of insulin. That's perfect. You make it every second of every day in your body. It's not perfect if you want to use it as a drug. People in the early 1980's, shortly after Somatostatin was discovered and the sequence of these amino acids, elucidated by a group called Gillyman and Brizeau out of Texas, started to use Somatostatin. The good news is Somatostatin, if you use it intravenously by a continuous infusion, will suppress peptides, serotonin, all the things that Dr. Kvols and I and Dr. Warner will be talking about. That's the good news. The bad news is, is that you have to keep that IV drip going. So people weren't able to get out of the hospital. They had control of their symptoms as long as the IV was running, and then the really bad news was, when you pulled the IV, not only did things go back to where they were, they actually got worse. It's something called a rebound phenomenon. Within two weeks of the discovery of the sequence of these amino acids, the story goes that a man named Yannos Pless designed a mini-Sandos, mini-Somatostatin analogue. This is what we now know as octreotide or Sandostatin and what Dr. Pless did was cut the middle out of the native molecule, kept these four amino acids that he thought was where this molecule bound to the receptor and added an alcohol on to the end of the molecule and amino acids come in D and L forms. The D forms are resistant to degradation in the body, so he added a D form here. That accomplished two things. The first of which is it made about 100-fold increase in its potency. That was pretty good, but that wasn't the really good news. The really good news is, this has a duration of action in the circulation measured in minutes, this has a duration of action or a half-life of about an hour and a half to two hours. This is one and a half to two minutes. So that you are 100 times more potent but you were also about a log order more able to keep it in the systemic circulation. That meant that you could give three or four shots of octreotide a day by sub-q and get out of the hospital and, you know, get back to the activities of daily living. While Novartis has sponsored this conference and has been a long-term supporter of all of us in here, there are other analogues that are coming to market. This one is known as RC 160 or Vapriotide. It is now close to being marketed, I hear, in Europe. And

another one that I don't have a slide of is called Lanriotide, which is on the market in Europe. Octreotide comes in two forms, as most of the people in this room know. A subcutaneous form called the aqueous form and one put into micro spheres that slowly release called LAR. How you structure one of these molecules is really critical. This is octreotide. This is a compound down here made by Dr. Pless and all he did was change this lysine to an ornathene and this is an important molecule to remember because I'm going to use it later in some of my studies to show that this molecule, while everything looks just about like this molecule, this won't bind to the receptor and is biologically inactive. Somatostatin came into the forefront because it suppresses peptides. Peptides are chains of amino acids and you heard Dr. Kvols talk about gastrin, insulin, somatostatin itself is a peptide, there are other ones—VIP, neurotensin, substance P, a list that goes on for about forever. When I was at Ohio State, one of my original teachers was a gentleman named Dr. Robert Zalinger. And Dr. Robert Zalinger is the surgeon who first discovered that tumors had functional capabilities. That is, they could make a substance that's normal in you and me. You and I make gastrin every minute of every day; it's what runs the acid-making part of our stomach. Gastrin binds to a cell called a parietal cell, and that parietal cell in the stomach makes acid. If you have a tumor that makes gastrin in boatloads, it is called a gastronoma—a tumor that makes gastrin. That's how all these tumors are named. It also is known by something called the Zalinger-Ellison syndrome. Just to give you a little insight into my life—I was the youngest member of the faculty at Ohio State after I finished my fellowship and joined the faculty, and Dr. Zalinger was the oldest. He was by that time in his early 80's. They didn't have any room in the Department of Surgery for Dr. Zalinger's office or my office, so we got moved right across the street to the Medical Library building. So it was Dr. Zalinger, myself, our two secretaries, and an empty floor. So Dr. Zalinger and I got to know one another pretty well. The first paper I ever wrote at Ohio State was taking one of this gastronoma tumors out in the operating room and chopping it up into single cells and then looking at what we could do to turn on and turn off gastrin release. So Dr. Zalinger nicely, after I got this manuscript all written up, agreed to review it for me. I gave it to him and the next day I am in my office sitting there talking to a family whose son is dying in the ICU and no knock on the door, the door opens, Dr. Zalinger throws the paper across the room and says "Woltering, you are the dumbest SOB I've ever met. If I could get your mother's phone number, I called her and tell her you are the dumbest SOB I ever met." So, with that, I got really interested in Zalinger-Ellison syndrome. And we started, when I went to Oregon, a study where we used Somatostatin analogues in patients with gastrin-producing tumors or gastronomas.

Now, let's just review a little bit of what won a gentleman named Sutherland the Nobel Prize. Dr. Sutherland showed that a peptide, or in his case amines, could bind to a receptor that sits on the outside of a cell. Imagine a cell as like a beach ball. And this is a revolving door in the beach ball. Well, at the time that Dr. Sutherland described this, the door didn't revolve, it was just a, you bind to it and it was like a light switch that turned on the lights inside of a cell. And those lights, or activities, are called signal transduction pathways. Big fancy doctor words for saying that when you plug in a lamp into the receptor, the receptacle in the wall, suddenly there's light. Well, it's the same kind of concept. A peptide binds to a receptor. Down deeper in the membrane there's an affecter. That affecter, in big doctor words, is called the G protein. When this complex all gets hooked together, this G protein tells complex pathways or signal transduction mechanisms inside the cell to do things. And those messages control things like secretion of peptides. Let's say this is serotonin or gastrin or whatever. Somatostatin binds to the receptor, the receptor talks to the G protein, the G protein talks to all these signal pathways, and they tell stop packaging and releasing the peptide, gastrin or serotonin. That's how Somatostatin was thought to work. That is a very small part of a story that is now getting much more complex that we'll go into more later, but Dr. Kvols alluded to it. Instead of it just being a plug in the receptor, what happens is this now is known to act like a revolving door. You put Somatostatin on its receptor, this receptor lygand the complex or peptide receptor complex, now can turn around like a revolving door and drop the Somatostatin inside the cell. For a long time everybody thought that Sandostatin went inside the cell and went to a lysesome. Now Dr. Kvols talked about that. A lysesome is another word for garbage disposer. Lysesomes give off enzymes that chew up the peptide into its amino acids and spit them back out so the body can use them to build something else. Recently we've discovered that there is another source of where these peptides go, and that is, they go to the nucleus, the nerve center of the cell, get inside the nucleus, and actually bind to DNA directly. There is a small section of DNA that the Somatostatin binds to.

