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Immunotherapies for Cancer

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Yale Cancer Center Answers

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Welcome to Yale Cancer Center Answers with Dr. Francine Foss and Dr. Lynn Wilson. 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 week, Dr. Wilson is joined by Dr. Cliona Rooney for a conversation about immunotherapies for cancer. Dr. Rooney is a Professor of Pediatrics Pathology and Immunology at Baylor College of Medicine. Here is Lynn Wilson.

Wilson Let's start off by having you tell our listeners a little bit about what it is that you do at Baylor?

Rooney I am an immunologist by training and I am interested in how T lymphocytes, which are white blood cells, recognize abnormal cells like virus infected cells and tumor cells and kill them, and I got interested in this field when I was working at St. Jude Children's Research Hospital and patients receiving bone marrow transplant were developing lymphomas caused by the Epstein-Barr Virus, and the reason that they develop these lymphomas is because after transplant they are very immunosuppressed and they do not have very many T cells, and it takes T cells a long time to recover after the transplant, and because T cells are responsible for controlling virus infections, that is why these tumors develop. During my post-doctoral studies in England, I learned how to make EBV specific cytotoxic T cells and these are lymphocytes that are able to recognize a cell that is infected with EBV like these lymphoma cells and kill it, and so I started to make T cells from the blood of bone marrow donors and we started to infuse them into the patients who develop lymphoma and what we found, to our surprise, was that a very small numbers of T cells after infusion would start to proliferate, or divide. So if you put in 1 million cells, you might end up with 10 million or 100 million or 1000 million cells in a very short amount of time and those T cells were able to eliminate the lymphomas in these patients and they were also able to prevent lymphomas from developing in patients at very high risk because they had large amounts of virus in their peripheral blood. This was very exciting research, and at that time, it was quite new and so we got interested in furthering this type of research to be able to control patients who are getting infections with other types of viruses and patients who are developing other types of cancer. The reason the T cell therapy is very interesting is because T cells are very specific for a virus infected cells. So, if you have one virus infected cell among a lot of normal cells, it will kill the virus infected cell and won't hurt the normal cell. So, patients who receive T cell therapies do not get sick, they do not lose their hair, they do not feel nauseous, and they do not have fevers, very rarely, and there are no long-term negative consequences like one sees with standard therapies for tumors and so it is a very gentle therapy and very tolerable for the patients.

Wilson For a patient who has developed lymphoma in the case that you described, is this a common problem for patients undergoing a transplant?

Rooney At that time, it was a cause of death in about 10% of patients who were receiving a bone marrow

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transplant from an unrelated donor and the main problem those patients have is a disease called graft-versus-host disease, so basically these patients are patients who have some kind of hematological malignancy, and so the patients are treated with chemotherapy and they receive total body radiation to kill all the tumor cells that they have and then they get bone marrow from a healthy donor whose HLA matched with them to reconstitute the immune system, but this reconstitution takes a long time and what the doctors does is remove the T cells, so the T cells do not recognize the patient as foreign and start to attack the normal lymphocytes, but because they remove the T cells, the patients are susceptible to virus infections, and EBV is one of the most problematic ones because it can cause lymphoma and can kill the patient, and at that time there were no antiviral drugs that you could use to cure the patients and so most of the patients died.

Wilson I see, and you mentioned EBV is the most common, what are some of the other ones that are concerning?

Rooney We all carry viruses and so almost all of us are infected with EBV, with cytomegalovirus, varicella-zoster virus, adenovirus, a lot of common viruses that we carry with us that we are frequently exposed to and we might have a short illness and then get better. Those types of viruses can be lethal for patients who do not have enough T cells to control it, and so one of the ways that were extending T cell therapies it to identify T cells that can protect against other types of viruses and we're interested particularly, in addition to EBV, in cytomegalovirus, and adenoviruses, and we're beginning to perform studies with a virus called BK virus which causes kidney problems and also human herpesvirus type 6, that also i a problem in those patients.

Wilson Can you tell us a little bit more, especially for our listeners who do not have any scientific background, what exactly are T cells and what separates them from some of the other fighting cells that we have such as B cells, what are the differences?

