New Concepts in the Treatment of Carcinoid Tumors: What's New, What's Not, and What's Hot

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INTRODUCTION:

Dr. Woltering is currently the James D. Rives Professor of Surgery and Neuroscience at Louisiana State University Health Sciences Center, the Chief of the Sections of Surgical Endocrinology and Surgical Oncology, and the Director of Surgery Research at Louisiana State University. Please help me to welcome Dr. Eugene A. Woltering.

Dr. Eugene Woltering:

For those of you who know me and for those who don't, I can only tell you what a great pleasure it has been to be associated with the California Carcinoid Fighters as a member of their board for the last year or so, and to help put together this conference. You have no idea how many hours the local people have spent putting together this conference, and my hat's off to them. Hopefully, you all will be getting information about Carcinoid 2004 that we're going to put on in New Orleans next year. The conference will be held September 23, 24, and 25, 2004.

Somatostatin is one of the key regulatory peptides in our bodies. You've heard about it today from other speakers. It's made by your body every second of every minute of every day. Native somatostatin is a 14-amino-acid peptide [slide], and when God designed this, he designed it very well. You eat, your blood sugar goes up, you release insulin, you use up your blood sugar, poof - your insulin has to go down. What turns off insulin? What turns off about anything that has to do with digestion? Native somatostatin. The nice part about it is because you make it every second of every day, it can have a very, very short half-life in the blood. Its half-life, which means the time it takes for half of it to be gone, is a couple of minutes. Perfect -- if your body makes it on an ongoing basis, but it's crummy as a drug because of its short half-life. You would have to use it as a continuous drip.

This [slide] is octreotide acetate, and it was the native somatostatin molecule that they cut the middle out of and made into a shortened form of somatostatin. The octreotide is about one hundred times more potent than native somatostatin, but what's really important about octreotide is that this drug has a half-life in the blood of several hours versus several minutes. Now, while we've talked about octreotide today, there are other somatostatin analogs coming onto the market in Europe or already available in Europe. This drug is known as vapreotide [same slide]. There is another octapeptide analog known as lanreotide. But all of them have, as a critical theme, these two amino acids in the site where the molecule binds to the receptor. If you substitute one amino acid in this binding site, you screw up the whole mess. If you make these changes the drug doesn't bind to the receptor, and it doesn't work. Somatostatin binds to a receptor, that receptor sends a signal to what's called an "effector". Indeed, that effector is called a G-protein, This G-protein then sends

intracellular signals that tell the cell to make or not make peptides through the release of secretory vessels. This is all old news.

This [slide] is what a somatostatin receptor actually looks like. It's part of a family of receptors in which the receptor protein winds back and forth through the cell membrane seven different times, and those areas are called the seven different transmembrane domains. The somatostatin receptor lives right down in this domain. While many of you take somatostatin predominantly for the control of symptoms, there is a building body of evidence that started in the early 1990s that says somatostatin does a lot more than just make your flushing and diarrhea and wheezing go away. Somatostatin blocks peptide release, it blocks tumor growth, it blocks angiogenesis (the growth of new blood vessels into tumors), and blocks the release of growth factors into the circulation that make a variety of tumors and blood vessels [on slide] grow faster.

One very common question that I'm often asked is what kind of data do we have on the effect of octreotide on tumor growth? We all know it makes us feel better, but so what? There are two sides of this coin. One, we want to feel better. Two, we want to live longer. In the late 1980s there was a NIH/National Cancer Institute consensus conference held which explored the effect of octreotide used at very, very low doses, on the percent of patient's tumors that became smaller, didn't grow (tumor stasis), or actually got larger. At this conference the oncologists looked at this bottom number - 13% of the tumors got smaller - and said "Ho hum, not a very good cancer drug", and all of them went home. But if you add these two columns up, 13% of them got smaller and 63% of them stopped growing, that's a pretty positive effect on tumor. But nobody knew how this worked. Remember, this is a drug that was designed predominantly to control diarrhea. Then a guy came along by the name of Dr. Larry Kvols. Dr. Kvols did a study at the Mayo Clinic where he looked at 66 patients treated with octreotide, and compared their survival to the survival of people treated with drugs that were commonly used back then to treat neuroendocrine tumors, streptozotocin 5-FU, and streptozotocin cytoxan. What he showed is that people on octreotide lived three times longer than those people treated with chemotherapy, and that was a pretty revolutionary concept back in the early 1990s. Again, nobody had a clue about how octreotide was lengthening people's lives.