That having been said, I'll turn back the clock to when I first went to Oregon, and Dr. Bill Fletcher, my partner, and I took patients with gastronomas and we gave them, we started them on 100 micrograms of Sandostatin three times a day and then we watched how their gastrin level was affected. And, as you can see, remember there's a break here, many of these people brought their gastrin levels down into normal ranges. Some very interesting patients. This patient had a very nice response, stopped her drug, we saw her back, we restarted her on the drug, when we saw her back, her gastrin level had normalized. This patient here had progressive metastatic disease and ultimately died of her disease. This patient here in the blue line did very well for about five years, and you'll see her in a minute when she had a single isolated recurrence in her liver. This patient here was a surgical cure at this point. So Somatostatin analogues, even in very low doses, had an effect on circulating peptide levels. Just like the people who had discovered Somatostatin would have predicted. However, like everything else, as you start to study people, looking for one problem or one effect, if you're smart and the gods look on you in a nice favorable fashion, they send you other observations that take you off on a whole new tangent in your life. One thing is very important and that is that we all recognize that none of us in this room who are physicians do this alone. We all have residents, fellows, nurses, and a whole bunch of support staff that I'll try to remember to acknowledge as we go along. This is a patient who weighed about 450 pounds. This lady had a gastronoma, she had been treated with everything known to man. She had been given renal failure with the drug Streptozotocin. She had been treated with every combination of anti-diarrheal medicines known to man. Her son was a pharmacist in Boise, Idaho, and he heard that we had the Sandostatin trial for gastronomas available. She came over to Portland from Boise, Idaho, and we started her on Sandostatin. We kept her in the hospital in the GCRC for a couple of days. She had been having 30 bowel movements a day with her gastronoma. While she was massively obese, she was very active in her church, and that's all she wanted in life was to get a drug that would stop her diarrhea so she could go back to doing her church activities. Within three days she was down to having a bowel or two a day, she thought we were wonderful, and she toddled back off to Boise, Idaho. We didn't see her back for about 10 months. Her son called me every month and said how she was doing. We had a physician there who was doing her follow-up studies, etc. And so, when she came back in 10 months, she was in wonderful symptom control, still having a couple of bowel movements a day and thought we were wonderful. I wanted to send her home. My fellow Everett Mozel, who is now a surgeon in Salem, Oregon, said, "Gene, we ought to get a CAT scan." "Why do we need a CAT scan, Everett?" "Well, we oughta because, you know, she's doing so well, you know, we ought to see how bad her tumor is." Because she had just boatloads of tumor in her liver the first time we CAT-scanned her. Well, I don't see why we should spend $1500 on a CAT scan, but Everett said, please, boss, get a CAT scan. Well, listen to your friends because that's why you're friends. I'm sorry that the lights are so bad in here but this is her original CAT scan; there's a tumor here, a tumor here, a tumor here, a tumor here, her stomach is very thick, and on other cuts of her CAT scan she had a primary tumor in the pancreas that was about nine centimeters. This was before therapy, again a therapy only designed to be like very high-priced limodal to stop her diarrhea. When she comes back, this is in 1989, this case was first published, she had a complete tumor response. Biochemically, she had a complete response as well, normalized her gastrin for five years. This lady, like I said, was of the large economy variety, and also had very bad heart disease. And ultimately died five years later of a massive myocardial infarction, and an autopsy had a single isolated tumor right here five years later. Her pancreatic tumor had completely disappeared. And this was our first evidence that Sandostatin or octreotide acetate could affect tumor growth. And the question then was how did that happen. And our first thoughts were that, if you trapped these vesicles of peptide inside of a cell, could you give the cell peptide constipation to the point that it would get impacted and sort of blow up. Well, we could never prove that but it was a nice sort of idea that, you know, as a retribution against diarrhea that, you know, we'd give the cells constipation. But that never panned out. And then there was this funny guy named Kvols that I met just about this time who came out with the slide that you have already seen earlier this morning. And that is, that patients treated with chemotherapy who had carcinoids did very, very poorly but patients who had been extensively treated, who were put on octreotide acetate, had three times more survival time than people treated with chemotherapy. Again, how does a drug that's designed to be a symptom drug have any effect on tumors? And, at this point, which was in 1993, and this article by Larry in Actaancologic in 1993, single best carcinoid paper ever written, my hat's off to Larry Eiten, to this day no one has ever written a better paper. But anyhow, this paper was the impetus for us to go a totally new way in my whole career and the research done in our laboratory, trying to figure out how the heck a drug designed to control diarrhea and flushing had anything to do with tumor growth.

About the same time that we published the article about Shirley, and Dr. Kvols was doing his seminal work at the Mayo Clinic, there was a consensus conference at the NIH. When the NIH doesn't know what to do with a problem, they bring together all the experts who don't know what to do with the problem, and I can tell you they didn't know what to do with the problem because the bottom line of this consensus conference was, yeah, there are some people that have tumor responses to Sandostatin but there are not enough of those to really make it worthwhile. And the bottom line here is, all oncologists, let me just tell you, the magic number is that 50 percent of the people don't have, or the tumors don't shrink by 50 percent, you don't have a "objective response." Well, only about 13 percent of people had objective responses when put on octreotide acetate. However, what we all missed, and raise my hand and say I missed it too, was the other column here. Is that in 63 percent of those people their tumors seemed to just stop growing. Well, 13 percent is not a very impressive number; if you add 13 and 63, now you're saying, maybe 70 or 80 percent of patients treated with octreotide, and this is in the dark days, the ancient days of octreotide, very low doses used in these days, 80 percent of people, 70 percent of people, had some kind of tumor effect, as Larry pointed out on the previous slide.

