

Dr. Peter Glazer, Understanding DNA Repair March 20, 2010 Welcome 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 he is 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, we are pleased to welcome Dr. Peter Glazer. Dr. Glazer is Robert E. Hunter Professor of Therapeutic Radiology, Professor of Genetics, and Chair of the Department of Therapeutic Radiology at Yale School of Medicine. He joins us this evening to talk about DNA repair. Here is Lynn Wilson. Wilson Let's start off by having you tell us a little bit about what DNA repair is? Glazer DNA repair is the process in a cell by which the cell tries to fix damage that occurs to its genes and chromosomes. DNA repair actually occurs through several different pathways in response to various different kinds of DNA damage. One of the most common types of DNA damage is what people experience when they go to the beach and get sunlight damage to their DNA, and that's why sunscreens are recommended, but the fact of the matter is that the DNA does get damaged by ultraviolet light in the sun and our cells, DNA repair enzymes, need to come in and fix that. You can have DNA damage from chemicals such as from smoking, or from diesel fumes, even some of the compounds that we eat lead to reactive chemicals in the body that cause damage to DNA and then other sources such as radiation can lead to breaks in the DNA that need to be repaired. Wilson What sorts of effects can chemotherapy have on cells, does that interact with DNA as well? Glazer Many of the chemotherapy agents that are used, and that are effective, have as their targets the DNA in the cancer cells. One of the most powerful chemotherapy agents is something called cisplatin and this agent acts by creating platinum adducts on the basis in the DNA and blocking the function of the genes. Wilson Tell us a little bit about what it is that you do within the Department of Therapeutic Radiology. Glazer As you mention, I am chairman of the department and so I have a fair amount of administrative duties which mostly consist of trying to keep everybody else happy so they can do their jobs, but I also do a fair amount of basic science, laboratory research, focused on cancer biology as well as some clinical care of patients receiving radiation treatments. Wilson What first got you interested in studying genes and cancer? Glazer Well, I was in college in the mid 70s, which really was the beginning of the molecular biology revolution, and I took a course in genetics that opened my eyes to the possibilities of the power of genetics and how that could be used to solve or address problems like cancer. 3:35 into mp3 file <http://yalecancercenter.org/podcast/mar2011-cancer-answers-glazer.mp3> Wilson Talk to our listeners about how DNA works and tell us more details about DNA repair. Glazer As you know, DNA is the material that makes up our genes and chromosomes. It consists of what our called bases, adenine, guanine, cytosine, and thymine (AGCT) which form the genetic code, and DNA actually is a double helix, and that's the famous discovery of Watson and Crick, and so DNA forms a double helix, but is subject to different

kinds of stresses in the cell and these can lead to DNA damage either from environmental stressors or endogenous factors. I mentioned some of the environmental stressors earlier, but DNA damage can occur in the cell also when there is a problem during replication, such as stalling during the period of time when the DNA is being replicated, so a cell can divide that can lead to breaks in the DNA strand. There can also be errors when the DNA is being copied, and if one chromosome is being copied into two, those errors are similar to spelling errors on the computer and you need a spell check, a program to come in and find the mistakes and then correct them.

Wilson What's the difference between DNA and RNA? Glazer DNA has the genetic blueprint that codes for RNA, which is expressed or transcribed from the DNA, and RNA can take many forms. The classical form is called messenger RNA which conveys the message of the DNA into a protein, so the messenger RNA becomes translated from an RNA sequence into a polypeptide protein sequence, and it is the proteins that form all the enzymes and structural components of our cells. It turns out there are many other types of RNAs that have been newly discovered, one of them is something called microRNAs, which were subjective of a Nobel Prize awarded recently and these seem to have a very fascinating regulatory role in the cells.

