“Immunotherapy in Prostate Cancer - CAR-T and the Tumor Microenvironment” (Andrew Rech) [#63]

Brad Power, Allen Morris
June 28, 2023

“What is going on with the cells in prostate tumors, and what do we need to change to see better responses to immunotherapy?” – Andrew Rech

“We're using spatial biology approaches, in particular combined with other things, to try to understand some of the big axes like, ‘What's different in bone mets?’ ‘What's different in black patients?’” – Andrew Rech

Meeting Summary

What can new technologies help us see about what is going on with the cells in prostate tumors, and what we need to change to see better responses to immunotherapy?

Advanced cancer patients see immunotherapy (a treatment leveraging the immune system) as offering one of the best paths to a durable response. Cancer vaccines (one immunotherapy approach) have a lot of potential because they offer a possible treatment option to nearly every cancer patient. And immunotherapy offers the promise of durable responses -- it is fighting a biological system (the cancer) with another system (the immune system), rather than the hit and miss, less durable paradigm of targeting a biomarker with a single drug.

Immunotherapy has demonstrated success in blood cancers, like leukemia and lymphoma. However, immunotherapy has had limited success in achieving durable remissions for advanced cancer patients with solid tumors. There’s one cancer vaccine approved, and it is in prostate cancer (Provenge). It's not great for survival, but it is a shift away from chemotherapy, a little more time, and for the most part, non-toxic time. Once in a while cancer vaccines have had safe and immunogenic results.

Patients need more potent T-cell-redirecting strategies. T-cell therapies are made by collecting T-cells (a type of immune system white blood cell, also called a lymphocyte) from the patient and re-engineering them in the laboratory to produce proteins on their surface called chimeric antigen receptors, or "CARs". The CARs recognize and bind to specific proteins, or antigens, on the surface of cancer cells.

Andrew Rech, MD, PhD, Post-Doctoral Research Fellow, University of Pennsylvania, Department of Pathology and Laboratory Medicine, Laboratory of Carl H. June is uniquely qualified to talk about the current state of next-generation immunotherapies in prostate cancer and the ongoing efforts to understand and enhance engineered T-cell therapies. He is a computational biologist, not an oncologist, a pathologist by clinical training. He studies the tumor microenvironment using new approaches in the context of CAR T-cell therapies.

In this discussion Dr. Rech reviewed current phase I trials (tests of the safety, side effects, best dose, and timing of new treatments, the best way to give them, and how they affects the body)

and the latest "bedside-back-to-bench" approaches (the process by which the results of research done in the laboratory are directly used to develop new ways to treat patients).

What is the state of key immunotherapies, especially for treating prostate cancer?

- **Immune checkpoint therapies**: Some immune checkpoint inhibitors (e.g., ipilimumab) have shown significant response in a small group of metastatic castrate-resistant prostate cancer patients, while others (e.g., PD-1 inhibitors) have not achieved expected treatment outcomes. Identifying additional biomarkers and individualized treatment regimens are crucial for enhancing the efficacy of immune checkpoint therapy in prostate cancer.
- **Bispecific T-cell engager (BiTE) therapies:** Have shown promise in treating refractory blood cancers and are being explored in metastatic castrate-resistant prostate cancer. Preclinical studies show promising antitumor activity and safety for BiTEs which target Prostate-Specific Membrane Antigen (PSMA), but clinical evidence is limited. We don’t understand what the pathways are. Future directions include combining BiTE therapy with immune checkpoint inhibitors and exploring alternative tumor antigens (such as prostate stem cell antigen, and Delta-like Ligand 3). In solid tumors it is extremely difficult to find good antigens, which is a challenge for BiTEs and CAR-T therapies.

- **Adoptive cell therapy, specifically CAR-T therapy**: Being explored for the treatment of solid malignancies, including prostate cancer. Phase 1 clinical trial results show some tumor responsiveness, but also toxicity, and no significant survival benefit for metastatic castrate-resistant prostate cancer. Challenges to enhance CAR-T therapy efficacy in solid tumors include physical interference by cells in the tumor microenvironment (stroma), and reduced self-replication ability of CAR-T cells. Future research may support the feasibility of combining CAR-T therapy with other treatments, such as chemotherapies (e.g., docetaxel).

How can a patient access CAR-T therapy?

It's very early days for CAR T-cell therapy in solid tumors, and access is very limited to a handful of academic medical centers where there are open phase 1 trials. It’s early days for selecting patients who may benefit from these therapies. And patients may be concerned that the process includes pulling out T-cells right before trying to activate them, the step of killing your T-cells to then re-implant the CAR T, and needing to be in the hospital for several days to monitor side effects.

What can be done to improve the tumor microenvironment for immunotherapy?

There are a lot of molecular candidates in the tumor microenvironment which could be targeted, but research right now has not answered which candidates are the dominant ones which should be prioritized, which is one of the things current research is trying to figure out. There are drugs that can be repurposed today (like sorafenib, which can change macrophages from bad to

good), but people don't know how to utilize these. It's not going to be perfect, which is a challenge.

What’s next in tests to understand cancer and predict an individual’s response to a therapy, such as an immunotherapy?

We have better technology that's been developed in the past five years, such as spatial sequencing and single cell approaches, that provide a higher level of resolution that may reveal insight that's more useful than what we have had. These technologies are being used in research labs to get a better understanding of cancer behavior through the tumor microenvironment, for example by counting cells (lymphocytes, TILs, CTL and Tregs) in the tumor microenvironment. At Penn they are using spatial biology and other technologies to try to understand questions like, “What's different in bone mets?” and “What's different in black patients?” But spatial tests are not available to individual patients to guide their treatment. A primary barrier is clinical actionability – patients have to understand what test results mean in a couple of weeks or a month; whereas researchers comb through the details for months.

The largest area of combinatorial investigative research for CAR T-cells in solid tumors is modifying the many suppressive factors. There are probably two dozen approaches that are in sophisticated preclinical models, or approaching or are actively enrolling in phase 1 trials across solid tumors. We don't know what the important pathways are, or if there is even one such pathway.

