“Personalized Cancer Vaccines” (Willy Hoos) [#29]

Brad Power and Brian McCloskey
October 12, 2022

“The goal of a cancer vaccine is to leverage the immune system's ability to see self versus non-self and leverage the fact that foreign parts of the tumor that don't look like self can be attacked by the immune system.” Willy Hoos

“The literature is evolving to indicate something is happening with these vaccines, but it's certainly unclear that they work.” Willy Hoos

Meeting Summary

Advanced cancer patients with solid tumors see immunotherapies as offering one of the best paths to a durable response. Personalized cancer vaccines have a lot of potential because they offer a possible treatment option to nearly every cancer patient.

Willy Hoos is uniquely suited to lead a discussion on personalized cancer vaccines. He is the president of the Jaime Leandro Foundation for Therapeutic Cancer Vaccines, which provides access to personalized cancer vaccines; a Cancer Collaborator Lead for the 1440 Foundation, which is a health learning network enabling collaboration on cancer; and an advisor to xCures, which provides software and infrastructure services to identify and rank the most promising treatment options for people with cancer who have exhausted the standard of care. He also advised Joanie and Scott Kriens on Joanie’s journey to treat her advanced pancreatic cancer.

What are personalized cancer vaccines and how do they work?

Personalized cancer vaccines can be used to introduce or stimulate selected T-cells (part of the immune system) to attack cancer cells. A personalized cancer vaccine leverages the immune system's ability to see self/normal versus non-self/foreign cells and attack the non-self/foreign cells through a tailored antitumor response to their tumor mutation signature. The vaccine is trying to get your body to produce enough of the right T-cells, and then combine it with things like checkpoints or whatever makes sense in a given cancer in a given patient to make sure that those T-cells can do their job and win the battle against the tumor.

How are personalized cancer vaccines designed, manufactured, and administered?

The way we make personalized cancer vaccines is complicated. You sequence the tumor tissue and healthy tissue to identify mutations. Then there are a bunch of algorithms that can choose peptides (protein fragments) that match the mutations, their likelihood of binding to the immune system, their likelihood to be immunogenic, and other characteristics. The result is a list of potential peptides that could go into a vaccine. Then those peptides are manufactured and delivered by injections.

How effective are they?

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The literature is evolving to indicate something is happening with these vaccines, but it's certainly unclear that they work. There are ongoing vaccine-related trials in prostate cancer. There's precedent that there's some potential benefit in prostate cancer. The peptide vaccines, from everything that's been seen, appear to be relatively safe. Most of the side effects are limited - similar to getting a flu vaccine or something where you get a sore arm, or you get a fever for a day or two. If you were to add checkpoint inhibitors, those have their risks.

What are other emerging immunotherapy variations?

There are other ways to amplify or enhance the behavior of the personalized vaccine approach. Tumor infiltrating lymphocytes (TIL) is where they take the tumor sample, find the T-cells that are in that sample and verify that those T-cells are the ones that are supposed to be there doing the job. If you just could boost those up in a variety of ways they would finish the job. Endogenous T-cell Therapy (ETCs) is taking the cells out of the blood and finding the T-cells that way. The hard part is now you've got billions of cells you're sorting through, instead of 1000s, or hundreds of 1000s in the tumor sample, so you must have a better way of figuring out which ones are the right ones.

T-cell Receptor (TCR) therapy is skipping finding a natural cell and finding a specific T-cell receptor.

A recent study in pancreatic cancer explored a vaccine co-administered with immune-modulating agents to enhance antitumor T cell activity as a strategy for overcoming the cancer’s resistance to immune checkpoint inhibitors. A personalized cancer vaccine from Moderna based on the messenger RNA (mRNA) technology used in their COVID-19 vaccine has been combined with Merck’s checkpoint inhibitor immunotherapy Keytruda to cut the risk of recurrence or death from melanoma, the most deadly skin cancer, by 44%, compared with Keytruda alone.

How is testing evolving?

Sequencing costs are going down, yet the standard of care is still doing sequencing panels (checking 50 to 400 driver genes) for most patients. With whole exome sequencing (about 20,000 genes) you could get HLA typing (human leukocyte antigens tell your immune system which cells are self or non-self/foreign) for free, to possibly prioritize some TCR-type therapies that are out there. You create the option to do a vaccine. It may pick up some mutations that weren't on the panel that turned out to be relevant, even though that's rare. Why isn’t whole exome testing the standard of care?

Who benefits most? How should this fit into a patient’s treatment strategy?

Most of the patients pursuing personalized cancer vaccines have been metastatic patients who are on various chemos, or have been on trials, and are trying to plan ahead, knowing that it takes three months to include this in one of their next therapies or to add it to their existing chemo regimen. Or if they have to take a chemo break, having the vaccine ready to boost their chances of something happening better than the known path.

One of the critiques of vaccines is that as a tumor gets larger, it starts to get more heterogeneous and more diffused and spread out across multiple sites in the body. The microenvironment is more suppressive to the immune system. Probably the less cancer you

have, or the earlier stage, the better. Can it work in the later stage when a lot of patients start to turn their attention to something like this? There's a case study of a patient with metastatic pancreatic cancer who had radiation and dual checkpoint inhibitors after they'd been on a vaccine for a couple of months, and they got a pretty profound response that's ongoing and durable a couple years later. There are late stage examples, but it's not clear. If you have a potential to respond to checkpoint inhibitors, and you don't have time to wait one or two or three more months to get through the vaccine manufacturing, administration and waiting period, you want to try and get the benefit of the checkpoints because they have potential on their own. You must take the primary treatment goal first, and then the potential that that undermines some of the vaccine's potential is just part of the risk.

And then there is the cost, and is it appropriate and ethical for a person or society to take on those costs? $80,000 is cheap for a cancer drug, if this were an approved drug.

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Meeting Notes

Wed, Oct 12, 2022

SUMMARY KEYWORDS

vaccine, patient, HER2, tumor, checkpoints, work, cancer, mutations, sequencing, cell, T-cells, immune response, whole exome sequencing, antigens, therapy, peptide, trials, target, proteomics, pancreatic cancer

SPEAKERS

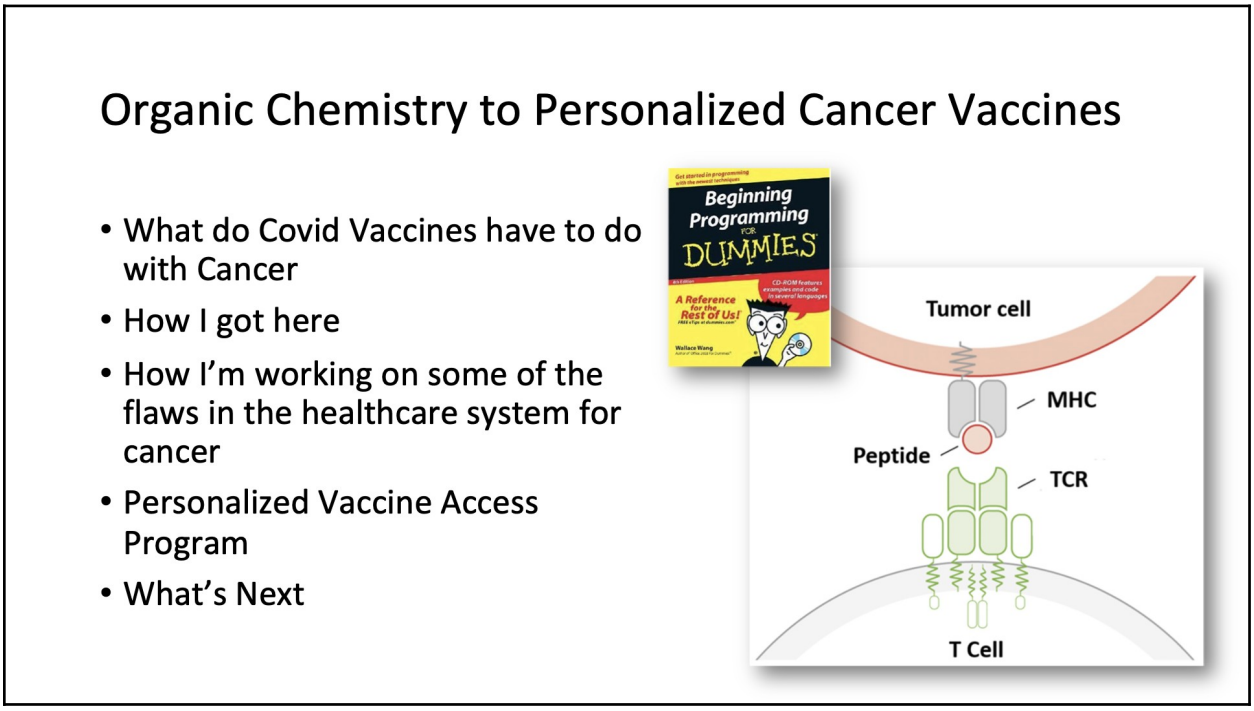
Eric Hall, Sheeno Thyparambil, Emma Shtivelman, Brad Power, Brian McCloskey, Willy Hoos

Brian McCloskey 00:04

Welcome everybody to today's Prostate Cancer Lab meeting. Today we have Willy Hoos, an advisor to xCures, which many of you know because they are a strategic partner of ours. He is also a Cancer Collaborator Lead for the 1440 Foundation, which I know has done some incredible work in pancreatic cancer. He is also the President of the Jaime Leandro Foundation for Therapeutic Cancer Vaccines.

