Dr. Jack Murphy, The Evolution of ONTAK February 27, 2011Welcome to Yale Cancer Center Answers with doctors Francine Foss and Lynn Wilson. I am Bruce Barber. Dr. Foss is a Professor of Medical Oncology and Dermatology, specializing in the treatment of lymphomas. Dr. Wilson is a Professor of Therapeutic Radiology and an expert in the use of radiation to treat lung cancers and cutaneous lymphomas. If you would like to join the conversation, you can contact the doctors directly. The address is canceranswers@yale.edu and the phone number is 1-888-234-4YCC. This evening Francine welcomes Dr. John Murphy. Dr. Murphy is Professor of Medicine and Chief of the Section of Molecular Medicine at Boston University School of Medicine. Dr. Murphy developed the first fusion toxin protein approved for clinical use in 1999 for the treatment of patients with T-cell lymphoma. Here is Francine Foss.Foss It is a tremendous pleasure to have Dr. Murphy here tonight to talk with us. It is not often that we actually get to talk to a person who invented a whole new technology that is very important for us in treating cancer patients. Dr. Murphy, or Jack, I will call you Jack, can you start off by explaining to us your work in molecular medicine? Can you explain what molecular medicine is and how that term relates to the term that we often use on this show, which is translational research?Murphy I would be happy to. Molecular medicine is brings to the field of medicine contemporary molecular genetics and molecular biology. In understanding the underlying pathways that govern how cells grow and multiply. It is through the understanding of these pathways and systems that allow us greater insight into cells that cause disease by understanding what the molecular basis is for many diseases now.Foss Jack, you developed the first fusion toxin protein. Can you tell our audience what a fusion toxin is?Murphy A fusion toxin is really a fusion protein, and a fusion protein is one molecular entity that is one protein molecule that might be thought of as having two different parts. In this instance, part of the protein comes from a bacterial toxin and the other part of the same protein that is assembled genetically comes from a human source, and in the case of the drug Ontak, that human protein is known as interleukin-2. It is a single protein that is part diphtheria toxin, part human interleukin-2.Foss So, you used genetic engineering to design this protein?Murphy That is correct. We started out many many years ago with an understanding of the basic biochemistry and mode of action of diphtheria toxin and what was abundantly clear thirty years ago, is that the first step in the intoxication process was when diphtheria toxin binds to its unique receptor on the surface of eukaryotic cells, or mammalian cells. Recognizing that the first step in the intoxication process was binding to the cell surface, many years ago, we asked the fundamental question, could one take away that portion of diphtheria toxin, which binds to its receptor, replace it with a molecular protein that is known to bind to its unique set of receptors, and in doing so bring the toxicity that is intrinsic to the toxin molecule toward only those cells that displayed the new targeted receptor? And the answer to that question happily was yes.4:25 into mp3 file http://yalecancercenter.org/podcast/feb2711-cancer-answers-murphy.mp3Foss That was really a ground-breaking concept at the time, to take a molecule like diphtheria toxin and reengineer it effectively

so that you could use the toxin part of that molecule to kill, in this case, cancer cells.Murphy That is correct, and while at the time, the work was considered to be a ground breaking, it really stemmed from our basic understanding of the science of diphtheria toxin as well as the science of the interleukins, in this case interleukin-2.Foss You had spent a long time studying diphtheria toxin in the lab. Can you tell the audience a little bit about diphtheria toxin? I think we all are familiar with it because we get a vaccine as children to prevent us from getting diphtheria, but I do not think that most people really understand the toxin itself.Murphy Diphtheria toxin is a protein that can be thought of as divided up into three different segments. So, if you were to look at your left hand and stick out your thumb, your thumb would be that part of diphtheria toxin, which when delivered into the cell causes inhibition of protein synthesis and actually eliminates or kills that cell. Your middle three fingers are that part of diphtheria toxin that forms a core in the cell membrane allowing your thumb to enter into the side of the cell. And your little finger, which is sticking out, represents the native receptor binding domain. So again, what we did many years ago was, in effect, take away the little finger, and replace it conceptually with a pen. In this instance, the pen was interleukin-2, so the fusion protein toxin, Ontak, then only goes to cells with interleukin-2 receptors. Again, the basic science behind the technology is rooted in the understanding of the basic biochemistry of the toxin itself.Foss There are plant toxins and animal toxins. Are there are other molecules or drugs that capitalize on this toxin technology?Murphy There are several that are in development for the clinic. Very