Wilson What role does DNA repair play in cancer therapy? Glazer Many of the agents that we use to treat cancer, such as radiation therapy, and many chemotherapies we have talked about such as cisplatin, cyclophosphamide and others, interact directly with the DNA and either form chemical changes in the DNA or actually break the DNA strands, which is what radiation can do, and that's where DNA repair comes in. One of the reasons that we are able to use these sorts of agents to treat cancer effectively is that cancer cells tend to be dividing more rapidly than our normal healthy cells, and as a result, they have less time to fix their DNA. They also tend to be a little sloppier in terms of how they repair their DNA. Some of these pathways are abnormal, in fact that's some of the area that we study in our own lab but this sloppiness leads to some increased susceptibility to agents that target the DNA. Also, cancer cells, in addition to dividing more rapidly, have some abnormalities in some of the processes in the cell that slow down the cell division when DNA damage is sensed. These processes are sometimes referred to as checkpoints, where the cell has a process of checking the DNA to see if it is intact before the cell divides. Normal cells will halt cell division to give time for repair, but cancer cells often lack that checkpoint and as a result would go on to divide with broken DNA, and again, the cancer therapy that targets the DNA takes advantage of that.

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Wilson It sounds like typically, normal tissues, skin, and other organs, are better at repairing this damage than a tumor is, for example, and that gives us a therapeutic advantage in some cases.

Glazer Right, in some cases there are subtle differences between the DNA repair capacity of cancer cells and normal healthy tissue that comes from a combination of the factors I talked about before, the lack of the checkpoints, the more rapid cell division, as well as some abnormalities in the repair pathways themselves.

Wilson Tell us a little bit about genetic instability, what is that in cancer? Glazer Cancer is not

only more rapidly dividing, but as I said, they are a little bit sloppier in terms of how they manage their DNA, they lack those checkpoints for regulating the speed of cell division and as a result they tend to incur many more mistakes and abnormalities in their DNA as they divide and the genetic instability can be at the level of broken or missing chromosomes, or too many chromosomes because the cell division does not occur properly, so the chromosomes do not segregate to the daughter cells properly. There can also be so called point mutations where single base pairs, those A, G, C, and T letters, are incorrect, but the cancer cell does not fix that properly. So there are many levels of genetic instability and this is a key feature of cancer cells. It is obviously not a good thing for normal cells to have genetic instability, but cancer cells actually can benefit from the genetic instability because it creates genetic diversity in the cancer and it sometimes allows the cancer cells to develop resistance to cancer therapy more quickly.

Wilson Explain for our listeners, Peter, what a mutation is? What does that mean? Glazer A mutation is a change in the sequence of the DNA. I mentioned earlier that the DNA is made of beads on a string with the letter A, G, C, and T and the Watson and Crick discovery in part showed that A binds to T and G binds to C and so the double helix has the sequence code and that sequence code is read during the transcription of DNA into RNA. A mutation is an alteration in that sequence code so it could be a G in place of what should be an A, it could be a rearrangement where a piece of the DNA is flipped around backwards, it could be an insertion of a string of letters from one up to hundreds. Any change in the sequence is a form of mutation.

Wilson And have some of these have been well identified in cancer? Glazer It turns out that there are a number of key genes that researchers have found are mutated in cancer cells, and many people feel a link to the causation of the cancer. One of the most famous is a gene called RAS, and another one is called RAF. Mutations in RAF are linked to melanoma. Mutations in RAS are linked to many types of cancers including cancers in the pancreas, lung, and other sites. More recent discoveries have identified mutations in genes linked to familial breast and ovarian cancers and those genes go by the name BRCA1 or BRCA2 and BRCA is lingo for BRCA1 or BRCA2 for a breast cancer associated gene, and individuals can inherit one of these genes with a preexisting mutation that inactivates the functions of the gene and what people think happens is 12:29 into mp3 file <http://yalecancercenter.org/podcast/mar2011-cancer-answers-glazer.mp3> that the good copy of the gene is lost in a particular cell and that creates an increased risk of that cell becoming malignant.

Wilson In certain mutations, do we have some medicines or drugs that might be more effective for the patient with that problem? I have heard some things regarding lung cancer mutations. A patient might have a certain mutation and they might be more sensitive to a particular drug than a patient without that mutation.