I know that you have many roles, but you're here to speak to us about the experience you've had with personalized vaccines and developing them for cancer patients.

Willy Hoos 02:14



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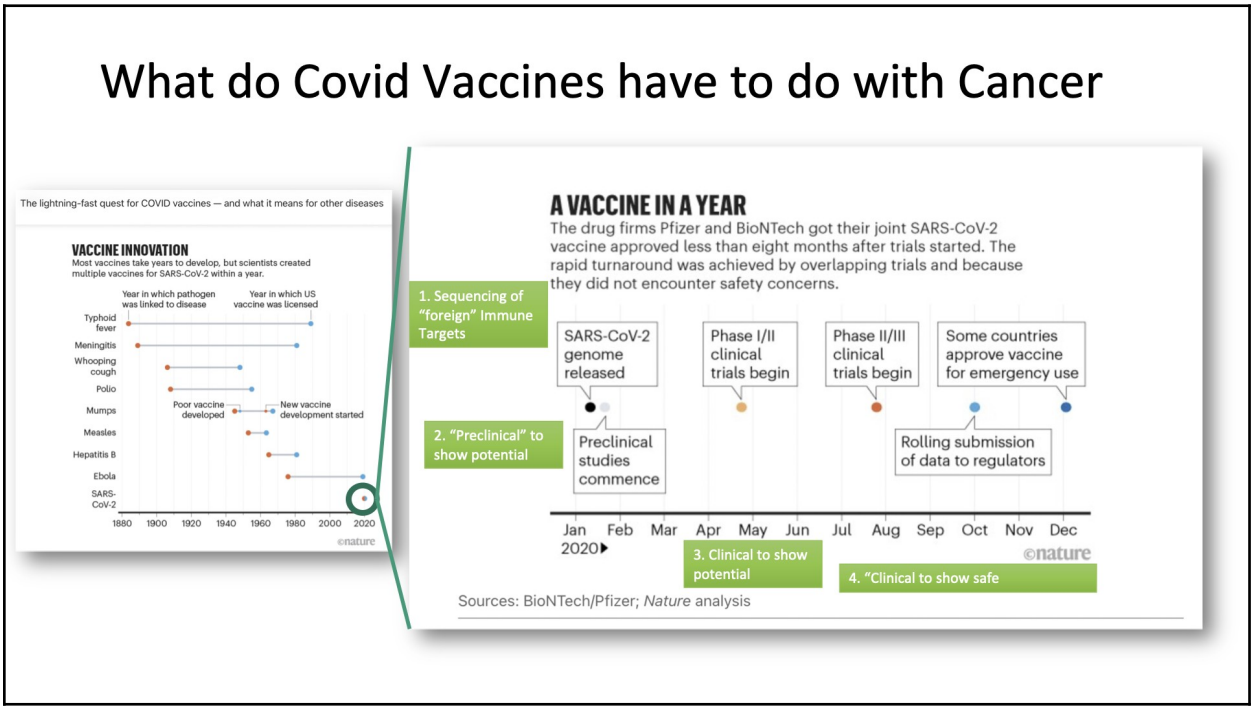
I include this slide on “Programming for Dummies” because early in my career, in the early 2000s I was building a software company, and I hadn't been a software guy. I'd been an organic chemist, and I had gotten my MBA and had an idea for a software company. I sat down and built the software myself and “Programming for Dummies” was literally one of the things that I used to get started.

On the right is a picture of the MHC - peptide - TCR complex, which is relevant to all of this.



What do COVID vaccines have to do with cancer vaccines? The main thing that I want to point out here is how long most vaccines take to make and why. The process starts with sequencing of the foreign immune targets, which in the case of COVID was a virus, but in the case of personalized vaccines is the cancer, which is unique and even heterogeneous within a patient. Then there is the preclinical step to show its potential, which was a bunch of work, then you have clinical trials to show its potential and to prove it was safe. That was where most of the energy and time went for the COVID vaccine. But when you get to a cancer that is deadly and urgent, you start to shift which of those things we have to do. Where can we take risks? Where can we add? Where can we leverage what we're pretty sure we know and make a risk/reward cost/benefit decision? The two months it took to make the vaccine components for COVID is all you need to do the same exercise for a cancer to the extent the rest of the thread makes sense. It takes a couple months of getting through the initial work.

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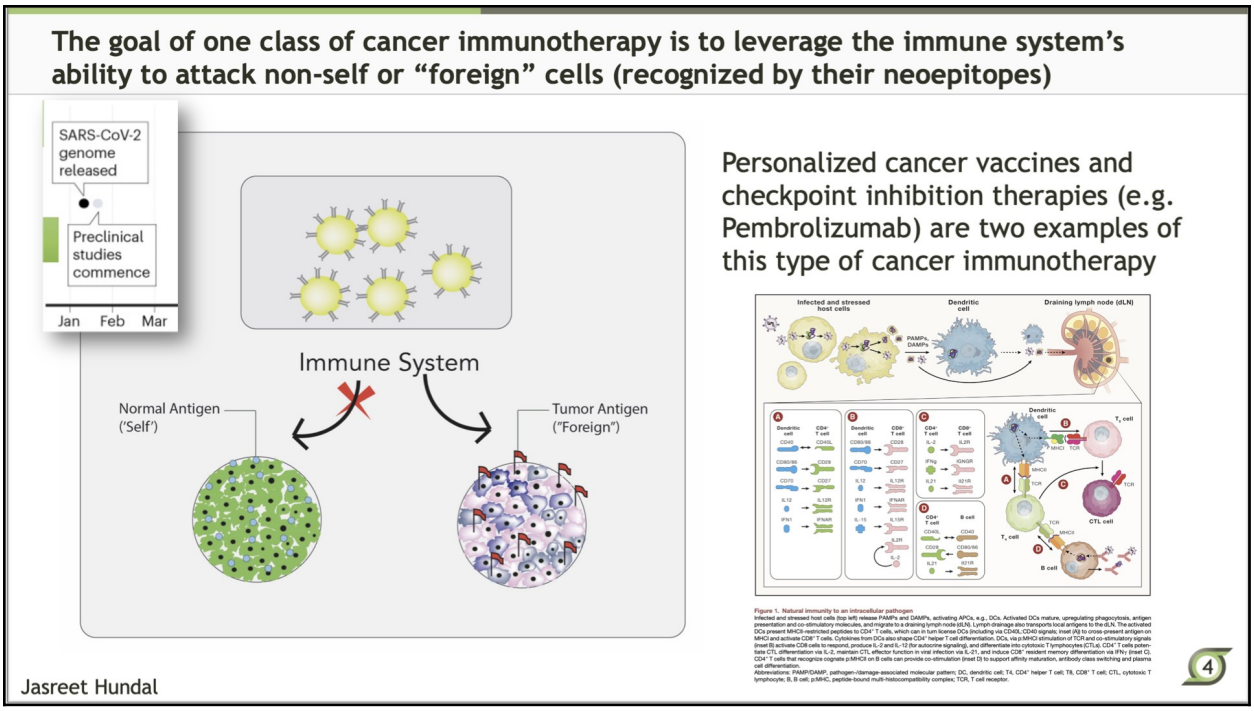
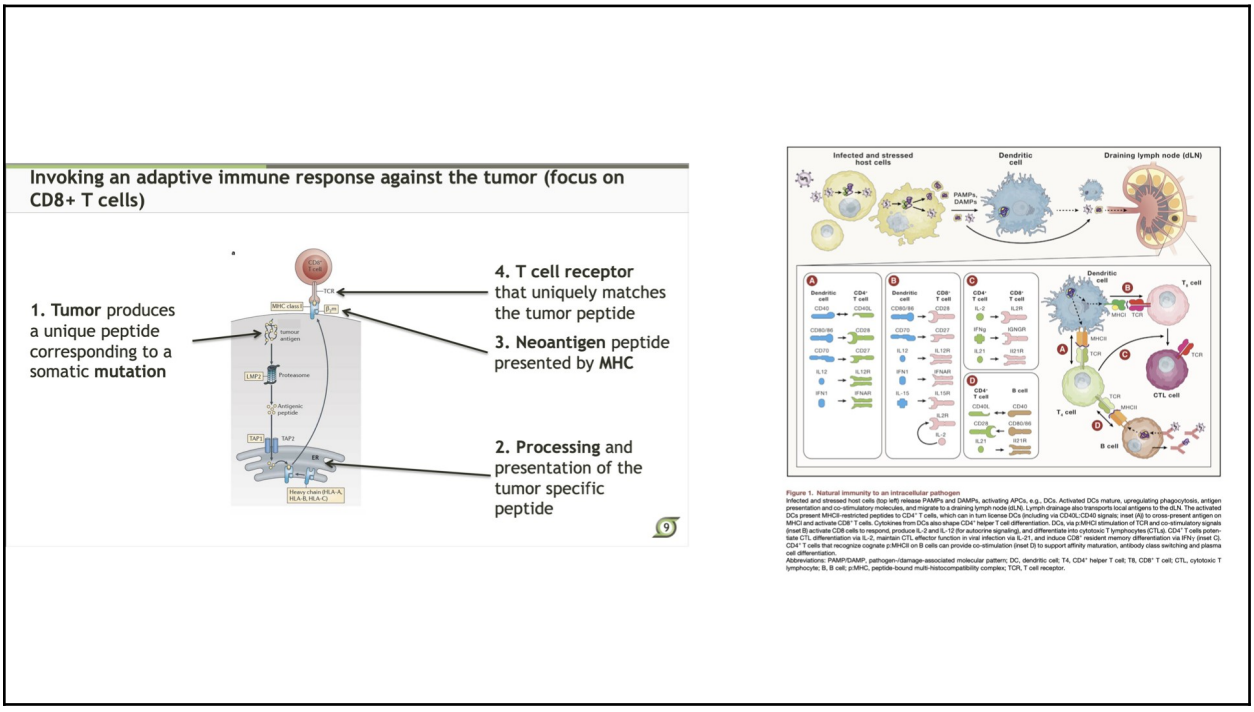