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Meeting Notes

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Discussion Outline

1. Introduction to today’s discussion.
2. Introduction to immunotherapy in prostate cancer. (2:36)
3. Immunity checkpoint therapy and anti-tumor vaccines. (8:23)
4. What are the roadblocks to the field? (13:54)
5. Introduction to CAR T-cell biology. (18:54)
6. Tumor microenvironment and metastatic prostate cancer. (25:09)
7. Tumor agnosticism and biomarkers. (30:52)
8. Tissue agnosticism and validation. (34:42)
9. What can be done to improve the tumor microenvironment? (39:18)
10. How can patients get access to spatial phenotyping? (45:31)
11. Spatial transcriptomics and the microenvironment. (51:15)
12. Tumor antigens in liquid and solid tumors. (56:15)

SUMMARY KEYWORDS

T-cells, prostate cancer, tumor, tumor microenvironment, patients, vaccines, cancer, immune checkpoint, understand, therapy, cells, approaches, antigen, solid tumors, car, microenvironment, work, pathways, immunotherapy, target

SPEAKERS

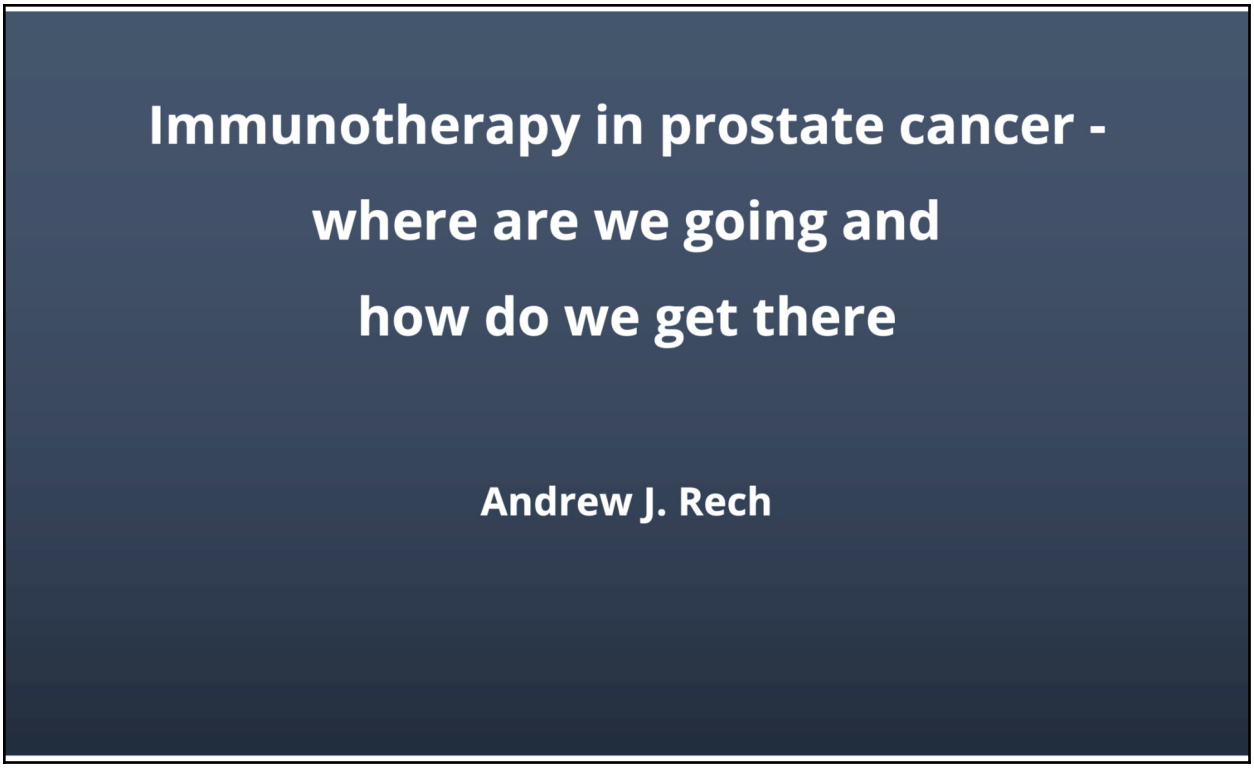
Andrew Rech (51%), Allen Morris (19%), Brad Power (16%), Robert Gurmankin (3%), Gitte Pedersen (3%), Ricardo Salgado (3%), Brian McCloskey (2%), Amit Gattani (1%)

Brad Power
We're honored to have Andrew Rech with us today. Our connection to Andrew was through Pete Kane. I forget where Pete originally met you, Andrew, but we were very intrigued. We're always interested in immunotherapies. I have lymphoma. There has been some success with CAR-T in lymphoma, leukemia, and blood cancers; not so much in solid cancers. But we love the idea of immunotherapies and cancer vaccines and CAR-T. We've had several sessions on cancer vaccines, with Lisa Butterfield and Willie Hoos, talking about what cancer vaccines offer as a treatment option. You're deep in the research and science side of it – the cutting edge of what's out there. So we're very intrigued to hear about it. I love the notion that if you leverage the immune system, you're having a system fight the cancer system, as opposed to finding a mutation and having a targeted therapy, which is like a specific part of the system fighting the system. It may work, but it will often work for only a short period of time; whereas an immune

system can get you a really durable response. Some people have gotten cures with CAR-T. So that's the context. We're really interested in and hoping to help advance your research, and hoping that you have great success in coming up with immunotherapies for prostate cancer.

Andrew Rech 2:46
Thanks for the introduction. It's really wonderful to be able to talk to all of you. I was just thinking on my way into work that through my MD PhD at Penn and residency I've talked to a lot of patients, and I've talked a lot about my research. However, I don't think I've ever talked about my research with patients ever in my career. That's probably unfortunate, and I'm happy that the pattern is changing. It's really an honor to get to talk to you.

By way of background, I'm a postdoctoral fellow in Carl June's lab at Penn. I'm a computational biologist. I'm not an oncologist. I'm a pathologist by clinical training, and I study the tumor microenvironment using new approaches in the context of CAR T-cell therapies.