Then our laboratory, along with Dr. Thomas M. O'Dorisio, started looking at the effect of octreotide on the growth of blood vessels. This [slide] is a fertilized chicken egg. This is a primary blood vessel, this is a secondary blood vessel, this is a tertiary blood vessel, and this fluffy gray stuff in the background is quaternary vessels. That's really important because in a tumor, the primary and secondary vessels belong to "the patient", and the tertiary and quaternary vessels belong to "the tumor". This experiment uses a small disc of methylcellulose that you can put drugs in. This disk slowly dissolves and releases the drug. This one is a control disk. The blood vessels around it grow normally. You can see right through these discs to see what happens to blood vessels. If, on the other hand, you put a disc containing the somatostatin analog octreotide on the surface of the egg then blood vessel development stops in the area under and surrounding the disk. Here is another picture where the disc is a little bit more visible; you can see that you have the complete loss of blood vessels in the area around the disc. This means that octreotide at least does two different things. One, it controls peptide release, and two, it controls, or stops, blood vessel growth. That is really important because without new blood vessels, tumors just stop growing. They don't necessarily regress. It's sort of like Dr. O'Dorisio talked about with diabetes; as long as you take care of your diabetes, you don't have to "cure" it to live a long life.

If we then look at the effect of different somatostatin analogs on their ability to inhibit angiogenesis, this line on the graph [slide] represents the antiangiogenic effect of octreotide, and this line is vapreotide (RC 160). You can see that they both are very potent antiangiogenics. At that time, the most potent combination of antiangiogenics known was a combination of a steroid and heparin. This combination of antiangiogenics was popularized by Dr. Judah Folkman, the guru of all gurus of angiogenesis. If you remember that first picture that I showed you, if you change one amino acid in octreotide, that's this compound right here - STZ-204-354 - this doesn't inhibit angiogenesis at all. This [slide] line is the effect of native somatostatin showing it's far less effective than the somatostatin analogs.

It turns out that there are a whole bunch of experimental angiogenesis inhibitors and we'll show you a lot of those today. One of the first things we did was we looked at a bunch of different

somatostatin analogs and compared their ability to inhibit growth hormone - (because we knew that it was the somatostatin receptor subtype 2 that was responsible for the inhibition of growth hormone release) - versus their ability to inhibit angiogenesis. You can see that it's almost a straight line; the greater the ability of the analog to block peptide release, the more potent the drug was in inhibiting angiogenesis. We then compared, using about 21,000 chicken eggs, the effect of a variety of compounds. Most of these you may recognize as chemotherapies: interferon, methotrexate, vinblastine, VP-16, mitoxantrone, and here's Dr. Folkman's most potent angiogenesis inhibitor combination that he'd come up with, there's octreotide. Now what's cool about octreotide is that unlike all these other drugs, there are no significant side effects or significant toxicity. Well, for an angiogenesis inhibitor to work, you have to be able to take it like insulin in a diabetic, every day for the rest of your life. Octreotide is a very non-toxic drug. People have made a mistake and given 50,000 times the recommended dose of octreotide with no loss of life and no significant side effects.

Octreotide is a funny drug; its effect on things isn't necessarily linear. Dr. O'Dorisio alluded to that earlier. These drugs work in a very narrow therapeutic window. More is not necessarily better. This [slide] is an example of work done by Dr. Roberto Danesi out of Italy showing the effect of octreotide on the proliferation of the cells that make up the lining of blood vessels called endothelial cells. As you can see, the drug starts to work as you increase the amount of drug. Its optimal effect is in this small area between 10-8 and 10-9 M. If you use more drug than 10-8 M, you actually lose the drug's effect. So more is not necessarily better. The trouble was that, at that point, there was no human tissue-based angiogenesis assay. It's important if you're a mother chicken that your eggs grow properly, but it doesn't make a lot of difference to you or me. What does make a lot of difference to you or me is what happens when you use a drug on genuine human tissue. So, our laboratory designed a human angiogenesis assay. Basically, what we did was we got placentas at the time the mother delivered them. If mom's okay and baby's okay, they throw the placentas away. They'd throw them in the garbage; we got them out of the garbage, dusted them off, and cleaned them up. We harvested their blood vessels, took small sections of blood vessels and embedded them into fibrin-thrombin clots. For those of you who don't know about fibrin and thrombin, imagine a blood clot without any blood cells in it. Sure enough, out of the cut edge, of these vessels started to grow new human blood vessels. This [slide] is an example: this center area is a 2 mm in diameter disk of human placental vein. You can see all these fuzzy things growing out are new blood vessels. The guys in my lab call these my "Chia Pets".