The goal of a cancer vaccine is to leverage the immune system's ability to see self versus non-self and leverage the fact that foreign parts of the tumor that don't look like self can be attacked by the immune system. Checkpoints are part of the story. Checkpoint inhibitors can help if the T-cells that need to go kill the cell are currently there, activated, and simply being inhibited by that checkpoint. Vaccines are considered if you don't have the T-cells there. Vaccines can be used to get the T-cells there, but then once they're there, you may still need to inhibit the checkpoints to unleash those T-cells.



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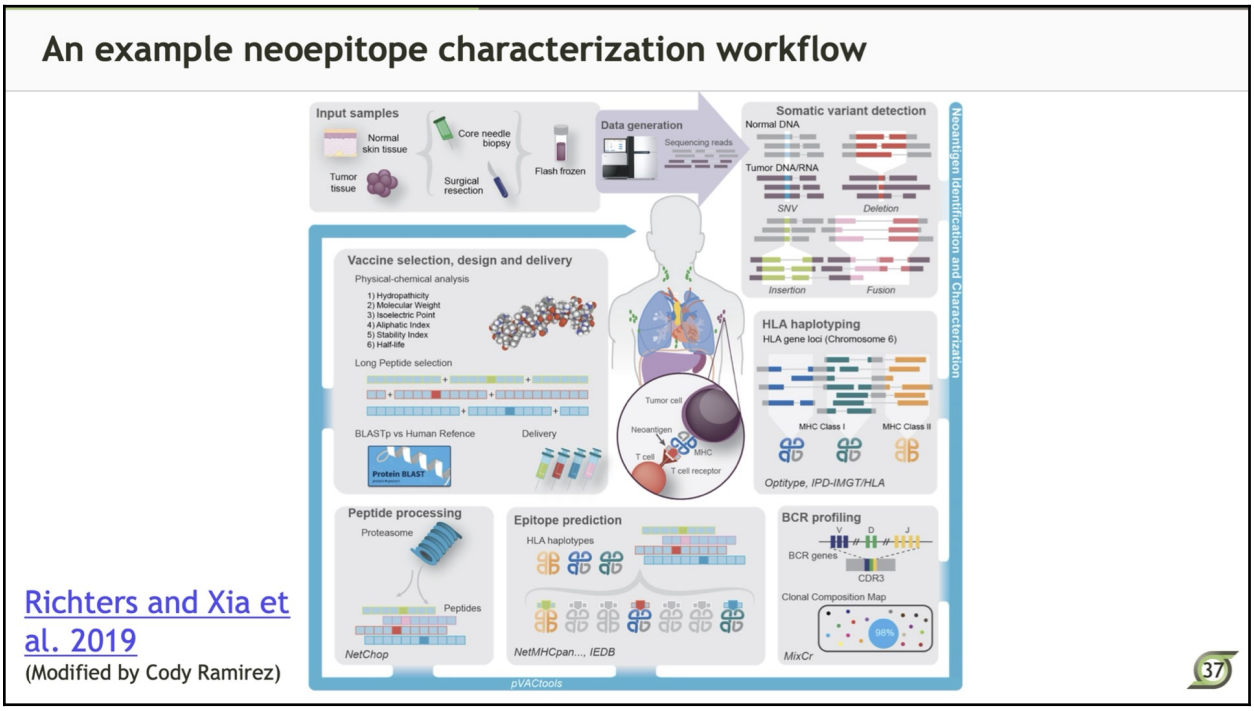
The way the immune system presents this is the tumor produces a unique peptide from a mutation. This is one of the ways to look at it, and it is not the only perspective. It is somewhat simplified. Then that antigen gets processed and loaded onto the “major histocompatibility complex” (MHC, a group of genes that encode proteins or peptide fragments on the cell surface for recognition by appropriate T-cells.) Then that unique MHC peptide complex has a unique binding partner in some T-cell in the body, which hopefully activates it, and all the rest of the things happen. That's where all the magic of the way vaccines can work and the way the immune system works independent of vaccines.



The way we make vaccines is complicated. You sequence the tumor. You also sequence the patient's immune system, which can come out of whole exome sequencing. It's often good to confirm it by another method. Then there are a bunch of algorithms that can process the match of the mutations, their likelihood of binding to the immune system, and their likelihood to be immunogenic with a bunch of other characteristics. All of that is what's happening to get to a list of potential peptides that could go into a vaccine or potential other ways to do a vaccine. Then those are manufactured and delivered in injections.