Rooney We have a lot of fighting cells in our blood, we have red cells and white cells, and the white cells are really the fighting cells and those cells patrol the body looking for trouble and looking for abnormal cells, and the T cells are really the troops, the affected cells and they go and recognize abnormal cells and kill them. B cells also recognize abnormal proteins and B cells secrete antibodies, so the antibodies are able to bind to the bacteria and help to eliminate bacteria. They can also bind free viruses and cause those to be killed, but the problem is when the virus gets into a cell, once inside the cell then it can become invisible to B cells and the antibodies cannot recognize an infected cell from the outside, but the way T cells can recognize a cell is that all cells have molecules on them called major histocompatibility molecules so that is a kind of molecule that carries fragments of proteins in the cell to the outside of the cell and displays them on the cell surface and they display in a way that antibodies cannot see them, but T cells can see them, and T cells are really amazing because each T cell has a receptor that recognizes one of these fragments

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associated with one of the molecules that carries it to the cell surface and they estimate that the number of possible different combinations of different receptors is about 10 to the 16th.

Wilson Wow!

Rooney That is a huge number of different types of receptors, but within a single individual probably the number of different T cells is about 2 million. So, the T cells are capable of recognizing 2 million different small fragments, and many of the T cells never get used and they die. But if the T cell sees a fragment that it recognizes, then it starts to divide and proliferates so it increases in numbers, and if it is specific for a pathogenic like Epstein-Barr virus, then it will increase to very large numbers. So as long as the virus is there, the T cell will increase in number so they can eliminate the problem, the cause of the infection, and then after that, most of the T cells will die, but a small fraction of the T cells will remain so they will be present in higher numbers than they were before you were infected with the virus, and in those types of numbers they are able to, if you get a second infection, to proliferate very quickly and prevent that infection from taking off and that is why if you have been infected with a virus once, then you are usually protected against it the next time and it is also the way vaccines work. What a vaccine does is increase the number of T cells specific for a certain antigen and so that if you get infected with that virus for the vaccine then you have enough T cells to control it and stop you from getting sick.

Wilson If someone got chickenpox, for example, when they were a child, typically it's reasonable to say they would probably never suffer from that again?

Rooney Yes, that is right, but chickenpox can actually reactivate later in life, and when people are stressed, it could be emotional stress and it could be just age, as your immune system ages it gets less potent and the chickenpox virus, which after you had your primary infection actually goes and hides in your neural tissues, it can reactivate and come out and cause zoster or shingles, which is a very painful form of disease and we're actually now trying to develop T cells specific for the chickenpox virus to protect patients who may develop it when they are older.

Wilson This activity of the T cells, it sounds like it is not as if they are dormant and only get revved up when there is a super special infection, but it sounds like they are doing work in the background all of the time, 24 hours a day.

Rooney They are, and it is very interesting, we study patients who have been vaccinated for smallpox, and nobody is vaccinated for smallpox anymore because it has been eliminated because of the vaccination, but people of my age were vaccinated as small children 50 years ago, and if we can take my blood and reactivate T cells specific for the vaccine virus, which is called vaccinia, and

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the T cells are still there ready to be activated if I was exposed to the virus, so our T cells last for a very, very long time when they have been activated in the first place.

Wilson Tell our listeners a little bit about why cellular immunotherapy is a good treatment option and tell us about the potential toxicities?

Rooney Cell therapy is a good treatment option because it is safe, it is so specific, and because T cells kill only the cells that are infected with the virus, or only the tumor cells and they do not kill normal cells. They are very good for the treatment of virus infections because we have been exposed to the virus and we have this type of memory that I told you that lasts for a very long time. It is very easy to grow those cells in culture and give them back to somebody who may be immunocompromised, so lack of toxicity and high specificity are the good parts but, of course, they are downsides, and it is much easier to target a virus infection or virus-induced tumor because those proteins that are expressed by the virus are foreign to the immune system, but when you have a tumor that is not associated with a virus, for example, melanoma, it is much more difficult to treat because there are no foreign antigens or proteins that the T cells can attack. So, what investigators have to do is try to trick the immune response into thinking that some of the proteins are foreign and trick the T cells into proliferating and attacking that protein but, of course, you have to select proteins that are exclusively expressed on the tumor cells, and when we started 20 years ago, people felt that there would not really be any proteins that the T cell could recognize. Because then it would kill a lot of normal cells. But people are beginning to identify more and more proteins that are expressed in non-viral tumor antigens that can be targeted and are not expressed in normal tissues for various reasons and so there are some proteins that are normally expressed during fetal development and then they shut down. But in tumors, because the regulation has gone slightly crazy, they re-express these fetal antigens and those are expressed for some reason that we do not really understand in many tumors, and so we can target those antigens, and Steve Rosenberg at the NCI has been very successful in targeting melanoma with these tumor antigens to the T cells that you can actually extricate from the tumor and then grow in large numbers in tissue cultures in the laboratory and then he infuses them back into the patient, but they are only effective if he first removes all the lymphocytes circulating white cells in the patients, so that when the T cells go into the patient, because there is an empty space, they start to divide because of what is called homeostatic expansion, where T cells fill up an empty space.