How does a blood vessel grow? Well, it turns out because nobody's really grown real human blood vessels before. Nobody really knew the answer to this, and it's sort of cool. If you look at the part that's growing way out on the tip of these, there are solid cords of these endothelial cells. As they start to mature, they develop a bunch of little fat globules called lipid vacuoles. Those, as they mature even more, these lipid vacuoles coalesce to form a lumen. So, you can actually follow each step of blood vessel development in our assay. Using this assay, we went back and we looked at the effect of octreotide acetate. We again wanted something to compare it to, so we went back to Dr. Folkman and he gave us a concentration of his heparin-steroid (the green line) [slide] to try. Unfortunately, this concentration of his drugs didn't work. So, we went up ten times more that what he recommended (the orange line); it didn't work either. Finally, we went up one hundred times his original recommendation, and that blocked angiogenesis. We then used octreotide in this model, and interestingly enough, from Day 0 - 6, wells began to become angiogenic, and then just stopped developing any more wells with blood vessel growth. Boy, this really put our laboratory on its ear. Why would it be that a drug doesn't work, and then all of a sudden starts working? The answer is: what if there are no somatostatin receptors present on the normal vessel when we put it in culture and then as blood vessels begin to grow, they develop somatostatin receptors? Could we prove that? Yes, we could. We took blood vessels from about thirty different placentas. We harvested a piece of the vein and immediately put it in a special solution so we could harvest its RNA. We then took the rest of that vein and put it in culture and made it angiogenic. Later, we harvested those wells with angiogenic blood vessels. The wells that had growing blood vessels had somatostatin receptor subtype 2, whereas the native tissue it came from did not have somatostatin receptor subtype 2. That means that in your body, normal blood vessels in your toes don't have somatostatin receptors. But, every blood vessel in your tumor does have somatostatin receptors.

This work [slide] represents work done by Drs. Douglas Balster, Thomas M. O'Dorisio and Sue O'Dorisio in collaboration with our laboratory. Tom and Sue developed an antibody specifically for

somatostatin receptor subtype 2. This [slide] is the normal blood vessel; there is no dark staining here. But the angiogenic blood vessels, or the growing blood vessels, have somatostatin receptor not only on the outside of their surface as everybody already knew, but in the nucleus of the cells, as well. This [slide] is another picture at a lower power; this center section of the well is the normal part of the blood vessel. This is where it's cut into a disc. That's where the angiogenesis occurs, or the new blood vessels start to grow. These are the new blood vessels growing out into the clot, and you can see that they all have somatostatin receptors. This is really good news because this result says that not only can your tumor cells be a target of a somatostatin therapy, but the vessels feeding your tumor can also be a target of this therapy.

That gave us the opportunity to ask some other questions. One of the questions, pretty logically, is "what does a tumor say to a blood vessel"? Does it say, "grow my way"; does it say, "start to grow"; does it say "grow faster"? We could ask some of those questions by looking at putting tumor monolayers on top of our cultured blood vessels or leaving some of them without added tumors. When you add tumor cells to the angiogenesis model, you can see that the number of wells that "initiate" an angiogenic response goes up. So, the message that a tumor sends to a blood vessel is "start to grow".

I've been known to bet somebody a bottle of wine, a case of wine, the deed to my house, on how much I believe in something. The question then was, does a tumor-stimulated vessel grow faster than a plain old blood vessel? And I would've bet you the deed to my house, or certainly given you all my children, if I was wrong. And I would've been wrong because a tumor-stimulated blood vessel grows at exactly the same rate as a non-tumor-stimulated blood vessel. This is really critical because if the message to "start to grow" is sent to the blood vessels, then you take the tumor out it doesn't make any difference. The chemical messages that say "start to grow" have already been sent.