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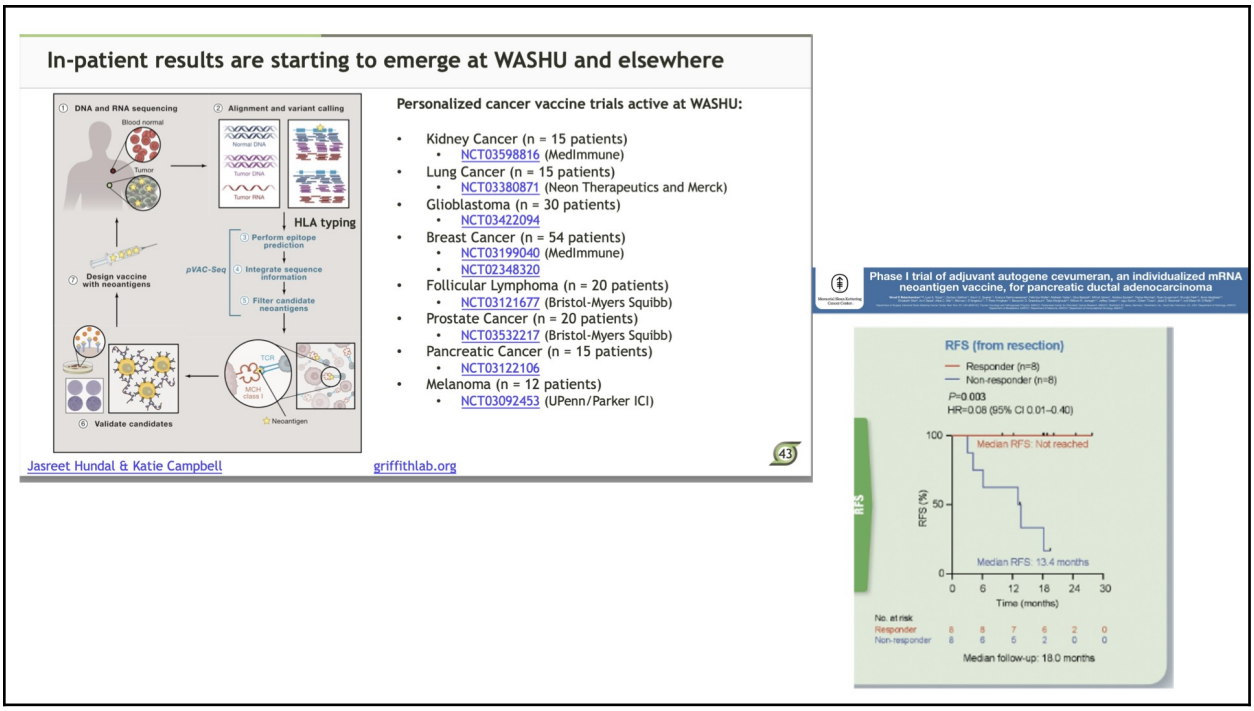

A couple things here. On the left are some references from trials that largely WashU has done. I'm just showing some of the potential and ability to elicit immune responses. It’s not just Washington University; there are a bunch of sources. The literature is evolving to indicate something is happening with these vaccines, but it's certainly unclear that they work.

On the right is from the recent BioNTech study that was done at Sloan Kettering that came out at ASCO where they had taken early-stage pancreatic cancer, and that scale on the bottom, by 18 months almost all of the patients in the group that didn't show an immune response had recurred, whereas none of the patients who showed an immune response to the vaccine had recurred. Now, that is not proof. This is not a randomized trial, comparing those two. The ones who had an immune response did a lot better, and the vaccine is thought to be part of that. But it could be the patients who responded to the vaccine had some other thing that predicted they weren't going to recur. There's a lot of critique of the available information out there.

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Vaccines – A Bridge to cure?

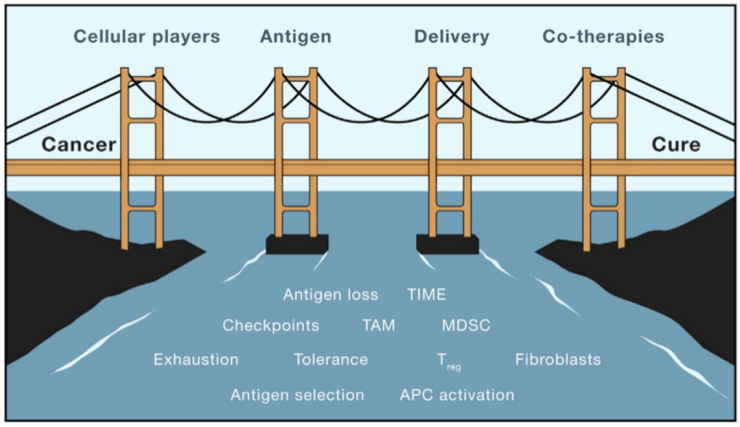


Figure 3. Vaccines, a bridge to cure?
While there are many barriers to natural immune responses to cancer, we propose that focusing on four pillars (innate and adaptive immune cells, antigen targets, delivery strategies, and co-therapies) will help vaccines become a bridge from cancer to immune-based cure.

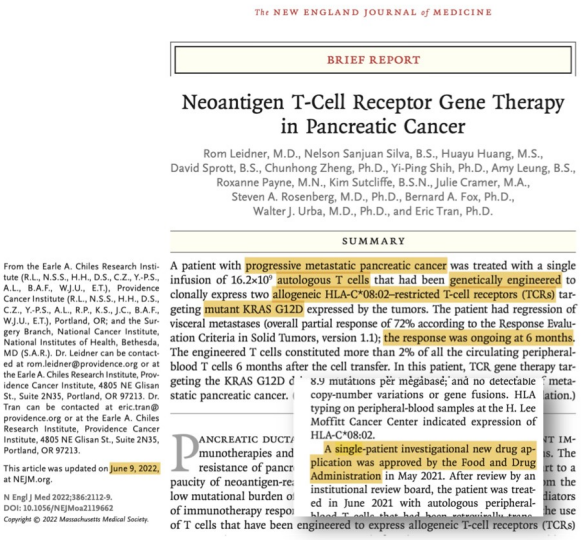
cells to attack malignant cells (Biswas et al., 2021). B cell-T cell interactions are important in two ways. First, antibody maturation and production are enhanced in the presence of activated $CD4^+$ T cells that recognize epitopes associated with the target protein, ensuring that antibodies are made in the context of a broader immune response (classically

https://www.cell.com/cell/pdf/S0092-8674(22)00787-5.pdf

There's a really good paper recently in Cell called “Cancer vaccines: Building a bridge over troubled waters” https://www.cell.com/cell/pdf/S0092-8674(22)00787-5.pdf. This paper lays out the things that are involved in the cellular players and the antigens, how you deliver it, and other things you do. Those are the key thoughts of the pieces of a vaccine that are needed to get that all the way through to lasting results. All this stuff in the rough waters underneath are the things that can get in the way of that. It's a great article.

What’s Next

- JLF Vaccine Access Continued
  - Sharing and research
- More advanced therapies
  - Tcell therapies building upon or complementing or replacing vaccine
    - TIL and ETC
    - TCR
    - CAR-T



The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

Neoantigen T-Cell Receptor Gene Therapy in Pancreatic Cancer

Rom Leidner, M.D., Nelson Sanjuan Silva, B.S., Huayu Huang, M.S., David Sprott, B.S., Chunhong Zheng, Ph.D., Yi-Ping Shih, Ph.D., Amy Leung, B.S., Roxanne Payne, M.N., Kim Sutcliffe, B.S.N., Julie Cramer, M.A., Steven A. Rosenberg, M.D., Ph.D., Bernard A. Fox, Ph.D., Walter J. Urba, M.D., Ph.D., and Eric Tran, Ph.D.

SUMMARY

A patient with progressive metastatic pancreatic cancer was treated with a single infusion of 16.2×10^9 autologous T cells that had been genetically engineered to clonally express two allogeneic HLA-C*08:02–restricted T-cell receptors (TCRs) targeting mutant KRAS G12D expressed by the tumors. The patient had regression of visceral metastases (overall partial response of 72% according to the Response Evaluation Criteria in Solid Tumors, version 1.1); the response was ongoing at 6 months. The engineered T cells constituted more than 2% of all the circulating peripheral-blood T cells 6 months after the cell transfer. In this patient, TCR gene therapy targeting the KRAS G12D d… 8.9 mutations per megabase, and no detectable …meta-static pancreatic cancer. …ation.)

…ANCREATIC DUCTA… …munotherapies and … resistance of panc… paucity of neoantigen-re… low mutational burden o… of immunotherapy respo… of T cells that have been engineered to express allogeneic T-cell receptors (TCRs) …FT IM-…is. The …rt to a …m the …diators …he use

copy-number variations or gene fusions. HLA typing on peripheral-blood samples at the H. Lee Moffitt Cancer Center indicated expression of HLA-C*08:02.

A single-patient investigational new drug application was approved by the Food and Drug Administration in May 2021. After review by an institutional review board, the patient was treated in June 2021 with autologous peripheral-blood T cells that had been genetically engine-

From the Earle A. Chiles Research Institute (R.L., N.S.S., H.H., D.S., C.Z., Y.-P.S., A.L., B.A.F., W.J.U., E.T.), Providence Cancer Institute (R.L., N.S.S., H.H., D.S., C.Z., Y.-P.S., A.L., R.P., K.S., J.C., B.A.F., W.J.U., E.T.), Portland, OR; and the Surgery Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD (S.A.R.). Dr. Leidner can be contacted at rom.leidner@providence.org or at the Earle A. Chiles Research Institute, Providence Cancer Institute, 4805 NE Glisan St., Suite 2N35, Portland, OR 97213. Dr. Tran can be contacted at eric.tran@providence.org or at the Earle A. Chiles Research Institute, Providence Cancer Institute, 4805 NE Glisan St., Suite 2N35, Portland, OR 97213.

