

# Introduction to the Basic Science of Carcinoid

Presented by

Eugene A. Woltering MD FACS  
The James D. Rives Professor of Surgery and Neurosciences Chief,  
Section of Surgical Endocrinology,  
Director Of Surgical Research  
The Louisiana State University Health Sciences Center  
New Orleans La 70112

From the Carcinoid Wellness Conference  
Memorial Hospital  
New Orleans, LA  
March 9, 2002

You are a rare group of people. Those people who have talked to me on the phone, and there are probably very many of you, know that I call you the “Pink Zebras with Purple Polka-Dots”. To give you an example, one out of about eight women get breast cancer in their lifetimes. One in a million women get carcinoid syndrome. So, you are truly the “rare squared” group of people. To be able to get almost 200 carcinoid patients who paid their own way, are willing to network, talk with, and support one another here in this auditorium is a major tribute to all of you.

First, I'll give you a little bit of background - and I do this to sort of get you comfortable with what I am going to be talking about today. We are going to talk about carcinoids but we are going to foray into other kinds of tumors to make certain points. Carcinoids probably have been around since the pharaohs but were not recognized as a specific tumor type until the late 1800s when the first ileal carcinoid with liver metastasis was described. The term carcinoid was coined in the early 1900s, and in the mid 1950s other functional endocrine tumors such as gastrinomas, VIPomas, and glucagonomas were clinically recognized. Dr. Thomas M. O'Dorisio from the University of Iowa calls this the “clinical era” of endocrine tumors.

Many of you already know what carcinoid syndrome is. You know about the tumors that cause it. Most carcinoids are usually very well differentiated. That means they look like the normal cells from which they arise. Carcinoid is the most common distant small bowel tumor. Its prevalence and incidence is very, very low. This tumor rarely metastasizes. Most of the time it is an incidental finding. It is classified according to its embryonic origin and there are two “flavors - chocolate or vanilla”, i.e. secretory (or functional) and non-functional, which means they do not make an overabundance of peptides or amines. They come in other “flavors”, too - foregut, midgut and hindgut. The foregut is represented by bronchial carcinoids - those of the respiratory tract, those of the stomach, the duodenum, the pancreas, and the ovary. Patients with these tumors can develop carcinoid syndrome. They can have all the classic signs of the syndrome such as flushing and diarrhea, but they can also have things like diabetes from oversecretion of glucagon or Cushing's syndrome from oversecretion of ACTH. The midgut carcinoids are what you're most familiar with. Those are the ones of the small bowel and the right colon and the appendix. It is more common to see carcinoid syndrome in those patients. Hindgut carcinoids occur predominantly in the rectum. In these tumors, the carcinoid syndrome is rare.

The peptides and amines that are hypersecreted by carcinoids are not “weird” or unusual substances. These are normal, everyday products that a normal person's body makes. Carcinoid patients just make a boat-load of these compounds rather than a Volkswagen-load. Serotonin, for example, is a neurotransmitter in your brain. It's something that, if you don't have it, you probably couldn't get out of bed in the morning. You have all heard about Prozac and the numerous drugs that are being developed to treat depression. Those drugs control the flux, binding or re-uptake of serotonin. There are other neurotransmitters such as tachykinin, neurokinin A, substance P, chromogranin A, and neurotensin. They are released from a nerve ending; they diffuse across a

## Introduction to the Basic Science of Carcinoid

Published on The Carcinoid Cancer Foundation (<http://www.carcinoid.org>)

---

small, neurosynaptic cleft to a receptor on the other side of the cleft. That allows you to put the substance and the receptor together. It is like having a lamp. You have the right plug, you have the right wall socket, and when you put them together, the lamp goes on. Occasionally, patients with carcinoid tumors produce an overabundance of substances like VIP which gives you diarrhea, gastrin which gives you ulcer disease, ACTH which makes you mildly diabetic, hypertensive and a buffalo hump, hirsutism or hairiness, and what is called Cushing's syndrome of effects. Carcinoid syndrome demonstrates that it is hypersecretion of peptides and amines and not the compounds themselves that are unusual. It is not the peptide that is unusual or a product that is not produced normally in people. It's the amount of secretion that causes the clinical symptoms. The carcinoid syndrome happens in about 10% of people with carcinoid tumors. What makes your syndrome worse or better than the next patient's is tumor size, where it came from, how much serotonin or other products it makes, and how many metastases you have. Here is a common problem. If there is a person with a carcinoid that is the size of a softball, and a person who has a carcinoid the size of a peanut, who has the worse syndrome? The answer is, "I don't know". The reason is, you can have a small tumor that is very hormonally active or you can have a large tumor that is relatively inactive from a secretory point of view. But, in any given individual, as your tumor grows, you will make more and more of the "stuff", whatever your "stuff" is.