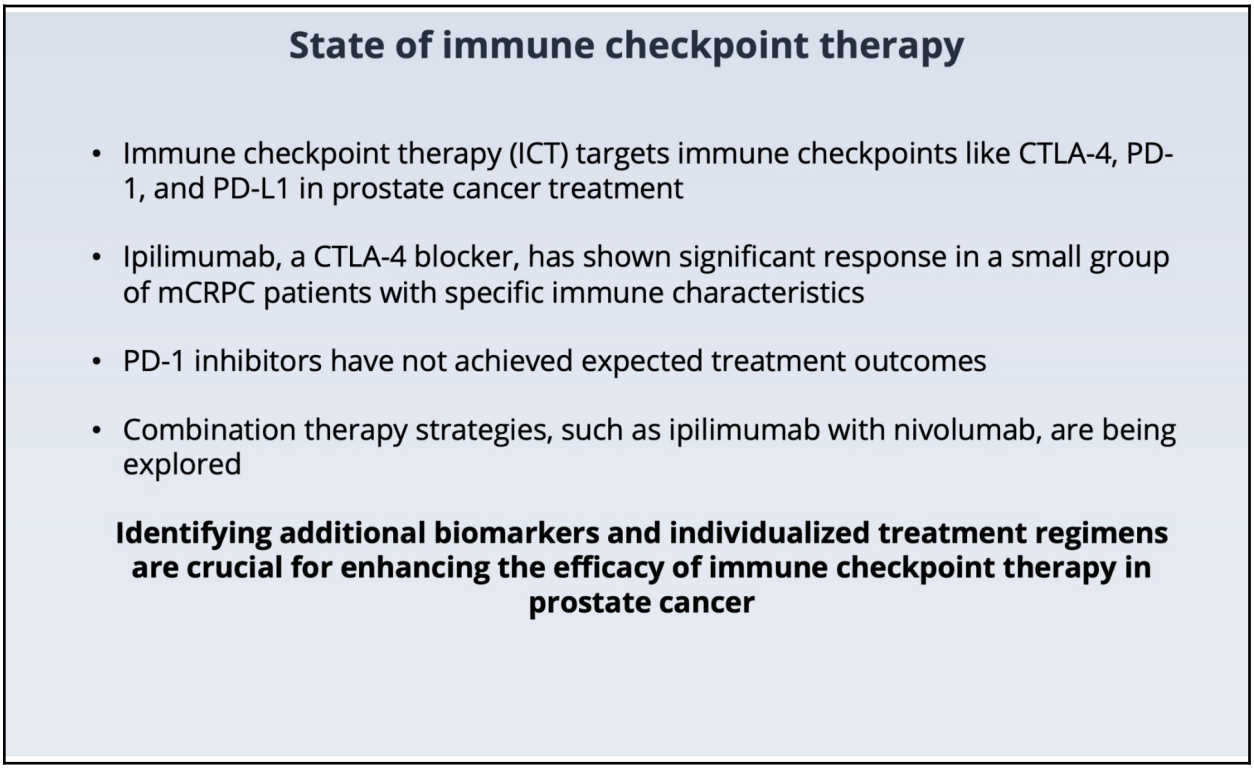


The clinical context for my research and perspective I'll be sharing in prostate cancer is trying to understand from some of our early phase 1 trials at Penn, what is going on with the cells in prostate tumors, and what do we need to change to see better responses to immunotherapy? I'm interested in approaching that from the perspective of trying to leverage the latest technology we can with the early clinical trials to try to make sense of what's happening with these cells.

The theme, as many of you know, is that despite our advancements in understanding many aspects of the immune system with respect to cancer, in many ways, we're at a complete

infancy in terms of understanding in prostate tumors and other solid tumors what is actually really key and important in the tumor microenvironment.

I was going to give a little bit of an introduction to immunotherapy and prostate cancer and then talk about CAR T-cell therapy, which is a form of adoptive cell therapy. I’ll talk a little bit about our work at Penn and our approaches and a little bit about my own work and perspective, and then hopefully, leave most of the time for questions in my area of expertise, which is tumor microenvironment studies in prognostics and diagnostics in this area, which is what I'm really passionate about, interested in, and what I'd love to talk about and answer questions about.



I wanted to start just with a little bit of an overview in 2023 with immunotherapies in prostate cancer before I talk specifically about CAR T-cells.

What is the state of immune checkpoint therapy? As many of you know, immune checkpoint therapy is a type of therapy that blocks inhibitory pathways on T-cells. And the common inhibitory pathways that have been targeted clinically and are FDA approved targets in other tumor types are CTLA-4, and components of the PD-1 pathway. The thinking behind these therapies is that you can block these inhibitory pathways on T-cells and thereby augment their anti-tumor response. In prostate cancer, anti CTLA-4 has shown responses, albeit in a small group of patients with very specific immune characteristics. And overall, with some exceptions, PD-1 inhibitors have really not achieved the outcomes that we hoped for a larger population of patients with prostate cancer. One direction for the field with respect to immune checkpoint therapy now is combination strategies. And furthermore, at a 30,000 foot view in prostate cancer, that’s what's needed and where we are.

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In terms of my interest in the area, it is trying to understand what it is about the minority, small percent of patients who respond to these therapies that's different about the tumor. And how can we identify those patients upfront if it's possible, and then learn from that. To summarize the lesson for CAR T-cell therapy from the immune checkpoint literature in the past decade, we really need to understand what makes patients different than tumors different with respect to immune function, and not just at the genomic level, but at the level of the tumor environment and how immune cells of the person interact with with tumor cells.

Brad Power 8:02
Would you just do the basics quickly on checkpoints versus vaccines? Those are both treatments leveraging the immune system, but this is the checkpoint angle?

Andrew Rech 8:14
Those are both strategies which are being investigated as cancer therapies. The idea is that for immune checkpoint therapy, these drugs work on T-cells, which already exist, and some of which may have anti-tumor function. By blocking these inhibitory pathways, the thinking is that those T-cells, which may not be functioning optimally for any one of a number of reasons or may not be effective against tumor cells, at baseline, could be made more effective by blocking these inhibitory signals. The thinking is that you have a gas pedal and you have a brake. It's very finely tuned because the immune system has this homeostasis all the time between protecting us from external threats and preventing autoimmunity at a broad level. So if you change that balance, potentially you can uncover an anti-tumor response against tumor cells by T-cells. CTLA-4 and PD-1 were among the first immune checkpoint pathways discovered on T-cells and those were among the first translated. After a long period of time this became among the first FDA-approved immunotherapeutics in tumor types which had a very high clinical need.