Well, every time, if you're like me, you try to read about the subjects you're interested in and I had a good friend, another surgeon, believe it or not surgeons do do research, this is a guy named Colin Weber, and he was in Philadelphia, he is now at Emory in Atlanta, and Colin got interested in octreotide but not in carcinoid, interestingly enough. His interest as a cancer surgeon was in breast cancer. And Colin took nude mice, and you'll hear the term 'nude mice.' A nude mouse is a mouse that has been bred not to have any immune responses. So I can put an orangutan tumor or a human tumor in a nude mouse and it will grow. It will not recognize human as foreign. So he put human breast cancers in nude mice. MCF 7, which had estrogen and progesterone receptors, and BT 20 that did not. And then he treated them with either a saline injection or two injections a day of octreotide acetate. What he showed was in the MCF 7 group that the ones that also had hormone receptors, that the volume of the tumor shrank and that the doubling time, the amount of time it takes a tumor to go from the size of one lesure pea to two lesure peas went down from 13 days down to 19 days. So the doubling time was slowed down. And the tumors without the hormone receptors, the volumes were no different but the doubling time was lengthened. This was a paper that was very provocative to me because this wasn't a neuroendocrine tumor paper, this was one of the first papers ever written on breast cancer. And, have you ever read something and you walk from it, and then you go – tag! There is something in that paper that I know I read that's not registering; I need to go back and reread that. And so I reread the paper. And I'd go away, and I'd come back and I'd reread the paper. And I kept knowing that there was something in this paper that I should be paying attention to, but it just wasn't registering in my brain. And, finally, after I'd read this paper about seven or eight times, there's one line, the proverbial comedian's one-liner. It said tumors treated with octreotide acetate were grossly less vascular. Meaning they had less blood vessels feeding the tumor. I sort of jumped up, ran down the street yelling Eureka, I have found it! And I finally started to put the story together that maybe, just maybe, instead of the Sandostatin having an anti-tumor effect on the tumor cells, maybe there was another part to the story. Maybe it was stopping the blood vessels that fed the tumor. Well, so we then split our laboratory into two sort of parts. Half of our group looked at the effect of octreotide on the tumor cell and the other half of the group went and started looking at the effect of octreotide on blood vessels. And this is work done by Darryl Kurosowa, it's in an article you can get from my office called Investigational New Drugs. What Darryl showed was that as you give these breast cancer cells that have hormone receptors, as you expose them to Sandostatin, that the number of cells in culture go down. At least until you reach a certain dose. But then, let me tell me, this is classic, just like everything else in the world, just because something's good, it doesn't mean that more is better. Insulin. If you give yourself the right dose of insulin, you fix your diabetes. If you give yourself too much insulin, you can kill yourself. Same way with octreotide acetate, it appears. There is an optimum dose and then if you go above that optimum dose, you can start losing effect. So, on these cells which didn't have any estrogen present, these are like a post-menopausal woman, octreotide slowed down tumor growth. What if you put estrogen in this situation? Well, number one is that you got a lot more cell growth. But number two, octreotide would still block the breast cancer growth. And, again, it did it at a very, very narrow therapeutic window. More is not necessarily better. You need an adequate dose, but not too much. Well, then Darryl asked the question, and again, thank God for residents and fellows who come to lab because they don't know enough sometimes not to ask the really good questions. And he said, maybe the way this works isn't directly on the cell; maybe the octreotide is

turning on or turning off something else in the breast cancer cell. And the logical thing would, does octreotide change the sensitivity of the cell to estrogen by altering estrogen or progesterone receptors. And, again, work that's published in the Investigational New Drugs, octreotide markedly up-regulates estrogen receptor. It's the idea that it makes breast cancers more sensitive to the effect of estrogen, and it does so a little bit to the receptor called the progesterone receptor. Estrogen does the same thing and if you put estrogen and octreotide together, you lose this unusual effect. So it looks like, if you could get rid of estrogen and use octreotide, there might be some kind of synergistic interplay between estrogen lack and octreotide. Well, how do we block estrogen's effect? There are whole compounds, groups of compounds now, called anti-estrogens or estrogen receptor antagonists. The biggest one you may have heard of is a drug called Tamoxifen. So the question was, if we use Tamoxifen and octreotide together, would that be a good thing. And what we did is again looked at the MCF 7 cells, used octreotide, used Tamoxifen, and used them together, they were more effective than either of the two drugs by themselves. About the same time a guy named Whetbecker from Novartis in Basel, Switzerland, decided that he'd do some experiments like this but use animals instead. And he implanted these animals with breast cancers, and then he either did nothing or he treated the animals for six weeks with either Tamoxifen, the red line, octreotide or their combination. And at the end of six weeks stopped their therapy and then watched what happened to the tumor afterwards. And these the number of tumors per animal. And you can see that, if you use octreotide and Tamoxifen together, you can block the development of tumors. And that effect, even after you stop treatment, goes on for awhile and then all of a sudden the animal starts developing tumors. This is again the number of tumors. What about the size of the tumors that do develop? And, again, the combination of octreotide and Tamoxifen when you stop therapy, there's this golden window when nothing happens and then all of a sudden, boom! it takes off again. Could it be that octreotide or Tamoxifen blocked blood vessel growth and when you stop treatment, it takes this long from there to there for the tumor to regrow a new blood supply? Well, if that's the case, the other way we can get rid of estrogen is take out your ovaries. And so what he did was waited until the mice had big tumors and in the yellow group he took out their ovaries, the red group he took out their ovaries and put them on octreotide. And here's what's really interesting. Again, this is the number of tumors per critter and what you see is oophorectomy and oophorectomy plus octreotide, the response rate during treatment was very similar. But when he stopped treatment, and the oophorectomy tumors alone regrew, and now he stopped the octreotide treatments, look how long this window before regrowth is. This is again number. If you look at the size of the tumor, it's even more impressive. Oophorectomy alone, taking out your ovaries alone, you got your response, but then after treatment, boom, they went right back up, whereas those just treated for six weeks with octreotide went for a very, very long period of time without evidence of tumor getting larger.

Well, we've showed you that the Somatostatin and analogues can directly inhibit cell growth and we've talked about what is a topic known as angiogenesis. If there is a hot topic in medicine today, it's angiogenesis. The idea that tumors require a blood supply to grow. Tumors have to gain access to the vascular blood supply to move from the gut or wherever it developed to the liver, the lung, the bone, the brain or wherever it's going. So angiogenesis not only controls tumor growth, but it controls the tumor's access to other parts of your body. But is there any evidence that Somatostatin and analogues can block blood vessel growth? Well, angiogenesis, or new blood vessel growth, is absolutely essential for an embryo to grow into a normal human being. We also use angiogenesis to repair surgical wounds or any kind of wounds. The three major forms of blindness, one of which is diabetic retinopathy, is caused by angiogenesis. Rheumatoid arthritis, what destroys your joint, is overgrowth of blood vessels. Even things like psoriasis are dependent on new blood vessel growth for their development. And then, finally, cancer. Dr. Judah Folkman, who is the father of all the concepts that you're hearing today, said in the mid-1950's that if a tumor can't generate a new blood supply, the maximum size it can ever get is two millimeters. Two millimeters is about a tenth of an inch in diameter. So you never know you had it. Now, why is angiogenesis an interesting target? Well, let me tell you what this whole slide says. It says that cancer cells are really smart. Cancer cells are smarter than the entire intellect of everybody in this room combined. Every cancer cell that I've ever met has figured out a way to get around every therapy that we've ever figured out. It's called drug resistance. Cancer cells and bacterias have figured out the way to get around all kinds of evil toxic drugs. You make adriamyacin; cancer cell figures out how to get around adriamyacin. If you figure out Cis-platinum. VP 16. Tumor cells develop drug resistance. On the other hand, if cancer cells are Albert Einsteins, blood vessels are dumb as a bag of rocks. They