early on, there were three toxin molecules that were used in a way to try to target cancer cells with a very high degree of specificity and selectivity, and those three toxins were diphtheria toxin, which has been the focus of our work, Pseudomonas exotoxin A, another bacterial protein toxin and work with Pseudomonas exotoxin A was largely centered in the laboratory of Dr. Ira Pastan at the National Cancer Institute, and the third toxin that was used was a plant toxin, Ricin, and the Ricin A chain was linked to monoclonal antibodies to make what we know as immunotoxins, part bacterial or plant toxin, part monoclonal antibody in the hopes, again, to develop drugs and biologic drugs directed toward particular forms of cancer.Foss So, there is a difference between an immunotoxin and a fusion toxin. Can you just go over that a little bit for us?Murphy An immunotoxin is really a conjugate, and by a conjugate I mean that each of the components are purified separately and then they are joined together chemically in the laboratory to make a8:28 into mp3 file http://yalecancercenter.org/podcast/feb2711-cancer-answers-murphy.mp3 conjugate molecule, and that linkage is generally made through a bond that is called a disulphide bond and one has to modify chemically each component and then mix them together so the disulphide bond will form between the two. That would be an immunotoxin because of the use of a monoclonal antibody for the targeting aspect of the drug that is ultimately formed. In contrast to that, fusion protein toxins are designed in the computer, assembled at the level of DNA, at the level of the gene, and that gene is put into usually a bacteria such as E. coli. And E. coli then makes the fusion toxin rather than having to assemble it outside of

a living organism. So E. coli makes a recombinant protein that is the fusion protein toxin that then can be purified and used for the development of a therapeutic.Foss Can you talk to our listeners a little bit about the whole concept of giving this diphtheria toxin molecule to patients? We know that we're all immunized against diphtheria and that diphtheria is obviously a bad thing. So there is a little a bit of concern, probably, on the part of some of our listeners in thinking about the fact that we're actually using this and giving it to patients.Murphy One has to step back for a moment and recognize that number one, diphtheria was an important disease until the development of the diphtheria vaccine, which is now given to virtually all children in the United States except those who choose not to be immunized for religious reasons. So diphtheria as a disease was an important disease up until the early 1940s into the beginning of the 1950s when mass immunization began. With the vaccine against diphtheria, that disease has been largely eliminated within the United States. There are less than a handful, that is less than 10 cases per year, and again most of the cases that are seen are seen in individuals who for one reason or another have not been immunized against the disease. There is a vaccine against diphtheria that is wonderfully effective. What we realized very early on was that the antibodies that are generated upon vaccination that prevent the action of the drug, or the action rather of diphtheria toxin itself, are antibodies that are directed against the little finger that I talked about just a moment ago and that is the native receptor binding domain, so neutralizing antibodies will bind to that little finger, if you will, and block the toxin from binding to its receptor, therefore neutralizing its activity. In the case of the fusion protein toxins, we have taken away the little finger and we have replaced it with another protein, usually a human protein, that is not recognized by the human immune system in an avid way, that is, to generate antibodies against it, and so as a result, anti-diphtheria toxin antibodies that are present in serum do not bind to the IL-2 component and therefore are unable to block the action of the fusion protein toxin. That allows us then to develop therapeutics that are directed in a cell receptor specific way that can be used to treat refractory human disease.Foss When you first came up with this concept to use diphtheria toxin as a way of killing tumor cells, you picked the interleukin-2 receptor as one of your first targets. Could you just go through how you actually made that choice?Murphy The first fusion protein toxin that we assembled at the level of the gene, we used a small peptide hormone called alpha melanocyte stimulating hormone, as the targeting ligand. When we put that gene into E. coli, E. coli recognized sequences and destroyed most of the protein that was made. 13:34 into mp3 file http://yalecancercenter.org/podcast/feb2711-cancer-answers-murphy.mp3 We then went to the next largest molecule which was interleukin-2, rather than being 13 immuno acids it was 133, that solved the problem of proteolytic degradation.Foss That is a really interesting story and we are going to hear the second half of that story when we come back after the medical minute. Please stay tuned to learn more about targeted therapies for cancer from Dr. John Murphy.Foss Welcome back to Yale Cancer Center Answers. This is Dr. Francine Foss and I am joined today by my