If you look at the effect of octreotide, and this set of data [slide] is in the "no tumor" model, then the percent of wells that started to grow went down as you increased the octreotide concentration. When you added tumor, the number of wells that started to grow went up significantly. And, again, there was a dose response to octreotide in the presence or in the absence of tumor. Again, this is the percent of wells that started to develop. If you look at the rate of vessel growth in "tumor" or "no tumor", you can see that they all look the same.

Well, what about other things that you've heard of today? Dr. Öberg is world famous for his use of interferon. We all use interferon to some extent or another. One of the things that interferon has been used for is to treat what are called "port wine stains" in young children. Port wine stains are those big, red blotches that you see on young children. Interferon causes those to regress in many of the children treated with this drug. So, with the sponsorship of Schering-Plough, we looked at the effect of interferon on angiogenesis. These lines [slide] are the percent of wells that became angiogenic, and as you can see, here is heparin-steroid, and interferon does inhibit angiogenesis. It does so by telling the vessels the message "don't grow" or by inhibiting the message that says "start to grow". Now we asked the question of whether you could start to get some kind of angiogenic index or quantitative measure of growth in our assay. Basically, we do this by visually dividing the discs into four quadrants, looking at the amount of blood vessel growth around the disc, and giving the growing vessels a score of 0-16. Interferon, in a very dose-dependent fashion inhibits blood vessel growth. The more interferon you add, the better it is at stopping blood vessel growth. Octreotide only knocks out the initiation step of the angiogenic response, the beginning of growth. In contrast, interferon looks like it may have its best effect on vessel growth. Unfortunately, the 4,000 units/mL drug concentration, where it's the most active in our assay, is not achievable in a humans. But the 400 units/mL level used in our assay is the peak interferon level in most patients getting treated with interferon. So, interferon can not only act at an immunologic level, but it can also help stop blood vessel growth.

I'm going to now take you on a journey that's been going on in our laboratories for the last 3 or 4 years. Because the title of my talk is What's New, What's Hot and What's Not, maybe some of you need to know what doesn't work in carcinoid. As you know, probably one of the hottest tickets in the angiogenesis world is work done by Dr. Judah Folkman on two compounds, one of which is called endostatin and the other one is called angiostatin. I won't go into a lot of detail, but I will just show you [slide] --- this data is the angiogenesis in our control and this data is the effect of our "old

fashioned" heparin-steroid inhibitor. This data represents the effect of endostatin at incredibly high doses. This data shows that endostatin doesn't work on initiation at clinically relevant doses, and it also doesn't work on subsequent blood vessel growth. Interestingly, if you haven't heard, the company that makes endostatin has now has now shut down production of both endostatin and angiostatin, and those drug trials will cease and probably never happen again.

One of the other things that we looked at in our human angiogenesis assay was angiostatin. EntreMed gave both endostatin and angiostatin to us out of clinical batches that were used in clinical trials. As you can see, angiostatin, at a dose that's even higher that we used in the endostatin trial (10-4 M) doesn't inhibit angiogenesis and has no effect on the subsequent vessel growth. It has basically no effect on angiogenesis at all. So, one of the answers to the question, "What's hot and what's not?" is, what's not hot right now are angiostatin and endostatin. That's a game that's been called on account of rain.

One thing that you'll hear a lot about is molecules that inhibit tubulin. As you remember from basic biology, as a cell splits during cell division, all of the chromosomes line up in the middle and they have to be ripped apart. One set of chromosomes goes to one cell, and the other set of chromosomes goes to the other cell. Well, what pulls those little suckers apart are things called microtubules. It's sort of like a car antenna that goes up and down. Think about it like that. These drugs either stop the antenna from going up or, once the antenna is up, it's like supergluing it in place - they can't retract. There are a lot of different kinds of tubulin binding molecules, one of which is called Taxol. You may have heard of it; it's a commonly used chemotherapeutic against breast cancer.