never learn. They never can develop drug resistance. They are very slow growing, but all the things that happen to cancer cells like moving from one type to a what's called phenotype, doesn't happen with endothelial cells. And so, they're also readily accessible to anything you put in one blood vessel ultimately ends up in every blood vessel in your body. Well, the first thing that we set out to do when we asked the question, will Somatostatin and analogues inhibit blood vessel growth, is to take the cells that make up the blood vessel in culture and expose them to various concentrations of the Somatostatin and analogue octreotide. And, as you can see, remember I said more is not necessarily better, you again have this very narrow therapeutic window where the Somatostatin and analogue concentration is most effective. If you use less than that concentration or more than that concentration, you'll lose your effect. If you put this Somatostatin and analogue on these cells, the effect starts to take place at 24 hours, peaks at 48, starts going away at 72, and is gone by 96. That's with a single exposure. What you have to do then is time your exposure so that you peak, give a new dose here, and so as you are starting to peak, you're getting a new dose, so that as this dose is going away the new dose is starting to come back up. This is work done, and the last slide was done on pig coronary artery, heart artery cells, and it was Dan Burley from Mallinckrodt was the person who got us these cells. This is work done by Roberto Dinizi out of Italy. And Dr. Dinizi showed that Sandostatin or octreotide would inhibit blood vessel cell growth, but in this case he used human umbilical vein, so when they delivered a placenta and they were going to throw away the vein that fed the placenta, they got cells out of that vein and grew them in culture. These are called huvex or human umbilical vein endophilial cells. And, again, as he added octreotide, he had a peak effect in this same range at 10 to the minus 8, 10 to the minus 9 molar, and more was not better. So, again, that very funny curve that says you better know your octreotide level or you may be getting too little or too much.

Well, all this stuff that you have just seen is in cell culture. Which is about as artificial a system as you can ever have. Cells talk to one another. Cells communicate with one another. Cells like, like people, like to be in groups. They are what are called contact inhibition. If you take cells and you have one cell over here and one cell over here like you do in culture, they can't talk and they can't interact normally. So what we wanted was a system that would be an intact living animal. And so I knew nothing about angiogenesis. So I pick up the phone and I call the father of angiogenesis, Dr. Judah Folkman, who believe it or not also is a surgeon at Harvard, at the Children's Hospital. And I say, Dr. Folkman, I am a dumb surgeon, I don't know anything about angiogenesis but I got an interesting idea I'd like to try. How could I do it? And he said, "it's very simple. This is even something a high school student could do." I said, "Well, maybe I have a chance then." And the idea is, you take a fertilized chicken egg, straight from the farm, you wash it off, you put it in an incubator, you let it sit till it's three days old. On three days old you sort of look at 'em and you can actually sort of tell which ones are going to make it and not many times. But by day six in the incubator you crack them and put them in a saran wrap hammock so that the whole egg with the fertilized embryo is sitting there and you put that whole piece of saran wrap in a piece of PVC pipe that you've cut off about that tall, so that it's sitting there, and you can look right through the open end of the pipe. On day six you can put little disk containing drugs on the chicken egg and on day seven you can see what happens. You just wash the egg, on day three put it in the incubator, crack it on day six, there it is in a piece of saran wrap, and there's a fertilized embryo in there. On day six you can see the embryo is developing, this is sitting in its piece of PCV pipe, and the microscope will look straight down through the chicken egg. This is what a chicken egg's blood vessels look like. Now, they're not black. And the reason they're black is because a woman who worked in my lab who is now a plastic surgeon in St. Louis named Tina Yura, injected India ink into the heart of these chicken eggs just before we sacrificed them. This is a primary blood vessel, a secondary blood vessel, these are called tertiary blood vessels, and the little gray stuff back in here are called quatronary vessels. This is the test drug, in this case this is nothing except this is made out of metal cellulose. But the nice part is you can see right through it and see what's going on. Now this is normal, okay. Sort of picture that in your mind. Tumors make substances called growth factors. Those growth factors are, as Larry said, the gasoline thrown on the fire. They're what make things grow. They're what make blood vessels grow. One of those things is called vascular endothelial growth factor, or VEG F. If you add VEG F to this disk, unfortunately it turns cloudy. But it also changes the way it looks. Notice now, this is called wagon wheeling. And all the blood vessels are growing towards the chemical messenger that says start to grow. What if I put in here instead of something that said grow, what if I put in Somatostatin and analogues that said stop growing. The answer is you get a big hole. Notice that the primary blood vessel is still intact, the secondary

vessels are intact, but you lose tertiary and quatronary vessels. That's sort of important. Because if I gave you an angiogenesis inhibiter and knocked off the primary blood vessels, all the blood vessels in your body would fall apart and you'd die. It turns out that primary and secondary vessels belong to the patient; tertiary and quatronary vessels, which are the hole here, belong to the tumor. So knocking out tertiary and quatronary vessels is important. Another picture. It's hard to see the disk is right down here, but you can see that all the blood vessels are gone but the primary and the secondary, these little faint things here are actually on the other side of the embryo. But we then put together a whole series of chicken eggs, believe me I don't eat omelets any more. We did 21,000 chicken eggs. A young lady in my lab named Susan Wright did about 19,000 chicken eggs herself. And again, we compared octreotide, the white line, to Dr. Andrew Shalley's compound, the Vapreotide or RC 160, and showed that as you increased the amount of Somatostatin and analogue you put in the disk, you inhibited progressively more of the blood vessel growth.