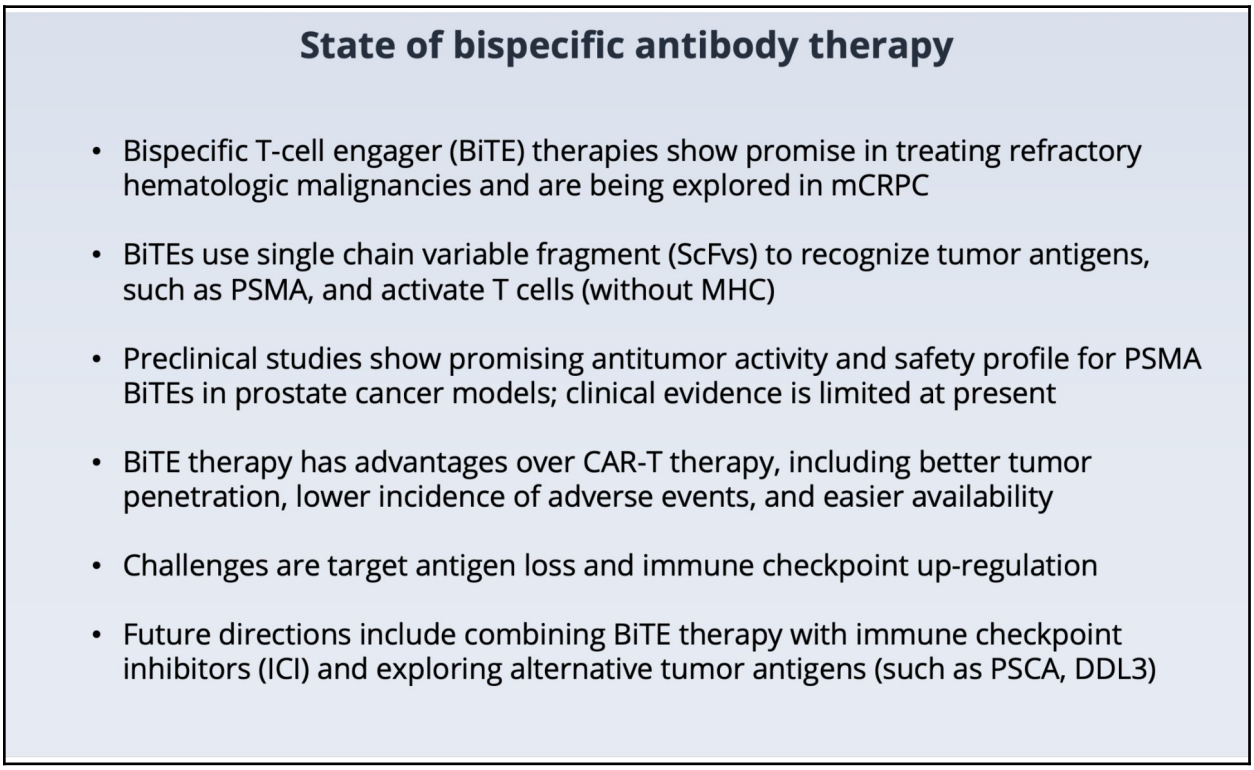
Vaccines take a different approach. Broadly, the idea with a vaccine is that you know something about antigens that the tumor expresses, and they could either be specific to tumor cells, because they're derived from mutations that are derived from things that are expressed by tumor cells, which are different from normal cells. Or they could just be selective in some way. That, for instance, are something called the cancer testis antigens (a category of tumor antigens with normal expression restricted to male germ cells in the testis but not in adult somatic tissues), which are these proteins, which are just not expressed everywhere in the body. In some cases, they can act like tumor antigens. In any case, if you know those things, you can design a vaccine in a manner that is broadly similar to how you would design a vaccine against a pathogen. And by administering that vaccine, the rationale is that you would uncover a T-cell response which is directed against that and act as a target on tumor cells. People have taken a lot of approaches, such as one called neoantigen vaccines. The idea behind this class of therapies is that each of us has a tumor that has different mutations because of random chance and variability. If you can sequence that tumor and understand what the mutations are that are different at a genomic level from normal cells in a given patient, you could design peptides around those differences and target tumor cells in that way.

The end pathway is the same, and that both are attempting to augment the cell type that we think has so much promise for targeting tumor cells. But these are actually quite different ways. And of course, people have combined them as well.

Brad Power 11:32
One common thing that everybody could relate to would be how this relates to COVID vaccines. That was mRNA. It's like a cancer vaccine, but it was going after COVID as the pathogen?

Andrew Rech 11:49
I'm not familiar with any work that has looked directly at the impact of COVID vaccines or COVID, frankly, on tumor vaccines in a in a direct way, if there's some interaction or something along those lines, if that's what you're asking, but broadly, actually, the technology and approach is similar. Many of the methods that have been investigated for devising tumor vaccines are similar to methods that have been used to target vaccines to pathogens.

So that's immune checkpoint blockade.



The other immunotherapy I want to touch on briefly, because it has some interesting comparisons to CAR T-cells in terms of thinking about the different approaches in prostate cancer that are being evaluated, is bispecific antibody therapy. Bispecific T-cell engagers, or BiTES, is a type of therapy, which is sort of related to immune checkpoint therapy and sort of related to CAR T-cell therapy, that's also being evaluated in prostate cancer. This is an antibody, but it has a unique twist on antibody-based therapies, which is that the BiTE consists of a portion of the antibody that recognizes a tumor antigen like Prostate Specific Membrane

Antigen, and a portion of the antibody that recognizes T-cells. The idea behind BiTE therapy is really a simple one. It's that if you can use this antibody to bring together a tumor cell which expresses the tumor antigen and a T-cell, you could elicit an immune response by doing so by bringing those cells in proximity and also activating a T-cell.