This photograph shows one of my favorite patients. She is a Native American, and was a patient in my Portland, Oregon practice. She was accused of being a drunk, yet she was a teetotaler. But, she had an undiagnosed carcinoid syndrome. Three or four times a day, especially at night after she ate a big meal, she would get this very characteristic carcinoid flush, mostly on the cheeks. As you flush more and more, you're constantly dilating blood vessels in your face. Those blood vessels are like a pair of socks, if you continuously stretch them out, one day your socks don't fit anymore - the elastic just doesn't spring back. So, occasionally you will see in patients with long-standing carcinoid syndrome, what are called telangiectasias, the classic picture of the "drunk" with large, dilated veins on his nose, etc. You can also get these dilated veins with carcinoid syndrome after years of repeated flushing episodes. Carcinoid tumors can come from the skin, the lung, the ovary, or the GI tract. They can affect your heart, your respiratory tract, your kidneys, your muscles, or your joints. Most of the people with carcinoid syndrome having flushing to some degree, diarrhea, or much less commonly, wheezing. If you have a significant amount of secretion of serotonin, your right-sided heart valves can be injured and you can have congestive heart failure. Some people will have fatigue, edema, some muscle pain, or finally arthritis.

The problem with carcinoid is both the good news and the bad news. The good news is that by the time you figure out you have the tumor, you've probably had it for up to ten years. Most people start their quest for a diagnosis after they have had vague symptoms for maybe up to ten years. That is what we call the "delay time". Dr. Zollinger, one of my old professors at The Ohio State University, did a very elegant study on people whose tumors make gastrin, called gastrinomas. He calculated that the average lag time from the onset of symptoms to the time they actually made the diagnosis was about twelve years. That it is the good news about neuroendocrine tumors in general (ie) your tumors grow slowly. But, the bad news is that they continue to grow and, if allowed to go untreated, can result in death. This disease has two components. When I start talking about the basic science of this disease, I want you to keep two things in mind; 1) you can die because of what the tumor makes, and 2) you can die because the tumor grows. If you have breast cancer, if you have colon cancer, you die of the cancer growth, not because the cancer is secreting a peptide, hormone or amine. With carcinoid tumors, you can die because of the tumor itself, or you can die of the effects of the carcinoid syndrome.

The most common thing I am asked when people call me on the phone is, "What is it that I should do to determine the location and stage of my tumor, and what should I do to follow-up on my tumor?" You will always hear me talk about the 5HIAA urine collection, a plasma Chromogranin A, and a substance P. You also have to perform an OctreoScan®. You also need a baseline echocardiogram to look at the valves in your heart and then baseline and interval CT scans of the appropriate area, whether it is the abdomen or the chest. You also occasionally need to look at other kinds of levels like 5HT or serotonin. These are very hard to do. A lot of people will call and say "Well, Dr. \_\_\_\_\_ asked me to get a serotonin level". I'll say, "Well, you don't need to tell me what that value is because I don't follow serotonins". For you to get accurate serotonin measurements, you need to have a blood collection site and laboratory committed to their measurement. Most laboratories do

not have the time or expertise to do these measurements reliably and accurately.

Why are we here today? There are three goals of therapy for patients with carcinoid tumors; 1) to control your symptoms, 2) to induce tumor regression or stop the tumors from growing, and 3) to increase your survival. However, survival without a good quality of life really does not “cut it” in this group of patients. What we are after with carcinoid therapy is increased survival with a good quality of life!