But you always want to compare this to a gold standard. Is this is relevant amount of inhibition of angiogenesis? So guess what? Back on the phone again. "Dr. Folkman, what is the best inhibiter of angiogenesis today" and this was about 10-12 years ago "known to man?" And it was a combination of two drugs—Heparin, which is used as a blood thinner, and Steroids, which, we won't go there, but are used commonly in a whole bunch of diseases. And this is Dr. Folkman's positive control for Heparin and Steroid. And as you can see, we are right in the ballpark which, at that time, was the most potent inhibiter of angiogenesis known. Remember, I told you to remember that molecule that had one amino acid changed. That's right down here. That's the same thing as Somatostatin and analogue octreotide with one amino acid change, no effect. And this is the native Somatostatin that your body and my body makes all the time.

Well, how does this compare in the same assay to the whole bunch of drugs that we all know and love or hate. All the chemotherapy drugs—methotrex 8, vinblasting, VP16, mitazantrone, adriamyacine, vincrystine, 5FU, Cytoxin, Cis-platinum, interluken, methotrex 8, era C. These are how those drugs work in this assay, and here's octreotide acetate. Here's Dr. Folkman's heparin hydrocortisone. And here's the chemotherapy. But here's the big difference. All the chemotherapies have a boatload of toxicity. Octreotide, there has never been a lethal dose of octreotide determined in rats or in humans. Early on, when people started using octreotide, there were some terrible mistakes made because people went and read papers about Somatostatin and used octreotide at the Somatostatin dose. BIG mistake. So people now have been given hundred milligram doses of octreotide. For the people in this room, if you get two milligrams a day, you're on a pretty doggone high dose. They have been given a hundred milligrams doses with no toxicity. So here's a drug that has very good effect but what's really important about it is, it has no toxicity. But all the stuff I've shown you either is cell culture or it's a chicken. I mean, I don't know how many chickens are sitting in this room, or how many of our spouses are chickens. But it's not human. And, if it was human, like Dr. Dinizi's work, it wasn't intact. Cells didn't talk with one another. So, there was a guy about 10 or 12 years ago who started taking blood vessels out of rats and instead of blowing them into a bizillion individual cells, he put them on a baloney slicer. And he sliced the blood vessels into rings. And those rings were then put in a clot. And that was like a blood clot without blood. It's called a fibrinthrombin clot. And he could get those rat aortic rings to grow. New blood vessels. So our lab said, damn, if you can get a rat's blood vessel to grow, why can't you get a human's blood vessel to grow? Well, there aren't many people in this room who are going to let me take a piece of their blood vessel even in a research project, so we thought and we thought and we thought, where was a source of blood vessels that were thrown in the garbage can? And the answer went back to placentas. When a woman delivers a baby, if the baby is healthy and wealthy and the placenta looks normal, it's pitched in the garbage can. So we went to our IRB, the people who guard human subjects, and we said, if the nurse would pick it up out of the garbage can and put it in a bag so we don't know what patient it came from, the patient's anonymous, and the tissue is discarded, can we use those placentas in research? And the answer was yes, we know nothing about the patient and the tissue was not needed for anything else. So what we did is, we brought the placentas back to our laboratory and, being surgeons, we dissected out the blood vessels, not out of the umbilical cord but out of the placenta itself. So now you have a tube. So what we do is we take that tube and we just run our scissors length-wise and so now we have a flat sheet of blood vessels. And then we just take a little punch, like a leather punch, and punch out two millimeter in diameter disks of real genuine certifiable blood vessel that's human. And we imbed it in the same kind of clot as Dr. Nicosia did with his rat aortas.

These are what the boys in the lab call my cheeapets. This is a human blood vessel and these are new blood vessels growing out of the cut edge of the human blood vessel. This out here is the fibrinthrombin clot. We tried a whole bunch of different things. We've tried ager, which is like jello. We've tried other things. Turns out that blood vessels need, like climbing roses, a lattice to climb on. The fibrin and the thrombin, as you are about to see, make a mesh and these blood vessels snake through that mesh and hold on like a climbing rose. Well, how do blood vessels grow? It turns out that nobody had ever grown a human blood vessel in culture before. So we started out at the beginning. And we said, how does a human blood vessel grow? Well, this is the fartherest part out into the clot. And that means it's the newest. And that says that there are endothelial cells and those endothelial cells are in basically solid cords. As these blood vessels begin to mature, they start to develop what are called lipid vacuals, our friend cholesterol, HDL, LDL, all those fats collect as lipid or fat globules interspersed in these cells. And then somehow by magic they all start to coalesce and the little septums between them all fall apart and there you go, that's the lumin or the whole in the blood vessel. And that's the most mature blood vessel. So these are real genuine human blood vessels, they have all the characteristics of blood vessels, they stain with all the appropriate stains to prove that they really are blood vessels. And they grow in culture in a very predictable fashion. If you. . . they require serum, and this is cow serum, to grow. If you don't have any cow serum, nothing grows. And over time the number of wells, if you plant a 132, 80 of them begin to grow, you lose a couple to infection, and now they start to grow, and down here even more of them grow over time. So, the incidence of angiogenesis increases in this culture over time for about two weeks. I am going to give you a very important concept, and this is a really hot new concept. Blood vessels exist in two states. Asleep or quiescent and then they undergo what's called the angiogenic switch. Somebody, the big guy, turns the switch, and I'll show you that that's maybe in certain cases tumor induced, and the cell changes pheno type or the way it looks. It goes from sitting there doing nothing to starting to grow. It's called the angiogenic switch. That's what the incidence does. How many of these went from resting to proliferative? And you can see it goes up in a very linear fashion. Once you've gone through this switch and you begin to grow, how fast you grow is also very linear. I'm really sorry that this room is so miserable for slides but this is the vessel on day one, on day five you can see that the blood vessels are about here, on day ten they are out even farther, and by day fifteen they almost fill the well. So that the blood vessel grows in a very predictable fashion. So two things that are important. One, taking resting and making it grow; and two, effecting the rate of that growth. That's called promotion. So we have initiation and then we have promotion. Here's how the length of a blood vessel grows over time. This is day seven, day 13 to 15, and day 21. And this increase is very very linear.

