Melanie Cole (Host): Welcome to the Podcast Series from the Specialists at Penn Medicine. I'm Melanie Cole. And today, we're discussing a novel CAR T-Cell medullary thyroid cancer clinical trial at Penn Medicine. Joining me in this panel today are Dr. Roger Cohen. He's a Professor of Medicine at the University of Pennsylvania and Associate Director of Clinical Research, Abramson Cancer Center of Penn Medicine and Dr. Donald Siegel. He's a Professor of Pathology and Laboratory Medicine and Director of the Division of Transfusion Medicine and Therapeutic Pathology at Penn Medicine. Gentlemen, thank you so much for joining us today. Fascinating topic that we're discussing.

So, Dr. Cohen, I'd like to start with you. Medullary thyroid cancer or MTC is different than other types of thyroid cancers. Can you tell us a little bit about this and why recurrent MTC is so difficult to treat?

Roger Cohen, M.D. (Guest): Medullary thyroid cancer is a rare disease. Most of it is referred to as sporadic, meaning we don't understand its cause. There are familial forms of the disease that are caused by mutations in the RET gene. Most thyroid cancer in the world originates from follicular cells. They are the cells in the thyroid gland that make thyroid hormone. Medullary thyroid cancer, however, starts in what are referred to as parafollicular cells. These are cells that live in the thyroid, but they are not thyroid follicular cells themselves.

And these cells make calcitonin. The function of calcitonin remains somewhat of a mystery in human physiology. There are only a couple of thousand new cases of medullary thyroid cancer each year. The mainstay of therapy is surgery. Surgery for this disease is potentially curative when the disease is confined to the thyroid manifesting most commonly as a lump in the neck. But medullary thyroid cancer can metastasize. It can metastasize to lungs, lymph nodes, bone, liver. And when it is metastatic, it's very difficult to treat. We don't completely understand why mechanistically it is so resistant to therapy, but it's not treated with chemotherapy because chemotherapy doesn't work. It can be treated with radiation therapy.

There are thoughts in the current era that maybe immunotherapy treatments with the anti PD1 antibodies may be useful. But most patients in the world are treated with what are referred to as kinase inhibitors. So, these are pills that patients take every day. Names of these drugs include vandetanib, cabozantinib, as well as some novel RET inhibitors that were approved by the FDA within the past year or two.

The problem is, that these therapies always stop working and while they can work well, sometimes for a period of years, they will eventually stop working.

And then our patients run out of useful options. And that's where this novel CAR T-Cell construct enters into the picture.

Host: Dr. Siegel, as Dr. Cohen just mentioned, there remains a compelling need for novel therapies to treat MTC. And you're currently conducting a clinical trial of a new approach based upon chimeric antigen receptor T-cells or CAR T therapy, which has been applied with great success to blood cancers at the Abramson Cancer Center and elsewhere. How would CAR T work for medullary thyroid cancer? Tell us a little bit about that.

Donald Siegel, M.D., PhD (Guest): Sure. Thanks very much for the question. So, chimeric antigen receptor T-cells is a type of therapy where we harvest T-cells, which are a type of white blood cell from a patient's bloodstream using a technique called apheresis. A patient sits down in a chair, is connected to a device, looks like a dialysis machine, but it basically removes cells from the blood.

And those T-cells then go to one of our laboratories. We do some genetic manipulation to endow those T-cells with the ability to target a specific type of cancer. Now the trick is to endow them with something that makes them go after a particular kind of cancer. Now, when it comes to the blood cancers, there's a target known as CD19, which is something on the outside of cells that lead to lymphoma or leukemia. And in fact, that's been known for a long time. And so the CAR T therapies for leukemia and lymphoma generally target the CD19 on those cells. The trick to applying CAR T therapy to other forms of cancer, requires one to know some specific type of target to go after, in this case on medullary thyroid cancer cells.

And so the trick here was to discover a target for medullary thyroid cancer and then develop an antibody to that target. The thing that directs a CAR T cell to the target on a cancer cell is actually a fragment of an antibody. So, after then developing an antibody to that, we needed to incorporate that into what we call a vector, which is what is used to transform a T-cell from a patient into a CAR T-cell.

We grow the cells up in the lab and then we infuse them back into the patient from which the original cells were harvested. And the hope is that these CAR T-cells circulate around, when they see this target on the tumor cell, in this case medullary thyroid cancer, they recognize the cancer cell, engage with it and kill the cell.

And that's how they would work in the case of medullary thyroid cancer. The target that we discovered is called GFRa4.

Host: Dr. Siegel, I understand that you have a personal connection to this effort.

Dr. Siegel: Well, yes. So, I've been here at Penn for almost four decades and I have a number of different responsibilities. Some of them are clinical in terms of running the blood bank and the cell collection facility in the hospital. And also running the laboratory that makes CAR T-cells. And my other responsibilities include my own research laboratory, which has focused for the last 30 years on antibody development. And those two areas in my life were largely unrelated to each other until a very close friend of mine, a scientific colleague from Scripps Research Institute. One year he said he wasn't going to be able to make it because of a series of tumors that were found in his neck area. And one thing led to another and it turned out that he had medullary thyroid cancer. And so, that's sort of how my two worlds collided unexpectedly.