Glazer That's right, so part of the elucidation of these mutations in these key genes has been understanding their function, how these mutations impact the function of the protein as well as the structure of the protein and how it behaves, and that has allowed people to design agents, small molecule drugs usually, or sometimes antibodies, the target those specific pro-

teins or mutated proteins and take advantage of that. In fact, there is a gene called Epidermal Growth Factor Receptor, which is a protein that is on the surface of many cells including cancer cells in the lung, and it turns out that if an individual's cancer has a mutation in the EGF receptor gene that cancer will be susceptible to a certain class of drug. Another example that is somewhat related to research in our own lab is as I mentioned, the BRCA 1 or BRCA 2 genes linked to breast cancer. Mutations in those genes actually impact the ability of the cancer cell to conduct DNA repair by a particular pathway. Those mutations give the cancer cell increased genetic instability and help turn it more malignant, but also create a little bit of an Achilles heel for the cancer with agents that exploit that deficiency in DNA repair and there are a number of agents that are now in clinical trials to take advantage of that deficiency. There are some trials going on at Yale-New Haven Hospital with those agents and in our own lab we are actually not only testing those agents themselves, but in combinations with other potential chemotherapy agents as well as radiation, and we have also been working to develop newer agents that may be even more selective or more potent. Wilson We are going to take a short break for a medical minute. Please stay tuned to learn more information about DNA repair with Dr. Peter Glazer.16:26 into mp3 file <http://yalecancercenter.org/podcast/mar2011-cancer-answers-glazer.mp3>Wilson Welcome back to Yale Cancer Center Answers. This is Dr. Lynn Wilson, today I am joined by Dr. Peter Glazer and we are discussing DNA repair. Peter, in the previous part of the show you mentioned antibodies, monoclonal antibodies, and small molecules, define for us what these things are, what they mean, and how they work on the cell?Glazer Antibodies are actually large protein molecules that our own bodies make as part of our immune defense mechanisms against infectious agents, and research almost 30 years ago found that antibodies could be raised in mice and the cells making them could be harvested such that the antibodies could be produced in a test tube and collected for use as a medicine, and the key there is that the mice are challenged with different proteins or antigens that might be related to factors that are important for the cancer cell to grow. So, in this way, monoclonal antibodies are developed and monoclonal simply means it comes from a single clone of an antibody producing cell, and I will not go into all the mechanics of doing that, but there are ways of isolating that cell and collecting the antibody for use as a medicine, and these are particularly promising types of cancer therapy agents. Some of the current ones that are being used in the clinic are Herceptin, which targets a protein on the surface of breast cancer cells. There are several that target the Epidermal Growth Factor Receptor I mentioned before, which is a protein on the surface of several types of cancer cells including those in the lung and the colon, as well as cancers in the head and neck region, and many more antibodies are being developed as cancer therapeutics, and how they work is still being studied, but part of the way they work is interfering with the normal function of whatever protein they stick to by binding to it and sort of mucking it up. They may also help to elicit an immune response to the cells that they stick to, so a combination of those actions is important for the effectiveness of an antibody. Small molecules are simply a term used to broadly

refer to chemicals that are the more conventional types of medicines that we have and these can be synthesized in a chemistry laboratory by pharmaceutical companies and they are similar to many of the medicines that we take everyday in pill form. Wilson Why are they called small molecules, is that because they need to get themselves inside of another cell? Glazer This is a little bit of scientific jargon to differentiate the molecules I just described from larger molecules like antibodies or things of that nature. Wilson Tell us a little bit about what hypoxia is and how that can play a role in cancer therapy? Glazer One of the key features of tumors is that their growth outstrips their blood supply, not so much that they do not grow, but to the point where many regions of the cancer do not get the kind of blood flow that healthy parts of the body get, and as a result they do not live in an area with the level of oxygen that is present in our healthy tissues. The lower oxygen levels in parts of tumors are referred to as regions of hypoxia, meaning low oxygen. What happens with hypoxia, if it is extreme it will prevent the growth of the cells, but it usually does not get so extreme that the growth is completely blocked, it may be slowed, but hypoxia profoundly changes the biology of 21:24 into mp3 file <http://yalecancercenter.org/podcast/mar2011-cancer-answers-glazer.mp3> the cancer cell. One of the main things it does, is it stimulates the cancer cell to send signals to nearby blood vessels to grow and expand so that more blood vessels grow into the tumor. The process of recruiting blood vessels into a tumor so that it can grow and expand and get bigger is called angiogenesis and is one of the fundamental discoveries of the last 20 or so years that has led to a lot of effort to develop medicines and strategies to interfere with angiogenesis. However, another thing that the low oxygen or hypoxia does is it changes the internal biology of the cancer cells. One aspect of that is, it makes the cancer cells more dependent on the sugar called glucose for their energy source and so that is in part the basis of why we use PET scanning today to identify tumors that take advantage of the high utilization of glucose in tumors. In addition, hypoxia affects the expression of many genes in the cancer and so it changes the constellation of activities in the cancer cell creating a situation where the cancer cell is somewhat different than healthy tissue and that is an area that our lab, and several labs at Yale, are interested in exploiting for cancer therapy. One particular way that hypoxia leads to a difference in cancer cells is by altering the DNA repair capability of the cell. It turns out hypoxic stress causes the cancer cells to decrease their capacity to carry out DNA repair in certain ways and our own research has discovered some of these steps and now we are designing new medicines that might be used to exploit that. Wilson How does hypoxia impact the efficacy of radiation treatment, for example? Glazer It turns out that radiation causes damage to tissue when the x-rays or photons pass through the tissue and knock electrons off of atoms in the tissue, and that is a process called ionization. That process creates radicals, so called ion radicals, which can then interact with other molecules in the cell including DNA. In the presence of oxygen, there are additional radicals and other species are produced that are longer lived and can cause more damage throughout the cell. When there is no oxygen present, there is less damage that is produced to the DNA. Wilson I