Well, how does octreotide do in this? Well, first you need a control. So I'm stuck again. Guess who I call? Back on the phone to Dr. Folkman. And Dr. Folkman then says, well, here's a dose of heparin steroid, the green line that I think will work. But it didn't. It looked just like our untreated group, which is the red line. So we went up ten-fold and lo and behold, nothing happened. So we went up another ten-fold to hundred times more than Dr. Folkman had guessed, and we were able to block blood vessel growth entirely. What about octreotide acetate? Well, after day six, nothing grew at all. But why did things grow between day one and day six and then nothing new after day six? Let me tell you, a lot of late night hours and arguments over Domino's pizza and coca-cola between, occasionally something other than coke but not me, I don't like beer. Anyhow, so between day zero and six we didn't know why the drug took effect on day six but there was something happening in here. So one of the guy's in my lab said, well, gee, maybe the Somatostatin and receptors don't exist on a normal blood vessel but maybe they do on a growing blood vessel. Another one of those Eurekas, I think you've got it. And so we started asking the question, do receptors for Somatostatin exist in normal blood vessels? You saw beautiful pictures by Dr. Kvols of OctreoScans. See any blood vessels? Didn't see the aorta, you didn't see the heart, you didn't see anything. It's because the receptor's not there. But I'll show you in a minute the receptors for Somatostatin and analogues develop as a vessel begins to grow.

Well, one of the critical issues for everybody in this room is what does a blood vessel say to the tumor and what does the tumor say to the blood vessel. Because they talk back and forth. That makes good sense. Well, the first one is pretty easy. What's the tumor say to the blood vessel? It says grow. But does it affect initiation, does it affect promotion, what about the direction? Can a tumor say to a blood vessel, I want you to go down to Miami Beach and then turn around and come

back. Can it say those kinds of things? So we set out to answer some of those. And the first question was pretty easy. You saw this picture before, all we did was take some breast cancer cells and other kinds of cancer cells and put it on top of our clot. And then said, will that change initiation or promotion. So, the first thing we showed was, is that it increased the number of blood vessels that began to grow. Now, this is sort of very interesting. So the tumor sends out a chemical message to the blood vessel that says I need you to wake up and start growing. Now, the question is, will a regular blood vessel that grows, does a tumor make that blood vessel grow any faster than a plain old blood vessel that you have gotten to grow with just serum. And the answer is no. But this is very scary. One of the experiments that I'm not going to show you today is a guy named Chris Watson who is now a cancer surgeon at Fox Chase, put the tumor cells on, but he put them in a baggie called a dialysis membrane. So he could take the tumor cells out after one day, after two days, after three days, after four days, and after three days the chemical message for initiation had been sent, and taking the tumor out at day three you might as well have left it there because the message was not a reversible message. Terrifying to me as a cancer surgeon because the basic tenet of surgery is that if I take out the tumor, I've done something really good. Well, I can promise you that those tumors have been there more than three days. That says if I leave one little cancer cell there, I leave it in an environment that has been stimulated to have a bizillion blood vessels growing towards that one little cancer cell.

Well, what about the direction? Is a tumor able to (tape has momentary gap). So, this is sort of a hard slide to envision. But imagine you've got a plate. And the plate is covered with jello. And I cut three holes in the jello, like ice fishing. One of them has tumor cells, this has the blood vessel, and over here on the wall that you can't see is well that has everything in it that this one does except the cancer cells. Would the blood vessel grow towards the cancer cell, would it grow over there? You can see the blood vessels over here, but what's happening over here, and you gotcha. They all grow on one side. They grow over to the tumor, they don't grow the other way. So, the tumor can now say two things. One, start growing. Two, grow towards me. A directionality of vessel growth. But what can octreotide do in the presence of tumor. Well, this is the slide that you saw before, but with some revisions. No tumor, the control, (tape reverses, some text dropped) . . . is they got there before the octreotide are growing, and they are growing at a rate that God predetermined and that, taking the tumor out isn't going to help. So if you leave one cancer cell, even though you've got octreotide on board, doesn't help. So you would hope that there could be something that would slow down blood vessel growth. Octreotide will prevent the recruitment of new blood vessels but not the old ones.

So, what does the blood vessel say to the tumor? What's the other side of the coin? And the answer is, I don't know. But we're working on that. Same kind of model, it appears that we can take a blood vessel and put it in this system and we can actually see the tumor cells starting to grow towards a well that has a blood vessel in it but it won't grow to the control well. So there is a cross talk between blood vessels and tumor cells and they both want one another to get together. It's sort of like two teenagers, boy-girl, somehow they figure out, no matter what the parents say, how to get together. Ask me, I've got 18, 17 and 16 year olds. So, you remember my cheeapets? This is the blood vessel again and here are the new blood vessels growing out into the culture. Well, remember I asked the question, could you show that arresting blood vessel didn't have Somatostatin receptors and new blood vessels did. And this is, I apologize, impossible to see, but this is the resting blood vessel and these are the cells here that are growing into the fibrin clot. This part here, as we come to find out, not all the blood vessel wakes up. The only part of the blood vessel that wakes up is the part that we traumatized by cutting right along here. The edge of the blood vessel. This is just what is called a hemotoxin and eason stain, this is to give you sort of an idea if we had a room without light, where we are. But now Dr. Otorizio, Dr. Tom and Sue Otorizio, and a gentleman working in Sue's lab named Dr. Doug Bolster, have developed an antibody towards Somatostatin receptor sub-type 2. And almost all the stuff you're hearing me and Larry and Dr. Warner, Monica, talk about today about the actions of Somatostatin have to do with this sub-type 2 receptor. So, this antibody you can actually now do stains to show where that Somatostatin receptor is. This is our, what we call, just our control, and you can see here the antibody stain is dark and you can see right on the cut edge the development of Somatostatin receptor sub-type 2. And these are the blood vessels out in the fibrin clot and they all have Somatostatin receptor sub-type 2. This is at a low power, this is at a high power. Again, right on the cut edge, where you induced trauma or hypoxia, etc., you can see the development of Somatostatin receptor sub-type 2, and these are the blood vessels out in that

fibrin clot with the expression of the Somatostatin receptor.

Well, if that's the case, you can show the receptor is a protein and you develop antibodies to proteins. But what about genes? Genes are where it's at. They're at the beginning. Remember, I told you Somatostatin went to the nucleus and bound to DNA, What DNA made out of? Genes. So, one of the things that we thought about is, now we have the blood vessel. The minute we take it out of the patient we can freeze it. And the little punches that we did will now grow. So, out of every individual in this room, I could take a piece of your normal blood vessel and your growing blood vessel and look at genes and the resting blood vessel and the proliferating blood vessel and figure out what genes changed during the angiogenic switch. And so what we did is something O.J. Simpson PCR, preliminary chain reactions, and this is the normal blood vessel. These are molecular weight markers in a gel, notice there is no band over here like there is here. No gene for Somatostatin receptor sub-type 2, but in the angiogenic blood vessel there is a gene for the Somatostatin receptor sub-type 2. So at a gene level there is a gene that is getting switched on that tells start making Somatostatin receptor sub-type 2 and that gene is expressed. It makes the Somatostatin receptor sub-type 2 protein, as we showed you with the other slide.