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We continue to make the vaccine available through compassionate access. But there's also more advanced therapies because what the vaccine is trying to do is get your body to produce enough of the right T-cells, and then combine it with things like checkpoints or whatever makes sense in a given cancer in a given patient to make sure that those T-cells can do their job and win the battle against the tumor. But other ways to do that are to go find out, or amplify, or enhance the behavior of that same general approach. TIL (tumor infiltrating lymphocyte) is where they take the tumor sample, and they find the T-cells that are in that sample and essentially assume and do some verification that those T-cells in the tumor are the ones that are supposed to be there doing the job. If you just could boost those up in a variety of ways that they would finish the job. ETCs, Endogenous T-cell Therapy, is one way of describing it (it has other words), but that is, instead of taking the cells out of the tumor, taking the cells out of the peripheral blood circulating, and finding the T-cells that way. The harder part is now you've got billions of cells you're sorting through instead of 1000s, or hundreds of 1000s in the tumor sample, so you must have a better way of figuring out which ones are the right ones. That's some of the emerging therapies that are out there. TCR (T-cell Receptor) therapy is skipping finding a natural cell and finding a specific T-cell receptor. You find the T-cell receptor that's unique to the target that you're looking for and then engineer that into someone else's T-cells so that it goes and does the job.

The New England Journal med paper (on the right) in pancreatic cancer showed one patient with a profound response and one with a response that developed resistance. It is something that they don't yet understand. But a couple points that are fascinating is (1) this was a TCR that was discovered in 2016 from a patient who did a TIL therapy at the NCI. Those TCR sequences were fully characterized there. They knew they were effective in a specific HLA matched KRAS-specific-mutation patient. Then they went and found patients with that same HLA and same KRAS mutation and engineered that same TCR into that patient five years later. They're now running a clinical trial of another 24 patients. They're adding in a new drug so they're not doing the exact same thing that was done here that had a profound effect. But they have a one year waiting list for that trial. It's backed up because they've had so many people who see this and want to take a shot at it. But this New England Journal paper was done with two patients who are treated on compassionate access, not necessarily in a clinical trial, even in the first place.

Brian McCloskey 13:47

One of the challenges with prostate cancer is that it's a cold cancer. We typically don't have tumor infiltrating lymphocytes in our cancer cells, I know for sure that I don't. With personalized vaccines, how successful are they in cold cancers?

Willy Hoos 14:29

What does “cold tumor" mean, why is it such, and is that changeable? Pancreatic cancers have always been characterized as cold. They don't have as many mutations as melanoma, which has this massive amount of DNA damage from UV rays. The answer is they just don't have the right targets. Therefore, it's “cold" because there was nothing to target. But then you have an example where you put the right TCR KRAS-targeted cell in there, and they can do their job. As we understood more, it's not just that they don't necessarily have enough mutations, but they also have this stroma, and other things in the microenvironment, that they recruit various cells or just excrete compounds and such that make the immune system not see it or not enter that area, and you have this tumor microenvironment that is immunosuppressive. Even if a T-cell went in there and recognized it, it's going to get shut down. Some of that suppression were checkpoint inhibitors. Those were the understandings in melanoma. What's the status with

prostate and checkpoints? The first CTLA (Cytotoxic T-lymphocyte–associated antigen 4 and programmed death 1 - PD1 - immune checkpoints are negative regulators of T-cell immune function.) breakthrough study came with the first CTLA drug that ended up getting approved. One of the first studies that everybody was getting excited about was in a prostate study at the Mayo Clinic where they saw evidence of some patients getting benefit. Then it shifted to melanoma where there was a much bigger response and much better. Melanoma was lower hanging fruit. There have been various levels of success in what percent of patients can get the benefit with prostate as an example. So that's the start of an answer. The punch line of that is "cold for what reason? Then what do you do to warm it up?"

Brian McCloskey 17:22

In prostate cancer, we hear a lot about Bi-specific T-cell Engagers, BiTEs, and CAR-T, Chimeric Antigen Receptor Therapy. Maybe talk about those? There's also another one called Provenge. There are a few different approaches to engaging T-cells. Maybe if you could help us understand the difference between BiTEs, CAR-T and personalized vaccines?

There seems to be more of a push for whole exome sequencing. I was just at an Illumina genomics forum a couple of weeks ago, and they were talking about the cost of whole genome sequencing going down significantly. Recently I had conversations with Weill Cornell about some work they're doing to integrate whole genome sequencing with RNAseq analysis. As the cost of sequencing is going down, access to whole exome sequencing is going up, which is what these personalized vaccines are based on. Should it be more available to people? If we move to whole genome sequencing, is it possible that that will unlock better vaccines for patients?

Willy Hoos 19:48

It's a good question. There's a company in the Netherlands where they're using whole genome sequencing as the input.

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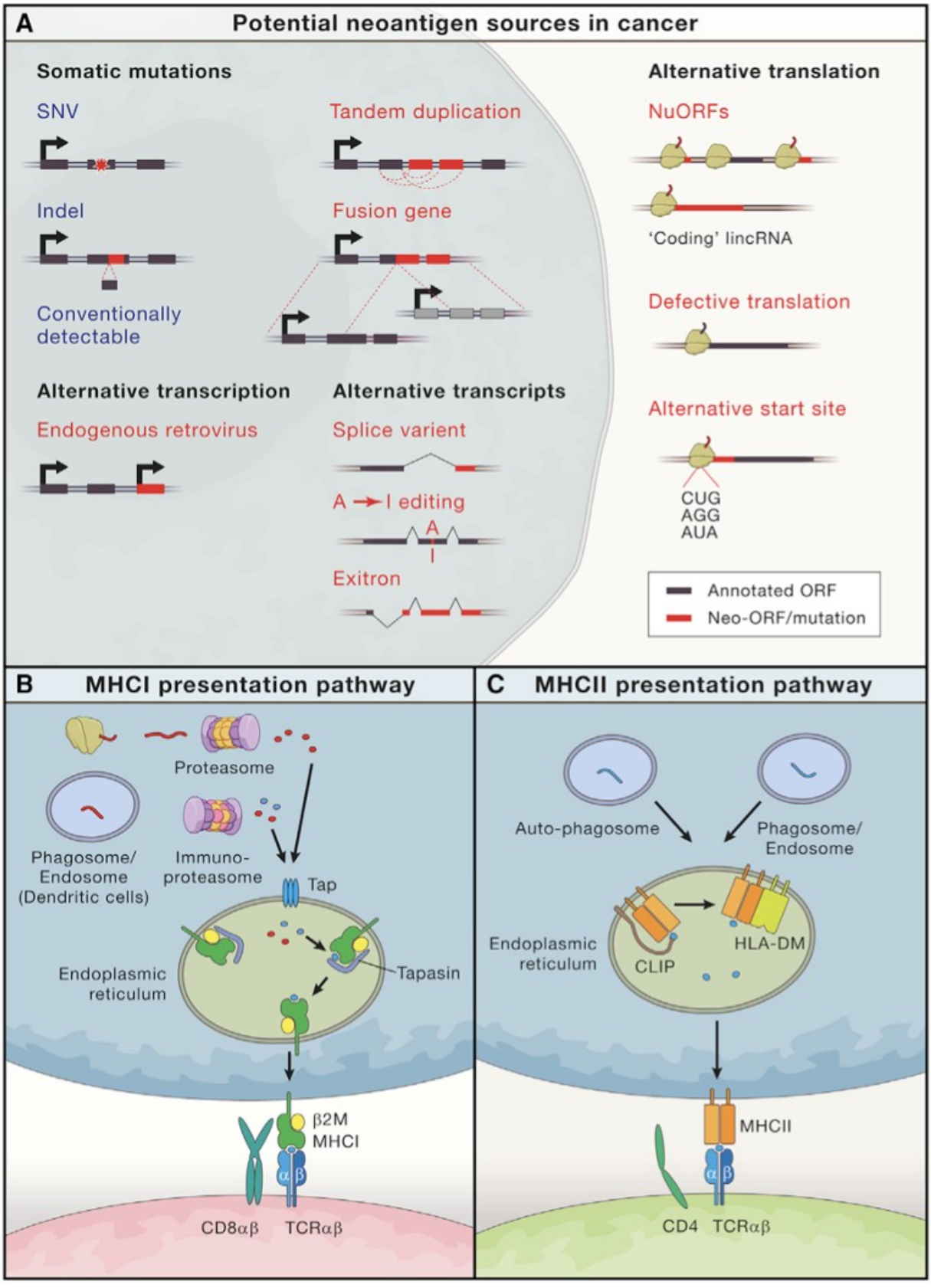