There is a clinical trial that was just completed using a product from Novartis called Epothilone-B. Epothilone is another one of the tubulin inhibitors that block the polymerization of these microtubules. In the early clinical trials, peak plasma levels were about 7 nanograms/mg of administered dose, which meant that the blood levels were about 25 nanograms/mg (5 x 10-8 M). What we're going to show you today is that a really effective angiogenic effect happens at drug doses that are about 1/5th of that peak achievable dose. This is a comparison of the effect of different epothilone doses in our human tumor angiogenesis assay. Not only can we take blood vessels from placentas, but now you can send us a piece of your tumor tissue at the time of surgery on wet ice, and we can now look at the effect of drugs on how many blood vessels grow in the control wells versus how many grow at epothilone doses of 1 x 10-8 M. And as you can see, epothilone is a very potent antiangiogenics. This, remember, is at a dose that is about five times lower than the peak plasma level that you would've achieved in the clinical trial. This slide represents the effect of 1 x 10-8 M Epothilone B on the growth of angiogenic vessels from human tumors. Again, this agent (in our assay) was very, very effective in several midgut carcinoids, in a thymic carcinoid, in a liver metastasis from an ileal carcinoid, in a breast cancer, in a kidney cancer, and also in our human placental vein model.

Let's give you some individual patient responses [slide]. These are patients who were entered into our clinical trial and we had surgical specimens from them. As you can see here, these are our controls. These bars represent data from low doses of epothilone. It is not effective at these very low doses. However, when you get to the clinically relevant dose (1 x 10-8 M) of epothilone, the percent of wells that begin to grow is totally blocked. This is the growth data form the same patient: not only is the initiation of the angiogenic response from those wells totally blocked, but since they don't initiate an angiogenic response it makes sense that they also don't grow. This slide represents data from another patient, this one with metastatic ileal carcinoid in the liver. Again, this is their actual plasma chromogranin-A level measured during the clinical trial. You can see that something interesting happens, and that is the chromogranin-A level goes up initially. At that point, everybody in this room would be going, "Get me the hell off of this drug!". Well, this is a phenomenon seen in antiangiogenic agents called unpacking. Imaging that you just peeled the outer layer off of the onion. So, what you see initially is that we unpack the tumor, allowing the tumor to get a spurt of growth as you're starting to kill the blood vessels. This occurs because the pressure in the center of an untreated tumor is high. This blocks blood flow and the delivery of effective antitumor agents to the center of the tumor. Antiangiogenic therapy initially allows the blood to reach the center of the tumor and this may cause an initial growth spurt in the tumor. After a while, when the blood vessels feeding the tumor begin to regress, the tumor either shrinks or becomes stable in size. Then the

epothilone progressively brings down the chromogranin A level over time. This is the difference between a chemotherapy drug that kills tumor cells and an agent that kills blood vessels. Agents that kill blood vessels may take awhile to demonstrate their effect. During this time you may have a transient increase in tumor growth or elevations in your tumor markers until that drug effect kicks in. Another slide representing data from a patient treated with Epothilone-B: this patient has a pancreatic neuroendocrine tumor. This is a guy who was literally on death's doorstep and who ascites (fluid in his belly). He made me look like a skinny dude, and those of you who know me know that I am anything thing but skinny! He had to have the fluid removed from his abdomen by a needle tap several times a week. When he was started on Epothilone-B, he was out of work and was on medical disability. Very shortly after beginning therapy with Epo-B, his pancreatic polypeptide dropped, his ascites totally went away, and he was back to work working full time. As you can see, again, this patient had the "unpacking phenomena", showed a transient little 'blip' in his marker levels, and then the levels went back down and stayed down. This patient response is still durable at 18 months of therapy.

Now we're going to look at work done on the effect of Epothilone-B on patient markers by Dr. Lowell Anthony and his clinical research nurse, Ms. Michelle Cronin. On this slide, I put all the markers together whether they were pancreastatin, whether they were chromogranin or 5-HIAA, and started them out at 100% of the "before treatment value". You can see that this patient [slide] clearly failed epothilone therapy. You see that this patient's markers went up and then started to come down. You have other patients that go up, start to come down, etc., but many of these markers if they don't go down, at least don't go up. Again, angiogenesis inhibitors can just keep your tumor from growing. Effective antiangiogenics don't have to shrink the tumor. Rather, they can just keep things in the status quo. I also looked at that data a different way. For a marker to represent a "positive result", we want it to drop by 50%. If the therapy is a failure, we want the maker value to rise by 100%. You see most patients represented on this slide have stable marker levels. You have a few patients whose marker levels go up. This one is a clear-cut failure. But here in this patient the marker levels go up initially. We would have considered them a treatment failure. After 12 months of therapy, the markers drop dramatically. Another thing that you need to know about angiogenic agents --- they're not at all like chemotherapy. They take a long time to get rolling.