Well, this is the concept that Larry was talking about. OctreoScan, octreother, somatother, and there are going to be hundreds of these coming out, is Somatostatin plugs into a receptor. It is very much like a lamp. You plug the plug into the wall, the lamp comes on. Nice part about a lamp is you can put in a six watt light bulb, you can put in a sixty watt light bulb, you can put a six billion watt light bulb. A six watt light bulb we're now using with a hand-held Geiger counter called a neoprobe or gamaprobe. It's about the size of my laser pointer. And we can take it in the operating room. We give the patient radioactive Somatostatin, we let it clear the background, and now the tumor glows. But it has so little radioactivity that if I'm just a little teeny tiny bit away, my probe won't find it. Give you an example. Let's say my middle knuckle here is a tumor cell. And I come along, beep, beep, beep, beep. I know right where the tumor is. And that's called probe directed surgery. And we're doing that with Indium Pentetreotide, we have our own product known as I125 landreotide and that's been published that you can find tumors that are so small that you can't find by any conventional way. If you go to a regular light bulb, you get an external scan. You saw beautiful pictures that Dr. Kvols showed you of OctreoScans. That's sort of the concept of the 60 watt light bulb. But if you put the sun, a six billion watt light bulb, plugged into the lamp, now when you turn it on it cooks everything in the room, and that's the idea of Somatother, octreother and all the other therapies.

So, and this is again a rip-off of Dr. Kvols' slide, with the circles, this is Octreotide, this is the linker that he was talking about, DTPA, and this is the Indium 111 that gives off the radioactivity, and that's OctreoScan. Boy, I wish this showed. Okay. So the question would be, could you put a tumor in an animal and know that the tumor didn't have Somatostatin receptors. And we can do that. We can now stain them, we can do binding experiments with radioactivity, and we can look for the genes. So we picked a cell line that doesn't have Somatostatin receptors. And we implanted those cells on the butt of a mouse and then we injected the mouse with radioactive Somatostatin analogue that looked for sub-type 2 receptors. And, believe me, this blank screen here, this is a mouse right here. And this mouse is laying down belly down on a piece of photographic film, and right here is his liver. And this dark spot that you can't see here is his liver, how much radioactivity is his liver. This is a living, breathing, genuine mouse and what we do is we cut the thumb, end of the thumb of a rubber glove out, put a piece of cheese there, and get him to run into the thumb of the glove, and sort of get trapped. So now we can turn him over on his back so he's now laying with his little feet up in the air and this is now the mouse here and this area right here that you can't see is the radioactivity going to a tumor that doesn't have a Somatostatin receptor in any of its cells. Where is the Somatostatin receptor? It's on the blood vessels that are feeding this tumor. So the cells don't have to have a Somatostatin receptor, the blood vessels can have a Somatostatin receptor. Well, if you can take radiation to a tumor and the blood vessel, you'd better know what radiation does to blood vessel growth. Remember, I showed you that Octreotide and a bunch of drugs that we've tested block initiation, block resting going to growing. So, when we asked this question, what does radiation do, you can bet that my bet was on initiation. And the answer to that question is wrong again, fat boy. No matter how much you gave, none or all the way up to 5000 centigray or rads, which is what we give to a breast cancer or a lung cancer or whatever, none of those doses changed going from resting to growing. Uh, oh. Now I'm really worried. So the bet in the lab is now, what

does it do to the rate of blood vessel growth. We've never seen anything to this point that affects the rate of blood vessel growth. Remember that's what scared the holy bejeebles out of me. So, the next experiment was the rate of blood vessel growth as we gave more radiation, the blood vessels grew less and less quickly. So now we have two things. We have Somatostatin analogues over here that block the switch, and now we have radiation that slows down the blood vessel growth once the switch has happened. What would happen if you put the two concepts together, put the Somatostatin and the radiation together. Well, first thing you need to know is that we wanted to ask the effect of the Indium Pentetreotide, the OctreoScan, and these are neuroendocrine cells here. And what we saw was, is that, yes, we could get internalization, but that the longer you exposed a cell to Indium Pentetreotide, the more bound to that cell and more went inside the cell. Remember, OctreoScan, everybody in the room has had one, you come in, they inject it in your arm, they pat you on the head, they scan you a few hours later, and come back tomorrow. What if you infused that, continuously exposed a cell to Somatostatin analogues. You would progressively increase the amount of radioactivity you could put inside of a cell. Remember, Dr. Kvols said that the radioactivity went to the garbage disposer, the lysesone. Well, this is to show you a different, maybe, perspective of this. Here we take cells, we expose them for various times, this is Indium Pentetreotide, OctreoScan, for various periods of time, and then we blow the cell apart and put it on a, what's called a density gradient. The heavy things go to the bottom, light things stay on the top. Needless to say, not to bore you, look for the peaks over here and the peaks over here. This is the plasma membrane, the plastic of a beach ball. This is the inside, the brains, the nucleus, over here. At one hour you see it starting to bind to the membrane. By four hours you are starting to get message over here in the nucleus, and by 24 hours all of it is there at the nucleus. That's how it's controlling gene expression, that's how, and I'll show you in a minute, can control the genes for things other than itself. And, yes, you can block this, if you add plain old Octreotide Sandostatin and you then give OctreoScan when you add the Octreotide, nothing happens. And this is why we try to get people off of their Octreotide before doing their scans or their therapies. But, sort of being a surgeon, I believe that nothing happens just willy nilly. You wouldn't take a peptide to a nucleus just so it could have a vacation in the nucleus. It has to do something. So, when it goes into the nucleus, it makes sense that it would go to DNA. The red line is just the Indium Pentetreotide. The yellow line is Indium Pentetreotide plus octreotide. And the green line is what's called specific binding to the DNA of IMR 32 cells over time. This is one day. Remember, it took a day to get to the nucleus. But it's not really in the DNA yet. It takes another day to get to the DNA. If you take cells, notice the counts here are about 1000 up here, if you take cells without the receptor, what happens? And the answer is, look, the green line, 50 counts. So, basically, nothing. Comparing it another way, these are the receptor positive cells, these are receptor negative cells, and the boys in my lab who always hold your feet to the fire and they say it's not binding to a gene, Gene, it's binding to things called istones, it's just binding to the protein. So, if you throw detergent on it or you throw a protein destroying enzyme called proteays on it, you'll just wash it right off. Well, the answer is, for once in my life, I was right and you can't wash it off. It's very, very sticky and binding to that sequence of DNA.