Wilson I see.

Rooney And so then he also requires the addition of growth factors which are proteins that make T cells grow.

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Wilson We are going to take a break for a medical minute. Please stay tuned to learn more information about immunotherapies for cancer with Dr. Cliona Rooney.

*Medical
Minute*

The American Cancer Society estimates that over 1000 patients will be diagnosed with melanoma in Connecticut each year. While melanoma accounts for only about 4% of skin cancer cases, it causes the most skin cancer deaths. Early detection is the key. When detected early, melanoma is easily treated and highly curable, and new treatment options and surgical techniques are giving melanoma survivors more hope than they have ever had before. Clinical trials are currently underway at Yale Cancer Center, Connecticut's federally designated Comprehensive Cancer Center, to test innovative new treatments for melanoma. The Specialized Programs of Research Excellence and Skin Cancer Grant at Yale, also known as the SPORE grant, will help establish national guidelines on modifying behavior and on prevention as well as identification of new drug targets. This has been a medical minute brought to you as a public service by Yale Cancer Center. More information is available at yalecancercenter.org. You are listening to the WNPR Health Forum on the Connecticut Public Broadcasting Network.

Wilson Welcome back to Yale Cancer Center Answers. This is Dr. Lynn Wilson and I am joined by my guest, Cliona Rooney, and we are discussing immunotherapies for cancer. Dr. Rooney, a question that some of our listeners may be thinking about is you have given some excellent explanations of the T-cell and how it works against viruses, but some listeners are probably wondering, you know, a common cold, isn't that a virus? Why if we have T cells and they are operational and I get a cold and it goes away, why do I get a cold again? Why does that happen?

Rooney That is because there are very many different viruses that can cause colds, lots of different virus families, and within those families there are lots of different strains, and some viruses like influenza change their proteins so that they can escape from T cells and that is why we have to have a new vaccine every year to try to protect against the influenza virus and there are millions and millions of viruses. So, I am afraid that probably we are still going to continue to get colds every year regardless of how many we have had previously.

Wilson And that sounds like pretty tricky business though, trying to predict what viruses might be happening next year and trying to come up with vaccines and so on. Can you tell our listeners a little bit about some of the science behind that?

Rooney It is very interesting because the influenza viruses is the main virus where we have a new vaccine every year, and where people have to try to go in and predict what the new influenza virus vaccine is going to be, and the way they do that is to study bird populations, because the influenza virus infects a number of different animals as well as humans, and it infects birds and they do not really get sick but the virus can replicate in them, so people will go and look for new bird viruses and test

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them. They have libraries of influenza viruses that have been around in the past, and if they find a new one that they find all over the world, and if humans have never been exposed to it than the idea is that humans, if they do get exposed to it, will not be immune, and therefore, they will become infected and then they will infect other people and then it will start spreading throughout the population. So, it is slightly hit and miss, they may not always get the right influenza virus.

Wilson What cancers do you primarily focus on in your work?