see. So, preferentially, we would not want to have hypoxic tumors if we want radiation to work as well as it can?Glazer That is right. Hypoxia is a double edge sword, or maybe a triple or quadruple edge sword really, because it affects the cancer cell in many ways, some to make them more susceptible to therapy, but some to make them less susceptible.Wilson Let's shift gears for a minute Peter, the NIH, the National Institutes of Health, is our governmental funding agency and for our listeners, that process by which we can obtain grants for research funding is extremely competitive. There are lots of different kinds of grants and recently you were awarded what is called a Program Project Grant, and you are the principal investigator of that program. Can you tell us the details about that and how important that is?Glazer The Program Project Grant is a type of funding mechanism from the National Cancer Institute, of the National Institutes of Health, that is designed to support cancer-related research of a team at a26:01 into mp3 file <http://yalecancercenter.org/podcast/mar2011-cancer-answers-glazer.mp3> particular institution, whereby investigators doing related work can get together and develop an integrated interactive research program that is highly focused on some aspect of cancer research. It is a little bit different than a research grant awarded to an individual investigator, which is a very important and highly valuable thing. This is a way to bring together investigators in a focused way.Wilson Tell us a bit of the details. Are there multiple projects within this Program Project Grant?Glazer There are four projects, and each is led by a project leader, not only myself, but three other faculty at Yale, Alan Sartorelli, Joann Sweasy and Patrick Sung, and these projects are focused in the area of DNA repair, with the ultimate goal of developing new cancer therapies. My particular project is looking at how DNA repair is different in cancer cells and how that can be exploited for therapy. Joann Sweasy is looking at the fact that some DNA repair genes are mutated in cancers and whether cancers with specific mutations and specific DNA repair genes may be more or less susceptible to existing therapies. Patrick Sung is looking at some novel interactions among DNA repair factors in a cell and has discovered that there are some key interactions that could be the targets for novel therapies, and Alan Sartorelli continues a long track record of developing novel agents that can be used specifically to target cancer cells. He is a pharmacologist and he has already synthesized a number of molecules that are in clinical trials and this Program Project is supporting his work in that area.Wilson Obviously important discoveries cannot be made without that support. Peter, what do you think have been some of the most important DNA repair discoveries say over the last ten years?Glazer It is interesting, when I started in this field 20 years or so ago, DNA repair was thought to be just a house keeping function of the cell and was not thought to be that important. Well, it turns out that as I said, many DNA repair genes are associated with inherited cancer such as the BRCA1 or BRCA2 genes and so our understanding of that has now opened up the possibility of exploiting these DNA repair deficiencies in cancer.Dr. Peter Glazer is Robert E. Hunter Professor of Therapeutic Radiology, Professor of Genetics and Chair of the Department of Therapeutic Radiology at Yale School of Medicine. If you have questions or would like to share your comments, visit

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