Here are all the possible sources of a neoantigen. Even outside of neoantigens, there's also some of the older vaccines and approaches. Those approaches suggest we must have a common antigen. You can never personalize things enough at scale to do individualized treatments. It's like looking for the keys under the light post when you are looking for the personal, or the non-personal kind, also called public antigens. What if that won't work? For example, what if you need to have three, four, five, or six successful targets that are personal to that cancer to really get enough of an effect? There are arguments on both sides of that. This is from that Bridges article in Cell. Up in the left, the single nucleotide variations and the Indels (an insertion or deletion of bases in the genome), those come out of regular normal sequencing. If there are other things that are splice variants and such, you can see that in RNA sequencing, for example, but fusion genes can be more difficult to detect if they're causing something that's not captured properly in whole genome sequencing, or whole exome sequencing, for example. There are other changes that aren't necessarily covered in an exome. There's a flip side of the exome/genome question. This is back to cost and quality. When we've had patients come to us for whole genome sequencing, the issue is that sometimes their whole genome depth is less, so some of your confidence in calling of mutations or having a clonal sub clonal mutation is less clear. It's not a target if it's not expressed. Part of our design process is not just getting the whole exome, but then using the whole transcriptome to confirm that a mutation is expressed. There's no perfect answer to quantifying that that amount of expression leads to enough peptide to expect an immune response. That's probably some of the failure modes for the algorithms. What are the extra things that come out of the whole genome that you can't get out of a whole exome that are driven all the way to expression? It's a little less clear exactly what extra things you get and how often that happens. I'm sure if my folks were on, they would have answers on that. They've shared some of those answers with me, and I kind of get it, but don't fully.

Why are we doing panels instead of whole exome? It was a cost issue, it was a stage of technology that, arguably, has passed. With things like that TCR sequencing, if you have a KRAS-HLA match, you should be seeking that out. It's a waiting list and all of that, but it's profound, and it's a reasonable risk-reward profile. It must be proven more, but I believe it's a priority. The challenge there is the world's still doing panels for most patients, when you could get HLA typing for free in whole exome sequencing. You start stacking up all the benefits of it and create the option to do a vaccine. It maybe picks up some mutations that weren't on the panel that turned out to be relevant, even though that's rare. You get the expression information, perhaps in a slightly different way than you get on a panel, and you get HLA typing to possibly go prioritize some other TCR type therapies that are out there. It starts to be a question of why aren't we doing that? I don't have the clinical equivalent answer for the whole genome, but I assume there are some.

Brian McCloskey 24:51

Super helpful.

Willy Hoos 24:54

In this article is an example of the binding of the MHC1 to the peptide. It's not maybe the best one. But the point is that there's a CD8 co-factor, a bunch of different checkpoints, and multiple bindings needed. Most of those BiTEs and such are trying to overcome that in some way to say, let's have a cell or a therapy that doesn't rely on a whole orchestra spontaneously coming together. Let's put the critical factor in, but let's add a couple other factors that increase the chance that it all works. The downside of that is that this is so complex. What are the unintended consequences of bypassing some of the less natural ways of doing things?

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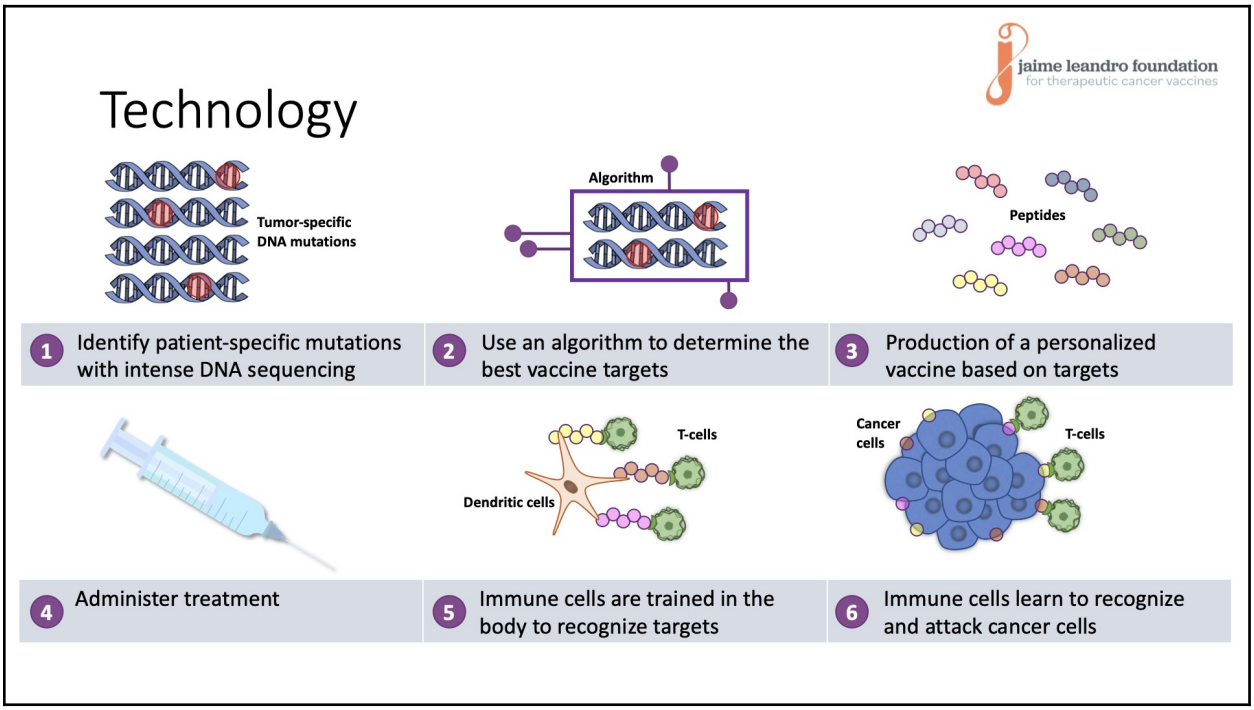
Bradford Power 26:24

I want to get into the mechanics, and you started talking about it. How should a patient think about this? Is it preclinical? Is it clinical? Is it ready for primetime? Is it phase one? Is it phase two? Most of our patients have a treatment options list. Should it be on the shortlist? If so, where should it be on the shortlist? What does it mean to access? I just want to translate from the chemistry or the science of it to the logistics and operations and implementation of this for a patient.

Willy Hoos 27:13



I don't have a slide on JLF’s process. I'll just put this up. At JLF, we're running a compassionate access program. We also have a research IND and a research study open. It's a patient pay program. It's $80,000 and it takes about three months to make the vaccine. It uses whole exome sequencing as the input - RNA and DNA. DNA for tumor and normal, RNA for the transcriptome. We've been using Boston Gene and Tempus. Both of those can work. Boston Gene specifications are more in line with what we need. There are other sequencing providers, but as to date, we have not been able to get the raw data that we need from them, or their test specifications are not sufficient. We run a compassionate access, which is also called expanded access. It follows a single patient Investigational New Drug (IND) protocol in most cases. The requirements are roughly that the patient has a serious disease where the benefits outweigh the risks of the vaccine. That's kind of an individual and patient/physician decision on what qualifies, and then the FDA reviews that. Most serious metastatic cancers without high potential, or long duration stability are likely eligible for the vaccine with exceptions. There are ongoing vaccine related trials in prostate cancer. You mentioned Provenge. There's precedent that there's some potential benefit out there for this strategy in prostate cancer, but this is where I hit my limits. We haven't had a patient with prostate go through this. I don't know where the data would suggest that this is a waste of time. The risks are relatively low. The peptide vaccines from everything that's been seen appear to be relatively safe. All of this is in the consent form and everything.

Most of the side effects are limited - similar to getting a flu vaccine or something where you get a sore arm, or you get a fever for a day or two. That's the main piece of the vaccine alone. If you were to add checkpoint inhibitors, those have their risks. Are those risks enhanced by the vaccine? We don't know that for sure. But it seems like the science supports that wouldn't necessarily be so. Hopefully, that partially answers your question, Brad, as far as the process. Then I started to go into prostate, and I don't have a great answer for where this should go on everybody's list. What we know is it can't get in the way of trying something that should be a higher priority. That's one of the things the FDA is worried about and is in the backdrop of how they evaluate things from what we've seen clearly. It has the financial implications and the risks of an unapproved drug.