Back to the radiolabeled somatostatin analogs: there are a lot of ways to skin a cat, and what you want to do is get the most radiolabeled analog to the tumor. How do you do that? Well, we asked ourselves whether we could somehow change the tumor uptake of the drug? We believe that the number of receptors on a tumor's surface is predetermined by the big guy upstairs. Your tumor either has receptors or it doesn't, and I do not believe that you can "desensitize" or downregulate the number of receptors on a tumor. Work done by Miss Mary Cox, Miss Susan Hughey, Dr Cathy Anthony, Dr. Sue O'Dorisio and myself using real-time PCR shows that exposure (long-term or short-term) to low-dose or high-dose octreotide doesn't change receptor number. One way to increase radioactive somatostatin analog uptake is give a lot more drug. That would put a lot more drug on receptors. Unfortunately, this exposes the body to more radioactivity in places that you don't need or want it. Potentially, you could change how you gave the drug to increase its uptake, or to increase a drug's therapeutic effect you could put more "energy" on the radiolabeled peptide. There have been many studies done with radiolabeled octreotide analogs. Many of you have heard these studies. The radiolabel we put on the somatostatin analog backbone can be anything from 90Yttrium to 177Lutetium to 111Indium. We put it on the somatostatin, and the somatostatin plugs into the receptor. You can use very, very low doses of radiolabeled somatostatin analog intraoperatively to help you find a tumor. You can use medium-sized doses like with OctreoScans to "see" a tumor on nuclear medicine scans. Or, you can "crank it up to notches unknown", to quote Emeril, and use it as a therapeutic agent.

In 1971, Dr. Sutherland got the Nobel Prize for his demonstration of how peptides and amines work. What he said was that as the blood level of a peptide or an amine goes up, the peptide or amine hops onto the receptor. This makes pretty good sense, right? The more drug in the blood the more likely for a molecule to "light" on a receptor. On the other hand, as your peptide levels fall, there is a movement of the peptide off of the receptor. This also makes sense. For example, if you eat three times a day, your insulin level goes up after each meal in response to increasing blood sugar levels. Your insulin level goes down, up, down, up, down to match your blood sugar levels. But, there's something funny about this curve. It's the part you never think about. If I bring you into the hospital

and plug you in to an intravenous drip of salt water without any glucose in it and I measure your insulin level from now until the day the cows come home, your insulin level will never go to zero. Your somatostatin level will not go to zero. Your pancreatic polypeptide level won't go to zero. Why would the big guy upstairs have us make peptides day in, day out, seven days a week if they weren't doing something? So, we asked the question, "Is there a difference in the way peptides are handled if they're by constant infusion versus bolus?"

A lot of people here are on LAR. LAR is a constant infusion where the aqueous Sandostatin is the saw-toothed portion of this curve. What happens when you constantly expose a receptor to a peptide is that it acts like a revolving door. It keeps putting peptides inside the cell. And those peptides that make it into the cell don't just get chopped up by the lysosomes, they actually translocate to the nucleus. How did we prove this? Well, we took our old friend OctreoScan, which is octreotide, a chelator (or a linker), and hooked it to 111Indium. Then, we exposed cells to that radioactive somatostatin analog. Now remember, for indium (since it emits such a low-energy particle) to kill a cell it has to be internalized in the cell. You're all thinking, "Wait. Tumors are heterogeneous." That means some of the cells have receptors, and some of the cells don't have receptors. But what about those angiogenic blood vessels? They all have somatostatin receptors - every last one of them. Therefore, the issue of tumor receptor heterogeneity is not nearly as big of an issue as you might first think. To prove this, we took cells and we exposed them for various times to the radiolabeled somatostatin analogs. We blew the cells apart and then we put them on a thing called a sucrose (sugar) gradient so we could take the light parts of the cell and separate them from the heavy parts of the cell. These over here [on slide] are plasma membranes - they are very light; they're full of fat. And over here in the heavy particles section is the nucleus. We can just count the radioactivity that is on the cell membrane. Four hours later, it's off of the cell membrane and it's starting to go into the nucleus. By 24 hours, the radioactivity has now transferred to the nucleus of the cell. This ain't the way it's supposed to work, guys. Sutherland would've never, ever predicted this.