Rooney I am very interested in Epstein-Barr virus associated cancers and it causes lymphoma that I discussed in bone marrow transplant recipients, but it also causes cancers in individuals who are not immunosuppressed. So healthy people like you and me can develop lymphomas, or a nasopharyngeal carcinoma and even these muscle cell cancers. It is not really clear why we develop them but the virus certainly plays a role in it because we know that the virus can cause cancer but it probably requires other factors and changes that happen in the cell that also contribute to the tumor. These EBV-associated tumors do express some viral antigens so it is very strange to understand why they can grow in somebody who has been exposed to the virus and controls the virus but it does not control the tumor cells and what we found is that we can detect in the circulation of a patient with a tumor, T cells that can recognize that tumor, but yet the tumor grows. This is because all tumors grow only if they can avoid immune response, because if they cannot, they are obviously not going to grow so we will not see them and tumors have got very clever ways to avoid immune response, they secrete proteins that shut off T cells and they recruit other cell types that are also inhibitory to T cells and one of the tumors that is associated with Epstein-Barr virus is called Hodgkin's lymphoma. EBV is not in all of the Hodgkin tumors but in about 30% of cases all the tumor cells express EBV antigens. But if you look at the bulk of a Hodgkin's tumor only one in 100 cells are actually tumor cells and all the other cells are cells that the tumor has recruited or has attracted and the way it does that is it secretes molecules that bring in cells that protect it. So, it brings in these cells called T regulatory cells which are inhibitory cells which protect us against autoimmunity and it brings in eosinophils, mast cells, all sorts of cells that really should not be there, but work to down regulate T cells and prevent the tumor cell from being killed, and so what we are trying to do is understand the ways that the tumor cells can inhibit T cells and then generate T cells that can overcome this kind of inhibition.

Wilson So if somebody is immunocompromised and they have EBV, for example, and develop a lymphoma and therapeutically we eliminate the virus, do we need to treat the lymphoma as well or by elimination of the virus in that particular situation could the lymphoma go away on its own if the viral stimulation is eliminated?

Rooney That is a very good question. What I have to explain is that when you get your first infection with EBV it infects your B cells and epithelial cells and you might not even notice you have been infected or you might have a sore throat, you might get infectious mononucleosis, but after you have controlled the virus it never goes away and you carry that virus in your body for the rest of

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your life in your B cells, and although the viruses has got about 90 different proteins when it is in your B cells it can shut them all down. So the virus DNA is in the B cell but it is not expressing any proteins and in that form it cannot be recognized by T cells but at the same time it is not hurting you in any way and in most cases most of us will carry the virus for our whole lives without even knowing it is there or having any problems with it, but sometimes a mistake can happen and there might be mutations in the cell that is infected with the virus and it can grow as a tumor and so you could eliminate that tumor cell but you will not eliminate the EBV infection so you will still be infected with EBV, but you could have eliminated the tumor. We don't really have a way eliminate the virus from our body with T cells because our T cells cannot recognize the virus infected cells.

Wilson I see, so it is complicated.

Rooney And that is very typical of Herpes viruses, that once they infect us, we might be sick for a short amount of time, but then we control the virus but we never eliminate it. So it lives in our body for our whole life usually without problems.

Wilson How has the research in this field advanced over the last five years or so? What are your thoughts about that?

Rooney In the last five years we have come to realize that for T cell therapy to be effective for a broader range of tumors outside of the bone marrow transplant setting, it is essential that the T cells proliferate or divide after you infuse them into a patient because every T cell that you infuse may have to kill thousands of tumor cells so it is not enough for the T cell just to kill the tumor cells or a few tumor cells and die, it has to proliferate and keep going quite a long time until all the tumor cells are eliminated, and then hopefully, it will reduce in its numbers but it will still be there in elevated levels so that they can reactivate if the tumor would recur but the problem is how do we make sure that the T cells proliferate after we infuse them into the patient, because the tumor cells are trying to stop the T cells from growing. So what investigators in a lot of different groups are trying to do is develop ways to make T cells grow better and one of the ways is to genetically modify them, and that could be modified in very different ways, but I guess the most popular way, is to modify them with a receptor. So a receptor is a molecule that sits in the membrane of the T cell and it sticks out of the T cell on the outside and also on the inside and on the outside you can put a receptor, that combine to a tumor cell and on the inside there are other molecules that tell the T cell to kill that tumor cell and also to proliferate. So we make what is called a chimeric molecule which has signals for the T cell not only to kill but also to proliferate and those types of therapies that are just starting to come into the clinic now, and recently the University of Pennsylvania has reported three complete remissions in patients with chronic lymphocytic leukemia who received T cells expressing one of these chimeric receptors on T cells, these are the types of the ways that we are going forward and I think we really have to make sure

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that T cells proliferate. Another way is to combine T cells with a vaccination so that if you can present the tumor antigen in an immunogenic way away from the tumor site then you might get T cell division and proliferation that is independent of the tumor but those T cells are to proliferate to come and go and kill the tumor and you can continue to vaccinate and increase the numbers of T cells so that you have got large numbers that can hopefully eliminate the tumor and then you can start vaccinating and that is something we have to evaluate in the near future.