As some of you likely know, there have been promising preclinical studies investigating BiTE therapy in prostate cancer. The clinical evidence is still quite limited. It's been a little bit of a rocky road towards clinical development. But BiTE therapy has a couple of advantages over CAR T-cell therapy that I want to mention, because I think it gets that later on. We talked about CAR T-cell therapy, what are the roadblocks right now. Immune checkpoint blockade drugs and BiTEs are both antibodies. They're small molecules. So they are able to penetrate tumors much more easily than CAR T-cells or an adoptive cell therapy just by virtue of the fact that those are cells. And tumor infiltration is a major barrier. There appears to be a lower incidence of adverse events, although it's obviously early days. And these drugs are much more readily available. And the reason for that is that BiTEs designed against something like PSMA don't have a patient-specific component like a neoantigen vaccine or a CAR T-cell therapy, which is designed from a patient's cells. And so these are universal therapies potentially, or at least universal in the sense that they're not unique to a single patient. So this is a huge advantage of this modality in terms of affecting the population.

Ricardo Salgado 14:53
Are these HLA restricted? (Human leukocyte antigens are proteins—or markers—on most cells in your body. Your immune system uses HLA to see which cells belong in your body and which do not.)

Andrew Rech 15:22
They're not. The BiTEs are not restricted to a patient's HLA or MHC. (The major histocompatibility complex is the same as human leukocyte antigen.)

The challenge for this class of therapies is the loss of the target, the thing that the BiTE is directed against. Theoretically, in the case of immune checkpoint blockade, you are augmenting an immune response, which is directed against a number of things potentially. And that's an advantage of that broader approach in some sense than BiTE therapy or CAR T-cell therapy for that matter where there's specificity for a single target.

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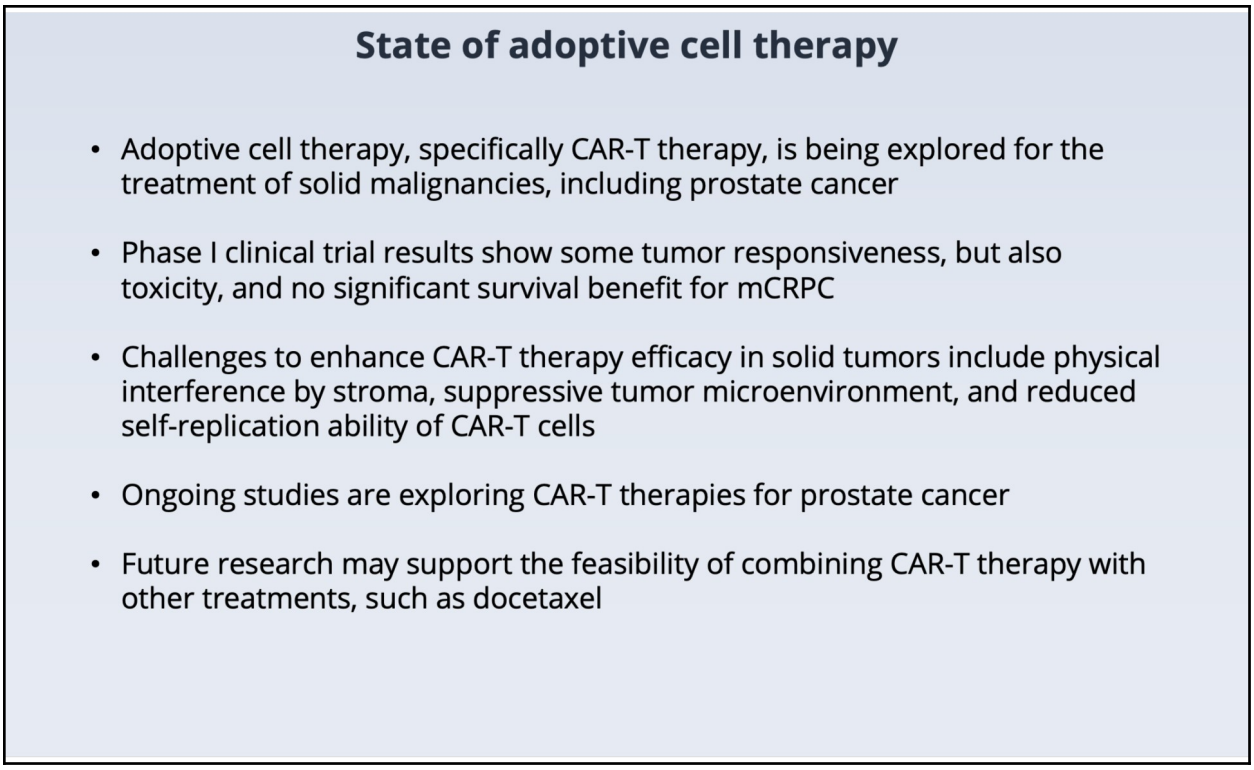
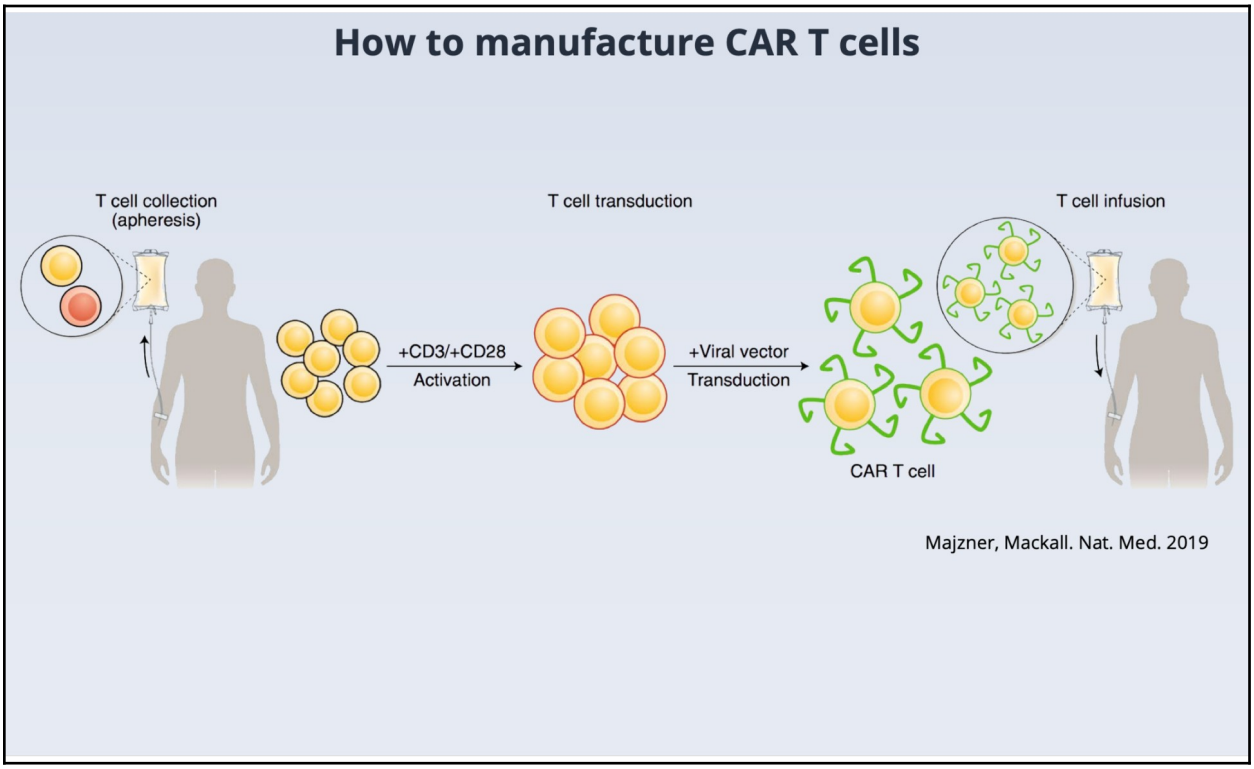