Could you actually show a picture of a somatostatin analog getting in a nucleus? Dr. David Coy, myself, and Mr. Joseph Fuselier created a series of compounds known as JIC compounds. Joseph hooked a fluorescein tag on these JIC compounds, and we incubated cell in these drugs. This slide shows the collection of the drug in the nucleus. After 24 hours of incubation, you can see that the fluorescing somatostatin analog is completely in the nucleus of the cell.

Well, if you're in the nucleus of the cell, it's sort of like being in the brain of the cell. I would have to ask everybody in this room what a peptide would do in a nucleus. To me, I have a simple brain, so I would think it has something to do with DNA. I think, "nucleus and DNA". So, what we did was we harvested the DNA of cells exposed to the radioactive somatostatin analogs. We saw that the DNA was radioactive. Had you exposed the cells not only to the radioactive somatostatin analog and also to high levels of unlabeled somatostatin analog, you could compete the uptake of the radiolabeled somatostatin analog into the nucleus. Not only could you compete this process, but when you took this DNA and washed the DNA with things that split off proteins (detergents), it was so stuck that you couldn't get it off. This is a very interesting concept that we're working on in our lab. If, on the other hand, you took cells that didn't have somatostatin receptors and put indium-pentetreotide on them, nothing got in the nucleus and none of them died. This is really critical because when you hear the next speaker, Dr. Kvols, talk about yttrium and lutetium and all those things that have a higher energy, the reason that people are interested in them is because they want them to kill cells without receptors. But that could also be your kidney or your bone marrow! So if you use the higher energy radioactive labels there's no free lunch. They may kill cells without receptors, but those cells might be normal kidney cells or bone marrow cells.

Finally, we said if exposing a tumor to an infusion of a peptide was different than a bolus, could we prove that? This [slide] shows one lady with an atypical carcinoid of the lung. She was given a bolus of the OctreoScan material, and this is the radioactivity in her tumor. It takes up very quickly, and then it loses it fairly quickly. In contrast this slide shows the same lady's scan after180 mCi. That's about 30 OctreoScans all at one time. If, on the other hand, you infuse that same180 mCi dose in 60, 60 and 60 mCi fraction each over 24 hours, the amount that gets into her tumor (what we call the area under this curve) is higher. We found out that the optimal time to infuse the radiolabeled analogs looks to be about 18 to 24 hours. We give most of the drug up front, then a little bit more and a little bit more. You can increase this even more, and if you double the dose you can increase

the area under the curve even more. This is really critical. It may mean that LAR on a milligram per milligram basis is more effective at controlling tumor growth than the same number of milligrams given as 4 injections per day of the Sandostatin aqueous.

We then compared the effect of external beam radiation to the ability of indium-pentetreotide to kill cells. What we found was that to get really good cell kill with external beam radiation you get about 5000 cGy or 5000 rads to the cells. We now can actually tell how much indium-pentetreotide you need to have in your tumor to give you significant cell killing as well. This is important because radiation also affects blood vessel growth, but very differently than octreotide. Remember, octreotide blocked the percent of wells that initiated an angiogenic response. Different doses of external beam radiation going from 0 to 5000 rads does not change the percent of wells that initiate an angiogenic response. You know what I'm going to say next. My bet was that if it didn't have any effect on initiation I would bet you the deed to my house (again) that it also doesn't affect how fast blood vessels grow in culture. And, again, I would've lost the bet! Radiation slows down blood vessel growth. Somatostatin analogs block the message that says, "start to grow", and radiation doesn't say that. It says, "You started to grow, now you're going to grow slower". You can create tumors in nude mice that have somatostatin receptors on the tumor cells. Remember, all of the blood vessels that feed a tumor have somatostatin receptors. And what happens to tumors that have somatostatin receptors in our angiogenesis assay? Following treatment with the radiolabeled somatostatin analogs? You don't see any blood vessels following treatment, and the tumor also gets killed. Now, if you made a tumor that had no somatostatin receptors on the tumor cells, the blood vessels would still have somatostatin receptors on them to act as a potential therapeutic target. Again, when we made somatostatin subtype 2 receptor- deficient tumors in nude mice, the tumor didn't get killed following therapy, but the blood vessels got fried.