This slide is a cartoon of native somatostatin 14. This is what you and I make every second of every minute of every day of our lives. This is made in the brain, the gut, it is made just about everywhere. I tell my surgical residents, “When you are asked a question on the Board exam that you don’t know the answer to and it has anything to do with gut physiology, answer ‘somatostatin’ if the question implies that it turns something off.” I call this somatostatin molecule the universal “off switch”. It turns off insulin, it turns off glucagon, it turns off serotonin release, it turns off VIP release, it slows down bowel motility. It increases absorption of fluid from the gut. It has an inhibitory effect on growth hormone release from the pituitary. It is truly the “universal off switch”. The good news is that you make it every second of every day. The bad news is that 1) it is not very strong, and 2) if you were to use it as a drug, you would have to give it intravenously 24 hours a day, 7 days a week. Within a couple of months of somatostatin 14 being described and sequenced, Dr. Janos Pless at Sandoz invented this molecule, which was called SMS 201-995. This is now known as octreotide or Sandostatin®. Notice on this drawing that these four amino acids in somatostatin 14 are the same four amino acids in octreotide. That is called the pharmacophore of the drug; that is the “kick butt site”, or where the molecule binds to the receptor. Notice that on octreotide, the middle group of amino acids is “missing”. So, what Dr. Pless did was cut the middle out of the somatostatin 14 molecule and kept it a ring structure. By doing so, he made octreotide 100 times more potent than the native somatostatin 14; but more importantly, octreotide “lasts” in your blood (has a half life of 1.5 to 2 hours), while native somatostatin 14 has a half life of 1.5 to 2 minutes. If you take the lysine in the pharmacophore of octreotide and you substitute it with an ornithine, you make the molecule biologically inactive. This shows that the D trp-lysine amino acids are required for the drug to “work”.

What does somatostatin do? Somatostatin is like the plug on a lamp cord. The plug has either a two-prong plug or a three-prong configuration, or it’s a 220 plug or a 440 plug. It has a specific configuration. Remember those four critical amino acids in the pharmacophore of octreotide or somatostatin - where do they plug in? They plug in to a specific receptor designed to fit their configuration. This is the membrane of a cell and these are a long chain of amino acids. This is called a seven transmembrane domain receptor. The somatostatin (or octreotide) comes in from outside of the cell and plugs into the membrane on a membrane somatostatin receptor. These are called somatostatin receptors. To date, there are five known subtypes of this receptor (called sst 1 - sst 5).

This is a set of cartoons. This is what happens to you when you eat and your blood sugar goes up. This cartoon represents insulin binding to a membrane receptor. This can also be somatostatin or octreotide after you give yourself an injection. The blood levels of the drug start to rise. As the blood levels start to rise there is a “pressure” for the drug to bind to the membrane receptor, so that as your blood levels rise, more and more of these receptors on the cells get “saturated”. Then, your blood sugar goes down and your body turns off insulin or your octreotide wears off, and the blood levels of the drug start to go down. What happens to the drug on the receptor? The “pressure” is now changed; to put it another way, the current of the stream has changed. The drug now starts to come off of the receptor. When somatostatin comes in, and it binds to the receptor, there are other structures in the membrane, called G-proteins. When these G-proteins are activated, it turns on (or off) many different processes in the cell. As an example, you don’t have to be inside of a room to turn the lights on in that room. Somatostatin binding to a receptor is like turning on a light switch from the outside of a room (i.e. the lights come on inside the room). One of the things originally described about somatostatin or octreotide, is that when they bind to their receptor, they turn on the inhibitory G-protein, which in turn blocks intracellular signals that are responsible for peptide and amine release.

This slide shows one of the first clinical uses of octreotide in a patient with carcinoid. Dr. Larry Kvols had a patient who was undergoing a surgical ligation of the hepatic artery, the artery going to the

liver. The surgical team had given the patient the usual anesthetic agents and the patient “crashed and burned”. The patient started to flush. Their blood pressure went down to 30 mm/Hg and, obviously, the patient was in deep trouble. They tried a variety of things to resuscitate this patient. Dr. Kvols received a frantic phone call in his office across the street from the operating room. He ran down six flights of stairs with a vial of octreotide in his hand, across the street, and up three flights of stairs. When he reached the operating room, he literally collapsed as he handed the vial to the anesthesiologist. The anesthesiologist administered the octreotide to the patient; thirty seconds later the patient’s blood pressure was normal and five minutes later the flushing cleared. More importantly, the patient survived. This instance (1986) was the first use of octreotide for “carcinoid crisis”.

The first group to use octreotide in the United States was led by Dr. Thomas M. O’Dorisio at The Ohio State University. The drug was used to control diarrhea in a gentleman with VIPoma in 1983. So, these drugs have been around for a very long period of time. Dr. Kvols was the person who moved the use of octreotide in carcinoid patients forward in the 1980s. He had a group of 25 patients at the Mayo Clinic that he treated with now what we would call “low-dose” octreotide (about 150 µg three times a day). He studied their clinical response to this drug. What happened to flushing and diarrhea? It responded very well. No toxicity was observed, and the effectiveness of the drug at low doses seemed to persist for at least eighteen months. Everybody at that time had the mindset that this drug was a very fancy, very expensive “Lomotil”. It would stop you from having diarrhea and “Oh, by the way...” would also stop you from flushing. This is what the world thought about octreotide at that stage in the drugs development- that it was a great, though expensive, anti-diarrhea medication. But then interesting things happened. Some people got a great symptomatic response and some people didn’t have any response to the same dose of drug. So, Dr. Kvols and Dr. Jean-Claude Reubi started looking at the relationship between how many somatostatin receptors (sst 2) were on a patient’s tumor versus the patient’s clinical response. The more receptors, the greater chance that octreotide can inhibit peptide or amine release. If a tumor didn’t have receptors on its cells, a few people still got relief from diarrhea because octreotide and somatostatin act at two sites. They act at the “tumor site” for control of peptide-amine production, but they also act at distant sites like in the small bowel, the stomach, etc., to decrease fluid production and decrease bowel motility. This is called a “secondary site of action”. Octreotide thus has the “double whammy” effect.