So, this fellow friend of mine actually was someone who developed the methods that are used throughout the world for engineering antibodies. And he taught them to me. So, using methods that he had taught me and using methods that had been developed here for CAR T-cells at Penn, put together a small group of people to first try to discover a target not just on his medullary thyroid cancer, but that would work hopefully for anyone who had the disorder, discover a target and then make an antibody to it, using methods that he had developed and taught me and that we were teaching every year in New York and then put that into a CAR T-cell and see if it would kill medullary thyroid cancer cells, first in a dish and then in an animal model.

And so, these techniques are very powerful and it actually only took us two and a half months from start to finish, to discover a target, make an antibody and make a CAR T-cell. At that point, unfortunately, my friend passed away. He had a very aggressive type of medullary thyroid cancer. Dr. Cohen mentioned people can have mutations in a protein called RET and he had very pathogenic if you will, type of mutation that led to a very rapid growth. And by the time he had been diagnosed, it had already spread throughout his body. So, unfortunately he passed away.

But through funding from his family, we continued the work which eventually led to putting together this clinical trial, having it reviewed by the FDA and getting it accepted, getting an IND for this new investigational drug. And then opening up this trial which just recently happened.

And hopefully this will have a very positive effect for patients who have a medullary thyroid cancer. Unfortunately my friend could not benefit from it.

Host: Dr. Siegel. Thank you so much for sharing your story with us. Just following up for a second. Can you tell us why that GFR target is unique for CAR T-cell? You mentioned it briefly, but can you expand just a minute on that?

Dr. Siegel: Right. So, what we initially did was to look at examples of medullary thyroid cancer cells and ask, is there something on these cells that is not generally expressed elsewhere in the body? And by doing a type of experiment called an RNA sequencing profile, and then sorting a list of proteins based on prevalence in the tumor versus presence elsewhere in the body.

The thing that was at the top of the list was calcitonin, which Dr. Cohen mentioned was a hormone that is produced by these cells. Now that's a soluble protein that's inside of the cells released into the blood, so that wouldn't make a target to go after. Cause you want the target to be something stuck to the outside of the cell that the T-cell can recognize.

But remarkably, the second thing on the list was something that we had no idea what it was. It was GFRa4. We saw the letter R in it. We thought, oh, maybe that's means it's a receptor and receptors are things that are generally on the outside of cells. So, we looked in the literature and found lo and behold that someone had actually studied this protein before, about 10 years earlier, there was a publication on it.

And it described the function of this protein in the medullary thyroid cancer cells. And they had done some experiments to show how it wasn't in any other tissue of the body, other than in this thyroid tissue. And so, we then did our own studies to confirm this and sure enough. We looked at all the different organs of the body. We could not find it expressed anywhere other than in these, in the cells that lead to medullary thyroid cancer. And at that point is when we said, okay, let's make an antibody to it. And then once we did that, we made the CAR T-cell and, and that's the rest is history, as I described earlier.

Dr. Cohen: And Don, if I could just jump in here, I think one of the most interesting aspects of this project is in fact that the expression of this receptor is limited to the medullary thyroid cancer cells. And you can live without your parafollicular cells that make calcitonin, you can undergo a total thyroidectomy and you can live a normal life, as long as you have replacement of conventional thyroid hormones, such as levothyroxin. So, the hope with this project is that the

specificity of these CAR T-cells for this one type of cell in the body, might help limit some of the potential side effects that could occur where the CAR T-cells to target inadvertently on other cells in the body. Now we won't know whether that occurs until we've treated people, but that certainly is one of the most salient and attractive and interesting features scientifically of this entire project.

Host: I agree. In my research, I was fascinated by that. So, Dr. Cohen, can you expand on the primary objectives of the trial and what is it you're hoping to achieve here?

Dr. Cohen: It is what's referred to as a first in human trial. So, the most important objective is to determine, to see whether the drug is safe. CAR T-cells do have some technical complexities. And as was the case with all of the CAR T-cells in clinical development, you have to also show that you can make them. So, that's referred to as feasibility. And you want to be sure that you have enough CAR T-cells to infuse into the patients. The third objective is in fact to see what happens to the patient's tumors after we treat them. Does their calcitonin, a tumor marker, go down? Do their levels of CEA, another tumor marker go down? And by means of various scans, do they also enjoy shrinkage of tumors and perhaps even improvement in symptoms resulting from metastatic tumors as a result of being treated? There are also within the trial itself, a number of biomarkers being explored by various blood tests, as well as the opportunity, if the patient consents and it's deemed safe to undergo biopsies and see where the CAR T-cells actually ended up. Do they proliferate in the bloodstream?