guest Dr. John Murphy. Today we are talking about fusion toxin therapy for cancer. Jack, we talked a little bit before the break about the choice of the interleukin-2 receptor as your target for this first fusion toxin that went into clinic. Can you talk a little bit about this whole process of bringing a molecule from bench to bedside, what were the hurdles that you had to overcome?Murphy It is an interesting question. The hurdles were really quite significant. At the time that this early work was done it was pioneering work and there were segments of the population who believed it should never have been done in the first place. This was in the early days of genetic engineering and recombinant DNA and so there was a lot of work that needed to be done in order to convince the public, as well as the funding agencies that supported the development of this work, that it was in fact worthwhile to do. The early experiments that were performed were actually performed under so called, Biosafety Level 4 containment. Remember, we were after all developing a brand new toxin, a toxin that the world had never seen before through recombinant DNA activity. Because of that, and I think prudently so, first experiments were done in maximum containment laboratories which would ensure that there was no release of organisms into the environment. After 18 months of investigation under maximum containment we then were able to demonstrate conclusively that working with these molecules in the laboratory strains of E coli did not, in fact, pose any threat to either the environment or to people and as a result those experiments were 17:26 into mp3 file http://yalecancercenter.org/podcast/feb2711-cancer-answers-murphy.mp3 downgraded from Biosafety Level 4 to Biosafety Level 2, which is the normal laboratory environment.Foss In your position at Boston University you were in the unique position to actually be able to not only develop this molecule, but actually see it go into clinical trials. Can you talk to us a little bit about that first clinical trial and how excited you were to see your molecule actually being given to a patient?Murphy It was really quite humbling to be honest with you. I remember very well the first patient who was sent to our medical center at Boston University from Massachusetts General Hospital. He was, at that time, a 53-year-old man with a five year history of cutaneous T-cell lymphoma. His disease continued to progress despite many different kinds of intervention, as you might imagine, of photopheresis, chemotherapy, and electron beam therapy, to name just a few. Because his disease continued to progress, he was sent over to our medical center and was one of the very first patients to be treated. I remember very well not only the excitement but also the anxiety of following this first patient, not knowing what this new recombinant toxin would do when given to a human. The transformation and the resolution of his disease was, in fact, remarkable. After a single injection once a day for five days he was sent home for a 21-day rest and when he came back his disease was already beginning to resolve. After his second course of therapy, he went almost into complete clinical remission followed then by his third and fourth courses of therapy where he achieved a complete clinical response. I followed this gentleman for the next 10 years and he remained free of disease very happily playing golf in Florida.Foss And that is a dramatic story.Murphy Very gratifying for the investigator.Foss In that

early experience I understand you noticed that there was some problems with the way the drugs was formulated in terms of the aggregation of the drug in the solution and you went back to the lab and you redesigned the drug. Can you talk a little bit about that?Murphy This is what we have done over the past several years now. One of the most concerning adverse effects that is associated with the drug Ontak is the fact that some patients develop a vascular leak syndrome upon being given the drug. This can range from very mild symptoms to life threatening pulmonary edema in these patients. So, we went back to the drawing board and identified amino acid sequences within the drug itself that gave rise to this vascular leak syndrome through again recombinant DNA in site directed mutagenesis. We have now been able to change those amino acid sequences and we have what I would refer to as the next generation of this first fusion protein toxin that we are trying to bring into the clinic as we speak. The hope is that this new generation will be safer, will be better tolerated by patients who receive it and best of all we will able to perhaps use a little more of this drug in treating patients and see even better outcomes in the future.21:48 into mp3 file http://yalecancercenter.org/podcast/feb2711-cancer-answers-murphy.mp3Foss This vascular leak that we see with Ontak is not specific to this particular molecule, it does occur with interleukin-2 as well.Murphy Yes, that is exactly right, and so there is a component that can be ascribed to interleukin-2 and there is a component that can be ascribed to the toxin component of it. The amount of Ontak that's given is generally much less than the amount of, for example, recombinant interleukin-2 and so the interleukin-2 component of the vascular leak that is seen, I think is less