This is a CT scan of a patient of mine from Oregon in the late 1980s. She came in with a gastrin-producing tumor (gastrinoma) and you can see that there are many tumors within this patient’s liver. All we wanted to do for this patient was to control diarrhea; at that time she was having thirty bowel movements a day. She came back nine months later, and I was going to send her home. But, my fellow said, “We ought to get a CAT scan.” I said “Why? We’re just treating this lady’s diarrhea.” We did the CT scan and she’d had a complete tumor response, which lasted for almost 5 years. Shortly thereafter, others began noticing that when you treated patients with octreotide for diarrhea and flushing, that occasionally their tumors decreased in size or stopped growing. One of the first people to note this response was Dr. Larry Kvols. He showed that about 15 - 20% of patients treated with higher doses of octreotide (500 µg three times a day) had a tumor response. Those were either complete responses (CR) or partial responses (PR), and the survival rate for these patients was enhanced. More importantly Dr. Kvols noted that many patients treated with these higher doses of octreotide experienced long time intervals of tumor growth inhibition. They still got great control of their flushing and diarrhea but this was clearly new information. This graph is in the back of a 1993 article in *Acta Oncologica* by Dr. Kvols. It shows that if you go on octreotide versus what is now “ancient” chemotherapy (5FU adriamycin or 5FU streptozotocin), your survival at three years is nearly tripled. Thus, octreotide has an anti-tumor effect in addition to controlling diarrhea and flushing.

Lowell Anthony, who will be speaking to you later, then did a critical experiment. He asked the question, “If a little bit is good, what about a boatload of octreotide?” He not only took the octreotide dose from 1500 to 6000 µg/day, he also used octreotide’s brother, known as lanreotide or Somatuline® and went up to 9 mg/day (9000 µg/day). What he saw was that, again, you had people who had partial tumor responses, people who had stable disease, and people whose tumors progressed. But the important thing was that there was no toxicity, even at these very high doses. This is not like chemotherapy where if you give somebody an overdose, they’re going to die because

their white blood cells all die. This drug has no lethal dose level ever demonstrated. During the FDA drug approval process, when they tried to kill rats by overdose to demonstrate toxicity, they had to give so much drug that the drug physically started precipitating out of the blood before they could make the rats die. So this may be the world's safest drug. As we were looking at articles about octreotide therapy, we started asking the question, "How does a drug that controls diarrhea also control cancer cell growth or tumor growth?" This slide shows work done by Dr. Darryl Kurozawa in my lab. What we showed was that octreotide would decrease the proliferation of breast cancer cells. Notice that this curve gives you a hint that there is a critical range of octreotide doses. Giving you either too much or too little is equally bad. This slide demonstrates the response of hormone-deprived (like post-menopausal) breast cancer cells to octreotide. If you treat them with estrogen (like a pre-menopausal state), then the number of cells goes up but you get this same biphasic drug response curve. Thus, there is a very narrow optimum therapeutic dose window. The optimum dose range happens to be that level of drug in your blood where all (90% +) of your somatostatin receptors are saturated. This drug level is about 10-8M or 10,000 pg/ml.