So how do we make up indium-pentetreotide as a therapeutic agent? A lot of people ask me this question. We buy a kit of OctreoScan from Mallinckrodt. We then get indium that is made as a radiochemical from a company called Iso-Tex in Texas. We have them put our extra indium on the kit and we then give doses of up to 600 mCi as a therapy. I have previously reported a pilot group of about 30 patients treated with high-dose OctreoScan in the past with a survival rate of about 18 months. Those people all had very advanced disease. Those were all treated at a dose of 180 mCi. Now our dose is up to about 500-600 mCi, and we've treated an additional 85 patients.

This [slide] shows work predominantly done by Dr Luis Linares at Memorial Medical Center, Mercy Campus, in New Orleans. The average age of those 85 patients was about 55 years old, and most of these people had widespread neuroendocrine tumors. There were 142 treatments administered: most patients received 1 treatment, 28 patients got 2 treatments, and 3 patients got 4 treatments. This [graphic] is the radiation reading emitted from the body over time. This determined how long people were in the hospital. Usually somewhere between Day 3 and Day 5, their radiation is cleared enough for them to go home.

This is actually a survival curve. When people talk about biochemical markers dropping by 50%, when they talk about partial tumor remissions, when they talk about things disappearing or reappearing on scans, that's all nice and good - we report the same thing. You have to ask, "Where does the rubber meet the road?" and that's survival. It's critical. In people who got one dose, two doses or three doses, our average survival in this group was about 22.7 months. What you see is sort of interesting: for those people who make it out beyond 22 months, the curve is starts to flatten out. So you lose some people early on, but those people who respond seem to do so for long periods of time.

Unlike yttrium, unlike lutetium, this therapy does not require any radioprotective scheme. With many of the lutetium and yttrium products, you have to have an infusion of amino acids to protect your kidneys from radiation damage but these infusions have a lot of side effects themselves. The indium product does not do that. You don't need that amino acid infusion. This slide shows kidney function over time: at time zero (before therapy) and after 1, 2 or 3 doses of the indium-pentetreotide. Dr. Krenning and other people have now developed a very nice rating scale so you can almost guess looking at a person's scans, their chance of responding to these kinds of therapies. If your tumor uptake is a lot more than normal liver, you have about an 80% chance of response. But if your liver has a lot more uptake than the tumor (it's black and your tumor's white), only about 25% of folks

respond to therapy. There are a lot of experimental therapies that Dr. Kvols is getting ready to tell you about. With indium-pentetreotide, about 300 patients have been treated worldwide. With OctreoTher, the yttrium product, the phase II trials are complete. The data is being analyzed, and it is my belief that Novartis has actually filed an NDA [new drug application] with the FDA to start trying to market this therapy.

You've heard several people allude to Dr. Eric Krenning's work with lutetium-177. This is still early data, and the early trials are very, very positive. It looks like it has some of the benefits of the yttrium and some of the benefits of indium-pentetreotide. I think it's too early to get survival curves out of those early trials. Indium-pentetreotide is available today. You can call Dr. Luis Linares and he will set up the therapy and bill your insurance company. This is indium-DTPA pentetreotide. Remember, this is an octreotide molecule, along with the DTPA chelator and indium - what you're about to see is that we can substitute the indium for other radiolabeled tags. You can also put lutetium on this molecule, but the lutetium is not as tightly bound to the DTPA chelator. A different chelator that Dr. Kvols is going to tell you about is called DOTA versus DTPA. The basic structure of the radiolabeled drug is the same octreotide, but it has a different chelator, and you can put indium or the lutetium on that molecule. And again, they've made some internal modifications to the octreotide molecule, but it's the same kind of concept. In the future, we will be reporting about head to head comparisons of these therapies so it is important to understand about the differences in the molecules.

I'd like to thank you all for coming today and I'd like to thank you for being so attentive. Thank you very much.

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