Wilson Tell us what a tumor antigen is? What does that mean?

Rooney A tumor antigen can be any molecule that allows a T cell or an antibody to recognize it and it is not expressed on a normal cell. It has to be something that is unique to the tumor so it could be foreign protein from a virus or it could be one of these fetal antigens that I have described or it could be a mutative protein. It is mutated in the tumor cells so it does not have the normal sequence that our T cells would recognize as being healthy cells and so anything that is abnormal can be called a tumor antigen and mostly they are proteins, but they can only be glycolipids or they can be carbohydrates so the chemistry can be very different.

Wilson This might be a complicated answer, but I'll try to simplify it if possible, how is it that my T cells can figure out what is part of me and they do not get fired up about that and how do they recognize something that might look sort of similar that is foreign to my body. I know it is incredibly complicated immunology, but how do you figure that out?

Rooney It is very complicated. T cells essentially have to go to school. So you make new T cells in your bone marrow all the time and they come out of your bone marrow and they go to the thymus, and in the thymus, they get educated about normal proteins and normal proteins are expressed in the thymus, all sorts of different normal proteins are expressed, for example, skin proteins and muscle proteins are expressed in the thymus and T cells that recognize them are killed in the thymus. So the only T cells that come out of the thymus are T cells that don't recognize our own antigens strongly, so what comes out are T cells with the receptors that do not recognize what we call self and they can only recognize a non-self and so the non-self are things like virus antigens and abnormal proteins, and we are lucky with the fetal antigens and this process is called tolerization so the T cell becomes tolerant to the cell. The T cells are not tolerized so much of these fetal antigens although it is true that the T cells, specifically fetal antigens, are often not as strong as the T cells specifically to the viral antigen. So there may be a degree of tolerance.

Wilson Tell us a little bit about some of the research going on in your laboratory?

Rooney In my laboratory we are trying to develop new ways to generate T cells, to have simpler ways to generate T cells, because we are very interested in now that we have developed therapies that are very successful, for example, virus specific T cells after bone marrow transplantation and T cells

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specific for EBV positive lymphomas, we want to try to make these T cells broadly available to all patients rather than the patients who come to our rather small studies, and so to do this, we are trying to make our T cell generation procedures simple and shorter and safer and so we are developing ways of growing T cells more quickly so that we can now, instead of taking about three months to generate T cell specific for viruses, we can now do it in about 10 days and this means that the patient does not have to wait so long because many patients with virus infectious cannot wait because they are too sick. Another thing that we have tried is again, because of the problem of growing cells in even 10 days that might be too long in a patient when they get really sick and they need T cells right away, and so what we have done is we have taken the T cells that we have grown up from healthy donors and we have characterized them, and that means we know what they recognize, what virus proteins they recognize and what tissue antigens they recognize and we can freeze them back in banks so that if you come to me and you say my patient has got an EBV associated lymphoma have you got T cells, I can go to my bank, and I can get the T cells that are hopefully tissue matched with your patient, and I will take those T cells and you can thaw them and infuse into your patient and that strategy has been remarkably successful and much more successful than we thought and that has produced complete remissions in over 50% of the patients with cytomegalovirus and adenovirus infections and EBV infections and with some partial term responses in over 70% patients. So that is a good strategy that they now give you a time window where you might be able to generate specific T cells for that patient.

Dr. Cliona Rooney is Professor of Pediatrics, Pathology and Immunology at Baylor College of Medicine. If you have questions or would like add your comments, visit yalecancercenter.org, where you can also get the podcast and find written transcripts of past programs. You are listening to the WNPR Health Forum on the Connecticut Public Broadcasting Network.