We published these observations, and very shortly thereafter other people made similar observations about the control of breast cancer with somatostatin analogs. In one of those papers (by Dr. Colin Weber from Emory University) there was a comment that breast cancers treated with octreotide were "grossly less vascular", i.e. they had fewer blood vessels in the tumor. We thought that maybe the tumor growth was inhibited because the octreotide was able to block the blood vessel growth into the tumor. This slide shows a chicken egg, and this area on the egg is called the chorioallantoic membrane or the respiratory membrane of a fertilized chicken egg. These are blood vessels, and this is a methylcellulose (dissolvable) disk that is 2 mm in diameter. You can see the blood vessels right through it. The nice part about this disk is that you can mix octreotide in this methylcellulose and put it on an egg. When you do that you get a totally different picture. Octreotide destroys (or prevents the growth of) the blood vessels (angiogenesis) in the area around it. This was the first observation that a somatostatin analog could have both a direct effect on tumor cells but it also could "choke" the tumor by destroying the blood vessels that feed the tumor. We published an article that compared the anti-angiogenic effect of octreotide to another somatostatin analog (RC160). You can see that the more of these drugs you give, the better the response. These somatostatin analog actions were also compared to the most potent inhibitor of angiogenesis known at that time which is a combination of heparin and hydrocortisone. Based on these comparisons, we determined that octreotide is a very potent inhibitor of new blood vessel growth.

But that model is a chicken. What about humans? To evaluate human blood vessel growth, you have to ask patients for blood vessel biopsies or you have to develop a human model that could study blood vessel growth. That model didn't exist. There was a scientist named Nicosia in Philadelphia who took rat blood vessels and got them to grow new blood vessels in culture. Clearly, if rat blood vessels would work, human blood vessels should grow also. The trouble is, not many of you want to come and donate your blood vessels to me. Investigators had to find a way to get a large number of human blood vessels. It turns out that we can get these vessels from placentas. Drs Chris Parish and Joanne Brown from Australia were the first to use these human placental blood vessels to study angiogenesis. After childbirth, if the mom is okay and the baby is okay, the placenta is discarded in the garbage can. We got permission from our Institutional Review Board to become "garbage men" and to salvage the placentas for our research. What we would then do is harvest the placental vessels, open them up, and take a skin punch (like a leather punch) to cut out "biscuits" of blood vessels. We would then embed them in a substrate like a blood clot without blood. In this photo you can see, coming off the cut edge of the vessel disk, new blood vessels growing out into the clot. How do human blood vessels grow? This wispy stuff in this photo is the fibrin-thrombin clot. You can see here the end of a blood vessel. It is like a broomstick handle coming up through this trelliswork of fibrin. This is a picture from our laboratory showing that these new blood vessels branch and they connect with one another like a normal circulatory system. They grow at the very tip of the blood vessel or the newest part of the blood vessel. On the tip of the vessel, the cells are solid cords of endothelial cells. As the vessel starts to mature you see little lipid droplets starting to appear. As the vessel matures even more, those lipid globules hook together and coalesce and that forms the lumen of a blood vessel. So these are real human blood vessels growing in a test tube. If you implant one hundred of these vessel disks into culture wells, more and more of them will begin to grow over time. That became a concept in our laboratory that we call "initiation". Initiation represents the changes that a blood vessel undergoes when it goes from a resting state to a growing

or proliferating one. Dr. Folkman calls this the “angiogenic switch”. Octreotide has an effect on the initiation of blood vessel growth.

Not only do these vessels begin to grow but they grow in a very orderly fashion. The length of each of these blood vessels and a number of blood vessels grow linearly over time in the culture system. That led us to ask the question, “If octreotide blocks the conversion of the “sleeping” blood vessel to the growing blood vessel, what does it do to the rate at which blood vessels grow?” The answer is---you’re going to have to wait a minute. There are a lot of other questions that you have to ask first. What does a tumor say to a blood vessel? What does the blood vessel say to the tumor? Do they talk to one another? The tumor says to the blood vessel, “grow”. Imagine that you have a plate and the plate has three round wells cut in it. The well on the left side has been loaded with tumor cells. The middle well has a blood vessel in it, and the right-sided well is a “control” well. What does a tumor say to a blood vessel? The answer is pretty scary. Tumors secrete growth factors that act as chemical messengers that direct blood vessel growth. Drs. Jeff Flattman and Yizarn Wang from our laboratories at LSU found that they say to the blood vessel “come here...grow toward the tumor”. What is interesting, and we’ll talk about this in a second, is that that may not take very long for a tumor to send that message. Conversely, what does the blood vessel say to the tumor? If the tumor is saying to the blood vessel “grow toward me”, does the tumor also answer the messages sent by the blood vessel? This photograph shows work done by Dr. Patty Masse, an LSU surgical resident. In this series of experiments, the blood vessel grows toward the tumors. However, the tumor is also growing towards the blood vessel. Thus, tumors and vessels have a two way bi-directional cross talk. The tumor “says” to the blood vessel “grow toward me”, and the blood vessel “says” to the tumor “grow toward me”. Scary, isn’t it?