That brings us to adoptive cell therapy. Adoptive cell therapy is the notion of taking cells, modifying them in some manner, and then re-infusing them back into a patient for therapeutic gain. Adoptive cell therapies, specifically CAR T-cell therapy, as many of you know, is being explored in phase 1 trials in prostate cancer. The “CAR” in “CAR T-cells” stands for “chimeric antigen receptor”. The idea behind CAR T-cells is that there is this molecule on the outside of the chimeric antigen receptor which is not expressed in nature. It's a combination of an antibody binding fragment that recognizes a known tumor antigen and then some signaling machinery that signals to the T-cell. T-cells normally signal to the T-cell receptor, recognizing a target in the context of HLA (human leukocyte antigens) or MHC (major histocompatibility complex). But CAR T-cells don't do that. They have this protein which is not expressed in nature that redirects them to the target that the CAR is expressed against. The thinking behind that is that you can control the specificity of those cells and direct them in a way that's therapeutically advantageous.

The phase 1 trials thus far have shown some tumor responses in terms of radiographic criteria, also a significant amount of toxicity, and there's been no survival benefit. To echo Dr. Sartor last week, thus far, CAR T-cells have disappointed initial expectations in prostate cancer. But it's early days, and we're trying to learn more about why that's the case, what the next generation of therapies should be, and how they should be engineered and designed.

There are several barriers for CAR T-cell therapy. The first is that unlike the literature with respect to B cell malignancies, where the the tumor microenvironment, so to speak as the blood in prostate cancer and other solid tumor types, you have a tumor environment which is very hostile to T-cells for a variety of reasons in terms of immune suppression, a lack of oxygen, a

lack of nutrients, physical pressure, in some cases that prevent the influx of cells. So it's a real challenge, unlike the blood, where it's essentially like a perfect incubator for T-cells, for CAR T-cells to actually actually be effective in a solid tumor. There are several ongoing studies for CAR T-cell therapies in prostate cancer at City of Hope, at Penn and other places. Another idea that's being explored, as you would imagine, is the combination of CAR T-cell therapies with additional modifications of the T-cells, or in combination with other therapies.



I'll give a little bit of more introduction about what CAR-T-cells are in some detail. I think the best way to do that is just talking about how they're made. Traditionally, the way CAR T-cells are made for therapies, T-cells are collected from a patient in a process called apheresis, where the patient's blood is taken and the white blood cells are enriched, and then T-cells are harvested. Those T-cells are activated against some proteins expressed on the surface of the T-cells. And then they're transduced with the CAR itself, which again is this foreign protein which is specific to a tumor antigen, which is not expressed in nature. Then there are many other steps that are highlighted here in terms of all the control and other things. Then this T-cell population would now express this new protein is infused back into a patient to elicit an anti-tumor effect. And so one aspect of CAR T-cell biology that I want to talk about for a moment is the fact that these cells are living cells. They have mechanisms of effect in a tumor, which go beyond their specific recognition of tumor cells that express a target antigen.