then that of the toxin component, and by changing those toxin sequences we retain the same potency toward targeted cells and at least in experimental animal we have seen markedly less adverse effects upon administration of the new drug.Foss Just to let our audience know, we are talking about a very minimal syndrome that we see in patients who develop some fluid retention, some of those patients put on weight, many of these patients tolerate that reasonably well with the existing drug, but what we were looking toward is trying to improve that for the future to significantly decrease some of these side effects.Murphy That is correct, and our hope is that if we are able to decrease some of these side effects, this particular targeted agent could be used in a variety of clinical circumstances, which continue to need new advances for therapeutic intervention.Foss Jack, after you designed the first molecule, the Ontak molecule, which was FDA approved back in 1999, have you thought about other molecules that could be designed on this diphtheria toxin backbone and where do you see that going?Murphy We have given a lot of thought to that. There are two molecules that we have been developing in the laboratory. The first is rather than using interleukin-2 as a targeting agent, using interleukin-3 as a targeting agent. And in this instance, patients with refractory acute myelogenous leukemia might be a population that could benefit from such an agent. The second generation, or the second molecule that we are working on in addition to the IL-2 and the IL-3 molecules is using epidermal growth factor as the targeting component. And in this instance, we envision that patient's with brain

cancer, particularly glioblastoma, might benefit from such an agent.Foss I understand there is also an expanded use of Ontak as a molecule that targets the interleukin-2 receptors. We have talked a little bit about its use in cutaneous T-cell lymphoma, but there are also potential uses in other diseases that are associated with these activated T-cells and also perhaps in tumor immunity as well. Could you talk a little bit about that?Murphy Sure, the drug Ontak targets the high affinity IL-2 receptor on the surface of cells. We know that there are several types of cells, T-cells, which have that receptor. One type of T-cell that displays these receptors are so called T regulatory cells and T regulatory cells serve to, if you will, suppress an immune response against a particular antigen. It is the body's way of maintaining a balance of the immune system. In many instances, solid tumors grow in patients because their own immune26:12 into mp3 file http://yalecancercenter.org/podcast/feb2711-cancer-answers-murphy.mp3system no longer recognizes them as being foreign. What this means is that there are T suppressor cells preventing an immune response against that solid tumor, thereby allowing it to grow. We have thought a great deal about using Ontak or this next generation IL-2 receptor targeted drug that we're developing to deplete or diminish the number of T regulatory cells with the hope that doing so would then allow the host, that is the patient, to develop their own immune response against a tumor, a solid tumor. That approach coupled with active vaccination against that tumor offers an exciting avenue of research for the future.Foss There has been some really exciting data recently looking at this molecule in metastatic melanoma. Can you talk a little bit about that?Murphy There has been and the results that have been published are really fueling our enthusiasm toward this approach. A metastatic melanoma, as you know, does not have the receptor for interleukin-2 on its surface and the use of Ontak then to eliminate or to deplete T-regulatory cells has been shown to be a really quite encouraging in that in early reports about 30% of the patient's that have been treated have had a clinical response following administration of Ontak through the development of their own antibodies against the tumor and some of those patients have gone on to do extremely well.Foss This story has come a very long way since that day in 1999 when this was FDA approved. My last question for you is if you could just reflect on how you felt back then in 1999, where you felt that this molecule was going and where it has gone today and give us your thoughts about that.Murphy The development of this agent over the years has been slower in fact than I would have hoped for. The commercial entities that took over the control initially were interested primarily in using this agent to make revenues for their company, appropriately so, but they did not seem to understand that drug development is an iterative process. One needs to look at results that are generated in the clinic and go back to the laboratory, solve problems that arise, and keep refining agents until one can have the best possible agent to use clinically. Dr. John Murphy is Professor of Medicine and Chief of the Section of Molecular Medicine at Boston University School of Medicine. Dr. Murphy developed the first fusion toxin protein to be approved for clinical use in the treatment of patients with T-cell lymphoma. If you have questions or would like to share your comments, visit yalecancercenter.org, where you can

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