Well what else do tumors “say” to blood vessels? What would happen if you put a tumor on top of your blood vessel-containing clot? What would the tumor do to the number of vessels that began to grow (initiation)? Does a tumor “say” “begin growing” or does the tumor “say” “grow faster”? Tumors make blood vessels begin to grow. Tumors affect the “initiation” step. When we implant one hundred pieces of blood vessel, half as controls and half with tumor, many more of them begin to grow in the tumor-treated group. Octreotide’s effect is to block initiation of the angiogenic response. I originally “guessed” that a tumor-induced blood vessel would grow faster than a normal blood vessel. I was wrong. Tumor stimulated vessels grow at exactly the same rate as normal vessels grow. So tumors “say”, “begin to grow”, then “say” nothing else. As part of that experiment, we had inserts that we could put in the test tubes. These inserts had a dialysis membrane bottom so the growth factors the tumor made could be released into the clot-containing blood vessel. After three days, we could take the tumor cells contained in the insert out of the system, and the vessels acted like it was still there.

Next, we started to look at how much heparin- hydrocortisone it would take to inhibit blood vessel growth in this model. We called Dr. Folkman (the “father” of angiogenesis research in the United States) and he gave us an estimate of an effective dose. Well, we took his dose and multiplied it 10 times, then 100 times. It took 100 times more than he originally guessed to really block angiogenesis in this model. The yellow line on this graph is the effect of octreotide on angiogenesis. This is one experiment that we were originally disappointed in, i.e. the octreotide had an effect but some vessels disks developed new vessel growth early on. After Day 6, the drug looked like it worked, but it didn’t seem to work from Day 1 to 6. So what was happening during Days 1 to 6? Remember, we use blood vessels right out of placentas so we can take one piece of them and freeze it. Then we can take the other parts of the vessel and put it into culture system. We let them get “hairy” and these two tissues then represent two different things, 1) resting or normal blood vessels, and 2) angiogenic or proliferating blood vessels. Now we have something. We have, from the same patient, the “same” blood vessel, but in both its “growing” and “resting” states. We started asking at a gene expression level whether somatostatin receptor subtype 2 was in the normal blood vessel. A normal blood vessel does not have somatostatin type two (sst 2) receptors. Octreotide treatment doesn’t work from Day 1 to Day 6 because there is nothing for it to “plug in” to until around Day 6. About that time, the model starts to develop angiogenic blood vessels and the angiogenic vessels have somatostatin subtype 2 receptors (sst 2)!

Could we prove that concept in other ways? This slide represents work done by Drs. Tom and Sue O’Dorisio and another investigator (Dr. Doug Balster) at Ohio State. We sent them a resting blood

vessel that was not growing and tissue-matched growing blood vessel. Brown stains on these slides represent the somatostatin subtype 2 receptor. On this slide, the “resting” normal blood vessel shows no brown stain. However, the blood vessels growing into the clot always exhibit a brown stain, and thus have somatostatin subtype 2 receptors. Somatostatin receptors (sst 2) are on the membranes of the cells in the growing blood vessels. To further investigate this, we implanted in a nude mouse a tumor that did not have somatostatin receptors on the tumor cells. We grew the tumor in the mouse’s flank until it reached the size of a peanut. Then, we injected the mouse with a radioactive somatostatin that went to the liver and, more importantly, to the tumor. Unlike some somatostatin analogs that are excreted by the kidney (OctreoScan®), the radioactive somatostatin analog (WOC 4) that we used is excreted by the liver. This scan shows us how much went to the liver and how much went to the tumor. Remember, the tumor cells didn’t have somatostatin receptors but the angiogenic blood vessels feeding the tumor did. Also remember, for the radiolabeled peptide to work, the “plug” has to match the “socket”, or receptor. When you hook low medium or high levels of radioactivity to a somatostatin analog, you can do all kinds of things with it. You will hear me talk many times about using low energy (sort of like a refrigerator light bulb) radiolabeled somatostatin analogs to help us find tumors in the operating room. OctreoScans®, in contrast, are higher energy, and can be compared to a 60 watt light bulb. If you just give 100 or more times than the scan dose (6 vs. 600 mCi) of OctreoScan®, you can do therapy with the same molecule.