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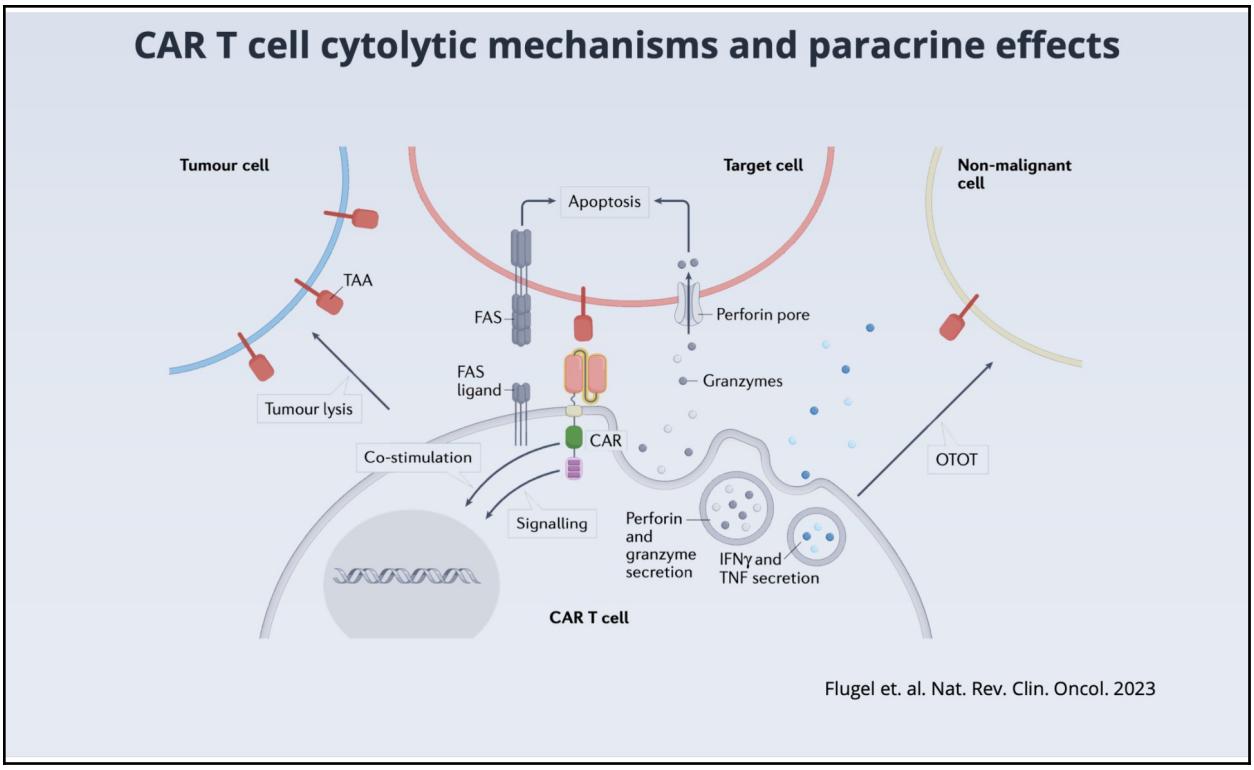

This slide shows a CAR T-cell on the bottom of the slide. This is the chimeric antigen receptor recognizing an antigen that the tumor cell or target T-cell expresses. The hypothesis behind CAR T-cells again is that this new protein allows this target antigen to be recognized. Then that elicits the cytotoxic effect, where T-cells can lyse and destroy this tumor cell. We will come to appreciate in our preclinical studies and in emerging clinical data that the CAR T-cells actually have many other functions of T-cells in their microenvironment, which are important to consider. One of those is that they secrete lots of pro inflammatory cytokines that modulate the tumor and modulate the microenvironment around the CAR T-cells and tumor, and those have effects which can both inhibit or augment anti-tumor responses, in addition to the direct specificity of CAR T-cells. Something we're trying to learn more about is understanding what those other effects are and how they could potentially be manipulated to improve therapies.

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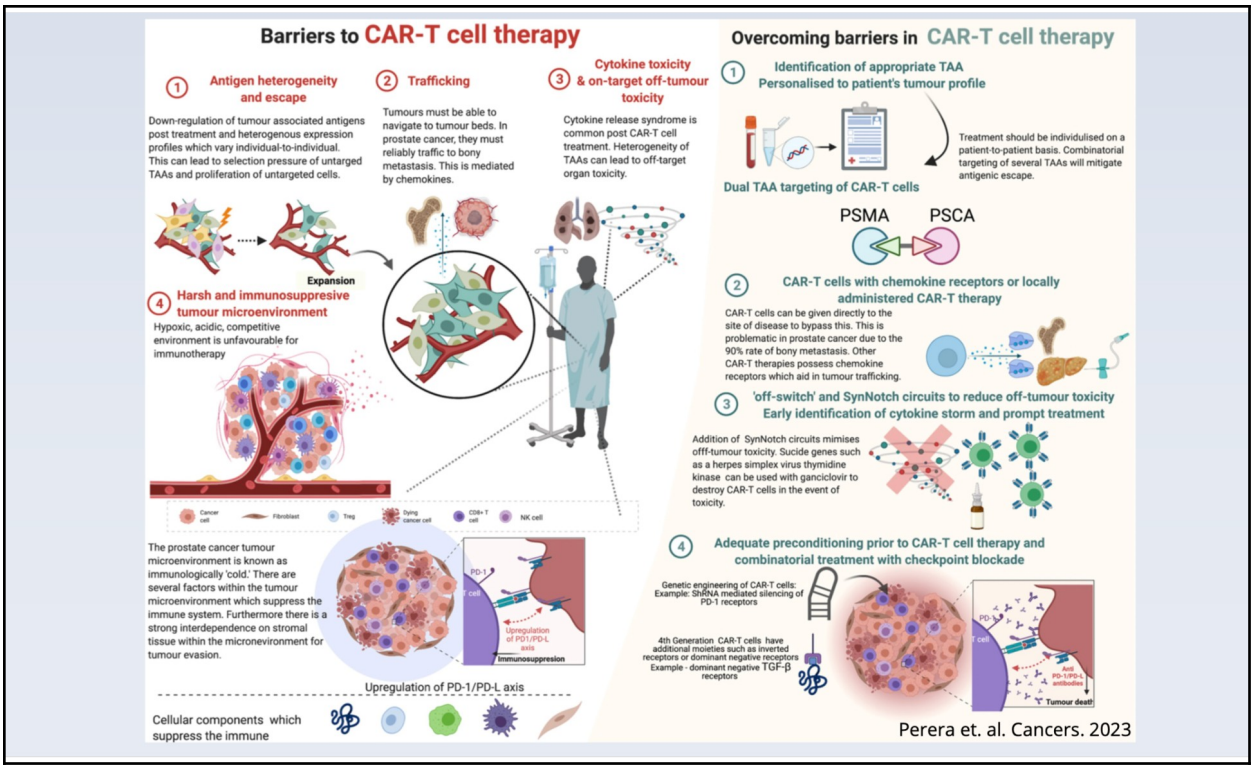

There are a handful of barriers to CAR T-cell therapy and a handful of approaches for trying to overcome them. That's summarized on this slide from a recent review, I think in a nice way. So I wanted to use it to go through some of these areas.