Bradford Power 31:26

Can you speak about it then in another cancer like pancreatic? What are the patients that are showing up? Who is selecting to work with you? What is their profile?

Willy Hoos 31:42

It's been mostly metastatic patients who are in various chemos, or have been on trials, and are trying to plan ahead, knowing that this takes three months to include this in one of their next therapies or adding it to their existing chemo regimen, or if they have to take a chemo break, having the vaccine ready to boost their chances of something happening better than the known path. But that said, one of the critiques of vaccines is that this tumor starts to get more heterogeneous as they get larger and more diffused and spread out across multiple sites in the body. They develop more of that microenvironment that's suppressive to the immune system. Probably the less cancer you have, or the earlier stage, the better. Where does this fit for earlier stage disease? It's not exactly clear. A lot of the trials have been done in the earlier stages of cancer, right after surgery, for example. From a clinical trial design of an experiment to answer a scientific question, that's where there has been a focus. Can it work in the later stage when that's when a lot of patients start to turn their attention to something like this? There's a case study out there from a patient who had radiation and dual checkpoint inhibitors after they'd been on a vaccine for a couple of months and got a pretty profound response that's ongoing and durable a couple years later with metastatic pancreatic cancer. There are examples where the whole of the efforts may point to some responses even in later stages of disease, but it's not clear. I don't have a crisp answer to that one.

Brian McCloskey 33:48

What about patients who have seen immunotherapies like pembrolizumab, or Keytruda? Do you see them come through and any insights into how their response is improved on a neoantigen vaccine versus an off-the-shelf immunotherapy?

Willy Hoos 34:14

Our experience is new and limited enough. We're mirroring the trial designs that had been done at Wash U in breast and in pancreatic and making that available to patients that it makes sense for. But we don't have the results of trials to say, in the patients who are in their post-checkpoint versus pre-checkpoint. This is how it works relatively. We don't know those things at all. Then you go to the science, and there is some scientific preclinical work linked with some analysis and trials that there's risk and data that the PDL1 can exhaust the T-cell repertoire that's there, and then perhaps undermine the emergence of new T-cell responses. The opinion of people

doing trials and who understand that science is that you're better off having the checkpoints after the vaccine has had a chance to start to do its job. We do five injections. The first three weeks is the priming phase. There's some science for why that is, and there's some just history, and then it shifts to monthly. Getting through that priming, primary series where there's at least an emerging set of T-cells that have been activated before expecting to unleash the checkpoint mechanisms is the current thought process, at least for the PDL1. CTLA4 is a little different because it may be involved in helping the T-cell response get started so that may not actually belong together if you're optimally doing it. I think the flip side is that the vaccines aren't proven. If you have a potential to respond to checkpoint inhibitors, and you don't have time to give one or two or three more months to get through the vaccine manufacturer, administration and waiting period, you want to try and get the benefit of the checkpoints because they have potential on their own. You must take the primary treatment goal first, and then the potential that that undermines some of the vaccine's potential is just part of the risk.

Brian McCloskey 37:00

It's a real issue. I've been on checkpoint inhibitors. I mentioned there other patients on here who've also had them. I can't remember for sure. I'm thinking about Provenge. Emma, do you have any thoughts in terms of sequencing these various treatments for prostate cancer patients?

Emma Shtivelman 37:30

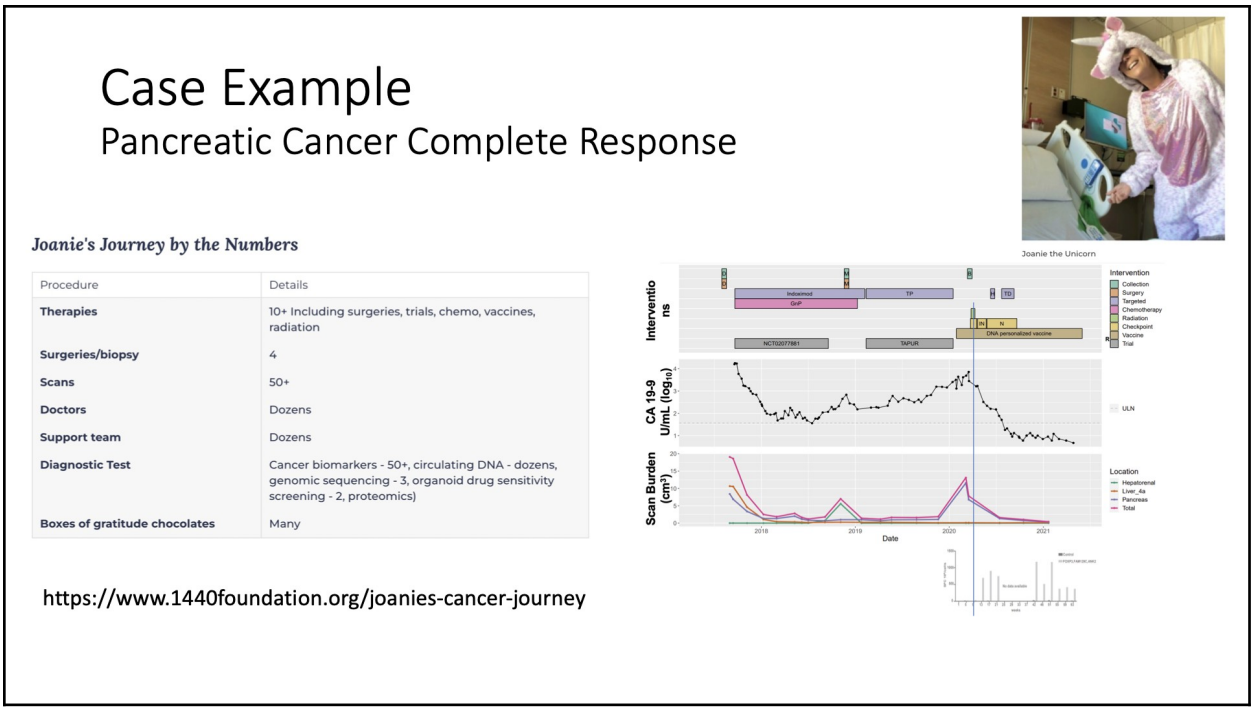
I think it very much depends on the particular mutational setup in each tumor, because prostate cancer is not considered to be immune sensitive. But combinations with PARP inhibitors in cancer that have mutations in homologous recombination pathways sometimes work. I didn't see much success in combining the newer generation androgen signaling inhibitors with immune drugs.

Have you had any successes with any cancers so far? Because in the literature, the reports are not very impressive so far. It works as you mentioned, in melanoma, of course, and lung cancer where there were reports quite a few years ago of good responses. Rosenberg's lab reported some, but their approach is less directed towards personalized vaccines.

Willy Hoos 39:01

The data is lacking on the ultimate question: Does this give clinical efficacy? There are case reports where there was response duration for the combination of things people were doing that were more than accepted, but that is not medical evidence. There is some of the literature pointing to successes. There is that pancreatic data. There's one interpretation of this that the patient's getting a significant immune response to the targets in the vaccine and not having recurrence. But there's another way to interpret this that it was essentially a proof of prognostic value, that patients who are capable of immune response are just the ones that were keeping the tumors in check and weren't going to recur with or without the vaccine part of it. The other thing that I was going to bring up is that it's prior to JLF.

“Personalized Cancer Vaccines” (Willy Hoos) [#29]



The patient that I alluded to, and her story and the case report are out there. But this was a pancreatic patient, and she had multiple lines of therapy. Then where the line is, they started their vaccine. There was a little blip of some immune response via ELISpots (enzyme-linked immunosorbent spot). ELISpot is looking for T-cells that are responsive to components of the vaccine, not necessarily proving that those T-cells can get into the tumor and kill the tumor. Because of progression, she added radiation and dual checkpoints, nivolumab and ipilimumab, because there was data saying that radiation and dual checkpoint look to have some immune response and some response rate higher than any of those three things together in metastatic disease. All three of those things, theoretically, help a vaccine response, help traffic T-cells into the tumor, and help with the activation by those two checkpoints in the expansion. The ELISpot level shot way up. CA19-9 normalized in the three months following that combination. We've continued to look deeper into that ELISpot response and find specific T-cell receptors that appear to be active, and it's still kind of ongoing work. There's a trial being designed right now to basically repeat this protocol, in a formal trial, to evaluate if it's a one out of five, one out of 10, or one out of 100 scenario. Or was it truly an anomaly and completely unrelated? Hopefully, we'll get those trials done. In the interim, the couple that went through this spoke at an event I was at this weekend, and the one point they made is that no inventions were required for them to do this. They were accessing things that had all been done before, and they were assembled logically together to yield something that's not scientifically clear exactly what happened, but the result is what was hoped for. That's this balance of where this compassionate access and where the state of the field is. If it turns out in 10 years, we figured out the exact story here, what regrets will we have in not pursuing these approaches?