This image is an OctreoScan®. You will hear a lot about this today. Again it’s octreotide hooked to a radioactive molecule called 111Indium. Remember the critical four amino acids? Remember the binding site of the octreotide molecule? This is the pharmacophore where the molecule plugs into the receptor. It has the chelator (sort of a hook), and you put the radioactivity on the “hook”. It is a very effective scanning agent in patients with neuroendocrine tumors like carcinoids. About 80 - 90% of people with metastatic carcinoid have a positive OctreoScan®! In almost all the other neuroendocrine tumors, 70 - 80% of patients will scan positive. This is what an OctreoScan® looks like. You can see tumor in the skull, the neck, the top of a lung, shoulder, and hipbone. At even higher (therapy) doses, you can see a number of the vertebral bodies (spine bones) light up and even a tumor in the knee cap.

Can we learn something about the way tumor cells and octreotide interact by using these radiolabeled compounds in our laboratory? When you take your somatostatin shot 3 times a day, your drug level goes up and the drug “hops” on the receptor. Every time your drug level goes down, the drug “hops off” the receptor. Thus, you are “turning on and off the lights in the room” 3 times a day. This is the way insulin works, gastrin works, glucagon works - almost all the peptides or amines like serotonin work in this same manner. When you eat, they go up. When you finish eating, they go down. You wait, nothing happens. You eat again, they go up again, then they go down again. You’re getting “pulses” of peptide. What about when you sleep, what happens then? Do your insulin levels go to 0 when you’re asleep? Or if you fast for three days do your insulin levels go to 0? The answer is no. Think about this, if you were going to design something that is energy efficient, why would you make peptides, hormones, and amines all night long when you don’t “need” them to respond to a food challenge? Based on those questions, we became interested in the effect of low-dose, chronic exposure to peptides. Do these constant low-dose drug levels behave differently than boluses of peptide? Is the receptor binding different? Because that may be very critical; there are people here today who have continuous infusion pumps or on sustained-release somatostatin analogs and people who take 2 - 4 shots (pulses) a day.

Remember somatostatin binds to the receptor, and when the drug levels go down, it “falls off” the receptors. What if the levels don’t go down? What if you just keep the drug level constant, and keep every receptor saturated at all times? What happens? Does the receptor just sit there and know that you can’t get any more drug on receptors? What if you could look at where peptides go inside of a cell? A group of people in our lab showed that the total amount of binding of a somatostatin analog progressively goes up in a tumor cell over time. From 6 o’clock at night when you stop eating until 6 o’clock in the morning, your insulin, somatostatin, and other peptides act like this. Somehow (a process we now know by the term “endocytosis”), these peptides get inside of the cell. To prove that concept, Dr. Conrad Hornick from LSU exposed cells to a radioactive somatostatin analog (111In-pentetreotide) (OctreoScan®) for an hour. Then he “blew” the cells apart, put them in a centrifuge over a sugar gradient, and divided them out by weight. After one hour, the 111In-pentetreotide is on the membrane. By about four hours the membrane is clearing and you

start to see a little peak in the nucleus, and by 24 hours, all of the radioactivity is in the nucleus of the cell! Now we've got real trouble in "The Big Easy" because now receptor binding is not at all like what we thought before. Now our observations show that somatostatin is not only "turning on" a membrane receptor and that turns on the lights in the room. But this new way of looking at receptor binding shows that somatostatin "knocks down the door (cell membrane), walks in (endocytosis) and "assaults" the nucleus where everything in the cell is controlled. Not only that, but Dr. Hornick took it one step further. He showed that when somatostatin went to the nucleus of the cell, it did something inside the nucleus and that "something" is it "combined" with or bound to DNA. Now this is really important! Following "pulses" of somatostatin, there is basically nothing that reaches the nucleus. But if you look carefully, with prolonged exposure to the somatostatin analog, it takes 24 hours of exposure for the drug to get to the nucleus, and to get to the DNA takes another 24 hours of exposure. So, a bolus of somatostatin analog isn't going to reach the DNA. We believe that a continuous exposure of tumor cells to the somatostatin analogs allows this to happen, but a pulse or a bolus of drug does not push the drug into the cell and the nucleus.

So, this is now what we think happens: somatostatin binds to a receptor. They cluster. This is called an endosome. It internalizes and this somatostatin is "dropped off" inside the cell. The receptor recycles to the surface and this becomes not a static system but rather a "revolving door". What happens with continuous exposure is that peptide gets into the "revolving door". Some of it goes through the lysosome for breakdown, and some goes to the nucleus and binds to DNA.