Do they end up in tumors? And when they are in tumors, what exactly are they doing there other than killing cancer cells? So, these are a series of standard objectives for a first in human trials, such as the one that we're conducting.

Host: Dr. Cohen, along those lines, then let's speak a little bit about patient selection and eligibility. Can patients on other therapies participate in this trial? Explain a little bit about eligibility. Tell us about patient selection.

Dr. Cohen: Patients eligible for this trial will be adults with incurable, either locally recurrent, or metastatic medullary thyroid cancer who have received at least one, or have declined to receive a tyrosine kinase inhibitor, such as cabozantinib. The patients have to be in good shape because this is a first-in-human trial, which means that they need a reasonable performance status. ECOG 0 or 1, and their organs need to function. Meaning heart, lungs, kidneys, and liver.

Host: Well then Dr. Cohen, sticking with you for a minute. A second objective for this trial concerns safety, particularly cytokine release syndrome, which can occur in CAR T-cell therapy. Tell us a little bit about cytokine storm and how this is addressed in this trial.

Dr. Cohen: So, one of the concerns about all CAR T-cells is cytokine release syndrome. That has been very well described in the medical literature, including for the registered products. So, when the immune system functions and functions extremely well, and of course that's the hope of this project is that these altered cells will do exactly what we want them to do and kill cancer cells rather quickly, but that can release a number of immunological mediators, cytokines into the bloodstream at very high levels instantaneously. This has been well-described for example, with COVID infection and is one of the reasons for the morbidity associated with that disease. So, these are immune substances that have enumerable actions within the body and cytokine release syndrome symptoms include fever, nausea, headache, skin rashes, rapid heart rate, low blood pressure and trouble breathing. These are immune side effects, and so they can be treated if they occur to an excessive degree with drugs such as prednisone or novel monoclonal antibodies, such as tocilizumab. We have a lot of experience at Penn Medicine in treating cytokine release syndrome. The syndrome was first described at our university and many of the paradigms that are currently used across the world for the management of cytokine release syndrome were developed here.

So our physicians, including intensivists within the intensive care unit, should that be required, know how to manage this syndrome and shut it down, if it gets really carried away.

Host: What a fascinating trial. And I hope you'll both join us again to update us as things progress. I'd like to give you each a chance for a final thought here. So, Dr. Siegel, starting with you, what do you want listeners and other providers to know are some end points of this study and what would you like them to leave this podcast with?

Dr. Siegel: Well first, I mean, specifically for those listening who are interested in medullary thyroid cancer, they understand how devastating a disease this can be and how there are not really many options for patients after some of the initial types of approaches. So, clearly having something like this that is potentially curative, is a major advance, as a general matter, and relating to one of your initial questions about the success of CAR T therapy in what we call liquid cancers or liquid tumors, hematological tumors, like leukemia and lymphoma. One of the difficulties has been to try to expand the use of CAR T

therapy into the world of solid tumors and medullary thyroid cancer, it would be considered a, solid tumor. There are many reasons why using CAR T therapy for solid tumors has some challenges that liquid tumors do not, but one of them is selection of target. Finding a target that's specific, and doesn't have what we call off tumor effects.

And here, I think we may have found something that fits the bill. And hopefully this may serve as a paradigm. And also the way in which we, as I said in two and a half months, came up with a therapy, serves as a paradigm for developing other types of CAR T-cells for other types of solid tumors.

Host: It's fascinating. And Dr. Cohen, last word to you. How can patients or clinicians reach out to you to participate in this trial? And what would you like referring physicians to know about all the exciting advancements that you're doing at Penn Medicine?

Dr. Cohen: I would encourage physicians or frankly, even patients to just contact me directly, via email. I will return your calls and your emails promptly and help figure out whether your patient or in the case of a patient, you are suitable for this trial. And whether it represents a reasonably safe option for you. I would also like to add a detail that we didn't get into in the earlier discussion, which is that this is designed as a single treatment. So, these cells are to be infused. They will stick around long enough, we hope, to substantially decrease or potentially even eliminate the medullary thyroid cancer cells in a patient's body.

So, this is really quite different from traditional cancer treatment for metastatic disease that goes on and on. And to echo what Don said, this is an absolutely unique target for what's referred to as a solid tumor. And so it's important, especially for patients with medullary thyroid cancer, but it also may be a pioneering, in the world of solid tumors, generally trying to get these fascinating drugs, CAR T cells to work as well in solid tumors as they do in some liquid tumors.

Host: Thank you both so much for joining us and telling us about the novel CAR T cell medullary thyroid cancer clinical trial at Penn Medicine. To refer your patient to Dr. Cohen, please call our 24/7 provider only line at 877-937-PENN. Or to submit via our secure online referral form, you can visit our website at pennmedicine.org/refer. That concludes this episode from the Specialists at Penn Medicine. Please remember to subscribe, rate and review this podcast and all the other Penn Medicine podcasts. I'm Melanie Cole.