Brian McCloskey 43:53

I was looking at her journey and I was, of course, paying close attention to the diagnostics, and I noticed a couple things. One is that it looks like they were using organoids. They were testing the vaccine on an organoid. Did they go through multiple iterations of the vaccine based upon the organoid response? Do you have any insight on that?

Willy Hoos 44:19

It is ongoing work to try and understand the organoid-vaccine linkage, but the organoids were done to do chemo sensitivity testing, and this patient also had HER2 amplification, which is uncommon in pancreatic cancer. It wasn’t a target of the vaccine because it wasn't a mutant HER2, it was just amplified. You couldn't target something like that necessarily. Whether the T-cells that appear to be the ones that were active to the vaccine and would be hypothesized to be the ones that hopefully wiped out the tumors, having that experiment to test that against the organoids has not yet been done, but is trying to be done.

Brian McCloskey 45:16

There's another diagnostic, and maybe Sheeno from mProbe can comment on this a little bit, but there were proteomics involved. Can you talk a little bit about how proteomics helped to improve, if it did, the development of the vaccine?

Willy Hoos 45:46

The proteomics were done primarily in the search for additional therapy priorities that were outside the vaccine. The confirmation of expression for the uncommon mutations, in theory could be done via proteomics, but it would be a lot of method development. I'm not even sure that the level of detection sensitivity that could be developed in rapid time would be at that level of detection useful for prioritizing or deprioritizing a given target. The short answer is that proteomics were not part of the vaccine part of that therapeutic journey. They were part of the HER2 part of the story, as well as the backup planning part of the story.

Sheeno Thyparambil 47:00

I agree. When you're looking for any peptides that are going to get presented into the MHC complex, the amount of material that you need sometimes is way more than typical proteomics will do. We must go through a little bit more method development to actually figure out whether that peptide got expressed or not. Outside of using DNA sequencing and RNA sequencing, we could find out what that peptide was. But at this junction, based on what we have with the sequencing and the informatics, you're basically taking a calculated risk.

Willy Hoos 47:50

Since someone may refer to this story, I'll make a point here. This TD and HER2 antibody drug conjugate HER2 drug that she got after the vaccine and the checkpoints. What's relevant in that is that by the time she went on that the CA 19-9, was almost normalized. Drawing down the line, it was at this point where it was at log two, which is CA 19-9 of like 100, down from this peak of about 7000. The three months following vaccine and radiation, and dual checkpoint, the CA 19-9 had almost normalized. There was a lot of debate in this case of whether there had subsequently been HER2 expression confirmed to still be there, and the sample that was taken at the time of the radiation. The sequencing had come back then because it was repeated. HER2 was still there. It looked on the face of it that they're getting what looks like a profound immune response. Where are the places where immune responses can fail? We can be fooling ourselves, and it's not really happening, and there's some tumor about to grow back, or there's an escape where they lose their MHC expression, and therefore the immune system can't target it anymore because it can't present the antigens and be targeted. The thought was using the HER2 drug in case there's some minimal residual or resistant disease to go in and do that. That

could be part of why this is ongoing. No evidence of disease two and a half years later, is that there was some resistance there that was knocked out by the targeted approach, or the immune system took care of it and is surveilling it, and the HER2 wasn't part of it at all. But it was all clearly happening prior to the vaccine, and prior to the addition of that HER2 drug because of how much the CA 19-9 had dropped, and how many other signals of things turned around. The last part of that slightly complicated story, which I think speaks to a lot of what this group is trying to do. Plan out a series of things that can be done and how you can inform it by diagnostics. We did all of this before JLF. It was some of the inspiration for why we're doing JLF. Back when she was clearly progressing on the prior HER2 targeted therapy, there was a question. There are now better HER2 drugs. Do you keep going after that, or do you switch strategies altogether if they're not compatible if you can't do them in parallel? The decision was that the vaccine could at least be done in parallel, but the checkpoints perhaps started to add risk at that time. There wasn't even knowledge about checkpoints in HER2 for example. They wanted to maximize the vaccine and add checkpoints, but they wanted to maximize the HER2 strategy. Could you do all that together? As part of the planning, she didn't have easy access to a biopsy at that time, or it didn't seem worth the risk with the complications from the biopsy and it was going to take time. We did a blood-based circulating DNA, and that came back and showed negative for HER2 amplification. Well, maybe there's evidence that tumors can lose the HER2, especially having what looked like it was a response on a prior HER2 therapy. That caused a change in strategy to prioritize radiation and checkpoints, along with the vaccine, rather than also continuing to go after HER2. It was while that was happening, and the response was then happening. The sequencing came back and showed that the HER2 was still there. That disconnect from those two methodologies may have been fortuitous, that the circulating DNA wasn't good enough for detecting HER2 amplification in her case, but it led us on a different path, that probably turned out to be the best path.

Brian McCloskey 52:24

Wow, that's a fascinating story. As prostate cancer patients, liquid biopsies can play an important diagnostic role. I have a very low PSA, and in some instances when I have a liquid biopsy, it will show no mutations, no biomarkers to go after. I must wait until my PSA is at a high enough level where it's going to shed enough cell free DNA. That's a fascinating story though.

Eric Hall 53:17

Willy, thanks for coming and giving us this. It's a glimpse maybe of the future of where treatment in medicine is going. What I'm wondering as a new patient, is that you mentioned it could be more successful earlier in someone's journey than later before there's more heterogeneity. I guess it's all research now. Is there any kind of access for someone like myself right now? Early in the journey? I'm three months into my journey. My PSA was 146 with Gleason 10. I'm on my first line of treatment being hormones, ADT Orgovix, with zytiga (abiraterone). I'm metastatic with a lymph node and stuff.

Willy Hoos 54:27

I don't have the answers here, but this is where you must consider: what's optimal, what's regulatory allowed, what's appropriate, what's financially the right time value of money for doing things earlier versus later and such. I don't know how to make this decision. I can go to the regulatory and get their specific language of what qualifies, and then I can share some of the feedback and pushback that we've had. There's been a few patients that are at a little earlier stage, and the FDA has pushed, "We want assurances that the patient is not foregoing

therapies that are proven for something that's unproven, and that they may not understand what they're getting into." There's a lot of nuance in multiple parts of that sentence or phrase. It gets to all kinds of libertarian perspectives and everything else that you could get into. But I think I absolutely agree that for early-stage breast cancer patients, I'm going to explicitly extract it to something that has 95%+ quality survival with no major long term side effects in a disease where 30 years ago, those results were nowhere near that. The therapies of radiation and chemo and such, and surgery, that are the accepted standards in that early-stage disease, result in very, very good outcomes. If a patient were saying I don't like those things, and I think these vaccines are really cool, I want to do that instead. That "instead part" is really a much harder thing to really support because they're arguing for an approach that significantly increases their risk, even if these turn out to work better than we expect. As you go later into it and you start to reveal that that patient is not likely in the 95%, without additional interventions, somewhere in there it switches. But even at that early stage, if it's possible for them to add the vaccine in the sequence somewhere, then it becomes much more what's the risk of doing that? There may not be that many risks, and then what's the cost? And is it appropriate and ethical and appropriate for that person to take on those costs? Then before somebody asked the question of should society take on that cost? I think $80,000 is cheap for a cancer drug, if this were an approved drug. It likely ends up being more if these ever get approved. But at the same time, maybe we could find a way to make these for $10,000. But even there, what's the threshold for society paying $10,000 per woman diagnosed with breast cancer for everyone right up front as part of that. That's a much broader topic than can a patient pay $80,000.

Emma Shtivelman 57:38

I totally agree with what Willy just said, especially regarding your case, Eric, which I'm working on now. I'm from Cancer Commons.