Why is this important? It is important because many of you are on Sandostatin LAR or on a continuous infusion pump. LAR or the pump gives you octreotide all the time. What is LAR? It is octreotide in spheres of polyglycolic acid, which is a polymer. It is sort of like those candies that have liquid centers - you bite into them and get a "squish". Here is an electron micrograph of some of these spheres that we made in our laboratory. You can see tiny beads, and you can see big beads. They rupture at different rates, thus giving you a continuous exposure of the drug for up to thirty days. However, it is interesting to note that when Novartis did their original study, they took people whose symptoms were well controlled on octreotide, they stopped all their somatostatin analogs, and this is the number of flushing episodes per day that they had on and off the drug. Then they started them on intramuscular shots of 10, 20 or 30 mg of the LAR/month. And, notice that there is no big advantage to getting 30 versus 20 versus 10 mg/month; a little bit, but not much. If you look at the number of diarrhea stools per day, doses of 10, 20 or 30 mg of LAR/month had the same result. Here is what you need to know. Do you remember the magic number for optimal receptor binding? That level is about  $10^{-8}$  M or 10,000 pg/ml. This slide shows the peak (mean) levels of octreotide from patients given 10, 20 and 30 mg of LAR/month. At 30 mg of LAR/month, patients reach blood levels of about 4,000 pg/ml. Remember that this release peaks at two weeks and then starts to go down so that at the peak, you're about half where need to be for maximum receptor saturation, and at the trough (day 30) you're about a third of where you need to be with Sandostatin 30 mg in the LAR form. This may be critical information - you may need 60 mg of octreotide per month for optimum receptor saturation. This dose-saturation phenomenon, however, has never been studied in a really good clinical trial.

We talked about how we could get the radioactive somatostatin analog  $^{111}\text{In}$ -pentetreotide inside the cell and we asked whether it would go to the nucleus, or if it would bind to the DNA. What we're really talking about is taking radiation into the cell. What happens if you radiate blood vessels? I would have bet that radiation affected the number of vessels that began to grow or initiate. However, when we radiated our human blood vessels in the lab, it didn't change the number of blood vessels that began to grow (initiate). Well, what about the rate of blood vessel growth? In this case, the more radiation you give, the slower blood vessels grow! This is the concept for you to remember: octreotide affects Part I (initiation), radiation affects Part II (vessel growth). What if you could put a somatostatin analog and radiation together? Would that affect blood vessel initiation and blood vessel growth rates?

To study this, we took human tumors and we put them into mice. These IMR tumor cells had somatostatin receptors, and the angiogenic blood vessels that develop also have somatostatin receptors. In other mice, we implanted tumors in which the tumor cells (MDA) did not have somatostatin receptors. But, we knew that all the blood vessels that are growing have somatostatin receptors. So if we then treated these two types of tumors in our angiogenesis cultures with

## Introduction to the Basic Science of Carcinoid

Published on The Carcinoid Cancer Foundation (<http://www.carcinoid.org>)

---

radioactive somatostatin analogs. We thought that we could kill the tumor cells with receptors but also thought that we could kill all the blood vessels regardless of tumor receptor status. This is work done in our lab by Dr. Seza Gulec, an LSU surgical resident. Basically, this work shows that almost all of the tumors (about 85% of them) begin to sprout new blood vessels. If your tumor cells have somatostatin receptors, the tumor dies and the blood vessels die. If the tumor cell doesn't have somatostatin receptors, the tumor doesn't die but the blood vessels do. Why is that important? Because, dead is dead. If the tumor cells are dead, the tumor is dead. If the tumor can't eat, drink or breathe, you've strangled it, and the tumor is dead, or in the worst case scenario, at least will not grow. Again, let me make this point: regardless of the tumor's somatostatin receptor expression, if you kill the blood vessels feeding the tumor, the tumor will die or, at the very least, cease to grow.

We now have ways of clinically telling you how many somatostatin receptors your tumor has. This slide shows work done by Dr. Eric Krenning in Rotterdam; basically, he grades your tumor somatostatin receptor status based on an OctreoScan®. If your tumor's uptake is equal to liver, it is a Grade 2 on the Krenning scale. A tumor that has an uptake a little bit greater than the liver is a Grade 3, and if your tumor is black (intense uptake) and the liver is light gray, that is a Grade 4 uptake. The best responses to therapy are seen in people with Grade 3 or 4 uptakes. Going back to that concept from the early 1990s, the more receptors you have, the better off you are. This is a critical concept! What we've shown you today is that in patients with carcinoids who are treated with octreotide, a) you ought to be treated early, and b) you ought to be treated with the right octreotide dose. These maneuvers will impact new blood vessel growth, it will affect your tumor and cell growth, it will also optimize your peptide-amine release and will optimize the control of your carcinoid syndrome.

**Source URL:** <http://www.carcinoid.org/content/introduction-basic-science-carcinoid>