Now, could you in the human blood vessel model, show that the Indium Pentetreotide could kill blood vessels? Well, first thing you learn is, again, every time you set out to learn something, you learn a couple of things. And here's the percent initiation in our control group that just has serum. Here's our group with Indium Pentetreotide, much nice blocking of initiation, but interestingly enough, here was Indium Chloride by itself. This is the radioactive thing, not hooked to Somatostatin, and it appears that even if the Indium doesn't get inside the cell, it can have an effect on growing blood vessels. If you look at the growth rate in millimeters per day, Indium Pentetreotide is different than the control, but even Indium Chloride will have an effect on the growth rate. And if you look at the total vessel area, which is done by something called digital image analysis that looks and draws a line around that whole thing, Pentetreotide is very effective at controlling the angiogenic response. But even the gamma rays given off by Indium, in addition to the ogeays, have a cytotoxic effect. It may be why, the argument for using Indium Pentetreotide as a therapeutic and the higher energy beta Octreother is that the Octreother can kill an innocent bystander cell, a cell that might not have the receptor. That's both the good news and the bad news. The innocent bystander might be another cancer cell that doesn't have the receptor, it might be a spinal cord cell, it might be a kidney cell, it might be any good cell as well. So there's no free lunch. When you increase the potency of the radiation's effect, you also have the potential for doing damage. Whereas, the ogeay electron that only works withinside the cell, the good news, it doesn't kill anything outside that

receptor-bearing cell. But, if one of your tumor cells doesn't have the receptor, it also doesn't kill it. But this is the first time in these experiments where we're seeing that the gamma energy from Indium might buy you a little bit of innocent bystander effect, although not nearly as much as Dr. Kvols' betas.

Now, something that our lab is just now starting to work on, and this has relevance to how we give octreotide normally, Sandostatin in the old days, three shots a day versus the LAR. In the old days, a year ago, or two years ago in our laboratory, we thought that binding to one of these neuroendocrine tumor cells was dependent on two things—temperature. If you chilled the cells down to four degrees, the stuff would bind to the receptor on the surface of the cell but it wouldn't go inside. Because you would shut down the machinery of the cell. We knew that it was concentration-dependent. The more radioactivity, the more drug you give to the cell, the more binds. But we hadn't put into the equation is the time factor. So what we did is design this series of experiments where we varied two things—we varied the time and we varied the concentration. The question was, if you gave a little bit of drug all the time for a very long period of time, was it the same as giving a boatload of drug for a short period of time. Concept: back to basic math. Drug concentration times time might be what, remember, boy this is a long time ago, a constant. In other words, a little bit of drug for a long time is the same as a boatload of drug for a little bit of time. So, if you had hundreds of hours, you could use a little bit of drug for a hundred of hours or you could use hundreds of drugs for just one hour. So, what we did, and again it doesn't show up very well, is down here are the product, what are called milicuri hours. Goes from 190 milicuri hours all the way up to 12,000 milicuri hours. And over here is the number of cells killed. And so what we did was, we varied things going from, like 32 microcuris for as low as six hours all the way up to 512 milicuris, a big radiation dose, for three hours. And what you see is, while there's some variability in each group, the white line represents the mean. And what you see is, is there is a very linear function here that says that cell kill is a product of the amount of milicuris that you have exposing that cell's surface times the number of hours that it is exposed. So that when we are now doing some of our high dose radio labeled Somatostatin analogue therapy, we're looking at can you infuse that to keep your blood levels very very constant over time and be able to use less drug, or make the drug more effective, than had you given that same dose by just a bolus injection and the body clears it very rapidly.

Finally, I don't know, Bill, could we tilt the projector? You've seen this slide at another forum before and this is Dr. Kvols' slide with the 5FU and the streptozotocin and the streptozotocin cytoxin, and this is Dr. Kvols' line with the octreotide acetate in patients with carcinoid. Dr. Lowell Anthony, Dr. Kevin McCarthy, Mr. Greg Espanan and myself have now done 30-some patients with high dose Indium Pentetreotide therapy and these patients, like Dr. Kvols had alluded to, were part of a pilot or phase one trial. These patients, the FDA required us to take patients who had failed all conventional therapy. They had failed octreotide acetate therapy, many of them had failed chemotherapy, many of them had had bones or whatever radiated, etc. And they had to have, by an outside physician's assessment, less than six months to live. And you see the light blue line is the survival of our patients treated with at least 280 milicuri doses or about 60 times a scanning dose, twice. And our median survival is about 18 months. While it's not as good as Dr. Kvols' line with octreotide, remember our line really starts where his line leaves off. So, these are people that we thought would live around six months that are averaging about 18 months of survival at a dose of 180. We have now increased our dose to 360 and then we've increased our dose to 540 milicuris. The good news is, is that none of these people have experienced any significant toxicity. No hair loss, no nausea, no vomiting, no diarrhea, none of the associated things that you would see with high dose chemotherapy and the other good news is we've seen nobody, and we've now given over in the world, Dr. Kretting has given over four curis, we have given over three curis of activity with no effect on the kidney, which is the big worry with the higher dose betas. So acutely or chronically, no toxicity and very nice survival data in this group of heavily pre-treated patients.

Well, I'm going to leave you with one last very quick series of thoughts, and that is on growth factors. We talked about them. Growth factors are one of the critical elements for tumor growth and this is the chicken/egg model, and the growth factor we were looking for is called epithelial growth factor or EGF. And this is work done by Jan Hess of the Children's Hospital in Seattle in cooperation with our lab. This is the disk that contained no octreotide. When you increased it to 25 micrograms per disk, the EGF level markedly decreased and when you went to 50 micrograms a disk, there was

nothing. Although these eggs were still alive and the amount of protein in these disks measured to be the same as all these. What's incredible, and this I might as well not show, is this line which basically is a control octreotide estrogen or octreotide plus estrogen in a group of breast cancer cells. This is the marker for a what's called a housekeeping gene. And what you can't see here is that there is an EGF message here, there is no EGF message here, there is an EGF message here, and one here for the gene level. So octreotide can also control other genes other than Somatostatin receptors in these cells.

Thank you very much. I look forward to talking to you all in the future. As you know, anybody can call me any old time.