The first is the notion of antigen heterogeneity or escape. This is the idea that CAR T-cells are directed against, for instance, PSMA, prostate specific membrane antigen. Of course, there's then pressure on that antigen that can be lost if the tumor cells don't express their protein that CAR T-cells are directed against. That's a potential issue for the efficacy of the therapy.

In terms of solid tumors, a second issue is T-cell trafficking. CAR T-cells need to influx into tumors, which is difficult for all sorts of reasons, including just a lack of nutrients or lack of ability to persist functions that destroy tumor cells within that microenvironment.

A third issue is toxicity in terms of cytokine release syndrome, or another entity called immune cell associated neurotoxicity syndrome. It's early days of understanding these but the thinking is that in a subset of patients, because of the cytokines that are released during this anti-tumor response, that can be associated with significant toxicity in terms of adverse events and other effects. So we have to understand that process in order to understand how to limit those effects while preserving tumor response. There is much ongoing research on this topic, trying to do that.

Brad Power 23:59

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Can you talk about where this is in the translation from the lab to bedside? Are CAR Ts in clinical trials? If you're a patient and you want access to some of these treatments, is that possible now or in one to three years? Can you handicap things in terms of access and timing?

Andrew Rech 24:43
There are a handful of open trials in prostate cancer in the United States. They're restricted to academic medical centers, and they're restricted to patient populations that meet specific criteria like expression of tumor antigens, etc. So, on a broad view, I would say that it's very early days for CAR T-cell therapy and access is very limited on that basis to a handful of academic medical centers where there are open phase one trials.

Allen Morris 25:16
I am also a pathologist. So to give everybody else perspective, we both speak the same language and no other field in medicine speaks pathology. I have a lot of questions for you.

Andrew Rech 25:47
Thanks for revealing that you're a pathologist before asking your question. That's handy.

Allen Morris 25:56
Brad was talking about bedside. Well, the most concrete bedside thing as far as a pathologist is concerned is a pathology report. What people may not know, is that there is a guru pathologist Dr. Jonathan Epstein that a lot of pathologists like me and you send our difficult cases to. Okay, this talk is about the tumor microenvironment (TME). Is that right?

Andrew Rech
Yes

Allen Morris
Have you ever seen an Epstein prostate cancer pathology report, and for that matter, any pathology report, that mentions the tumor microenvironment (TME)?

Andrew Rech 26:35
That's a good question. Maybe, literally, a few times. But I take your point.

Allen Morris 26:44
I'm absolutely curious, because I've never seen one. What exactly is the pathologist saying in the pathology report in the few instances that you've seen mention of a tumor microenvironment?

Andrew Rech 26:55
In the context of phase 1 trials for CAR T-cells, there are some diagnostics that we do up front in terms of antigen expression that are important. We're at the infancy of trying to select patients who may benefit from these therapies.

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Allen Morris 27:13
Phase 1 is experimental. That's not in practice, at the bedside, the pathology report. I'm actually getting somewhere with this. It turns out that the only TME concept, but you can correct me if I'm wrong, because we're both pathologists and speak the same language - the only tumor microenvironment (TME) concept that has hit the bedside are Tumor Infiltrating Lymphocytes (TILs) and only in one cancer, and that's melanoma. Are you aware of any other cancer, where the NCCN guidelines suggest that pathologists should report anything about tumor microenvironment (TME) other than TILs in melanoma?

Andrew Rech 27:48
Yeah, I don't think so. I think you're right about that.

Allen Morris 27:51
Okay, great. And also for background, I want everybody who is not a pathologist to know, the original reason for the choice of the word “hot” in regard to cancer reflects that the cancer is inflamed. And “hot” was chosen because of one of the five cardinal signs of inflammation: heat (Latin: calor, 1st described B.C by the Roman writer, Celsus - of temperature scale fame). And these hot/inflamed cancers, specifically melanoma, in the 1980s, were recognized as such by practicing pathologists looking down the barrel of a microscope at a melanoma whose individual tumor cells were not only surrounded by lymphocytes, but showed varying degrees of cell death. And thus it was believed by pathologists, in the 1980s, that they were witnessing with their own eyes not just any lymphocyte, but a Cytotoxic T Lymphocyte (the CTL), the sentinel lymphocyte in the adaptive immune system. The sentinel cell that a vaccine is aimed at, to activate/prime it to kill cancer. And sure enough, when IHC (immunohistochemistry) tests for markers (such as CD3+ and CD8+) were developed for bedside use in the 1990s, these cells that surrounded and killed individual cancer cells were in fact the CTL; not B cells, etc.
If the cancer is densely inflamed by CTLs, as opposed to Tregs, the cancer is termed hot.
In other words, density of TILs (CTLs) in the TME is the basis for why a cancer is called cold or hot.

> [*Allen Morris background: Another nuance is that TILs are divided into tumor killing lymphocytes (CTLs) and tumor promoting lymphocytes (immunosuppressive Tregs) and the good guy and the bad guy look the same under the microscope, unless the CTL is caught in the act of killing*.]

My next question to you is: Do you know of any study that shows what the distribution of TIL density is amongst the most advanced state of prostate cancer patients: mCRPC (metastatic castrate-resistant prostate cancer)? Admittedly, in the future, this final advanced state will be subdivided into even more states, as prognosis gets better, but currently, that's the most advanced state. Do you know of any articles out there?

Andrew Rech 28:54
I don't actually, and I think that's missing in literature that certainly exists as you know in other solid tumor types. It does not exist, as far as I know, for metastatic prostate cancer. There are plenty of studies that assess the tumor microenvironment and assess T-cells and T-cell function, but not in a way that you're suggesting, to the best of my knowledge.

“Immunotherapy in Prostate Cancer - CAR-T and the Tumor Microenvironment” (Andrew Rech) [#63]

Allen Morris 29:14
I recently did a study. The case series study was courtesy of Brian McCloskey. Brian revealed to me that three Prostate Cancer Lab members had their TILs assessed. So I have a study of three random advanced prostate cancer patients. They're very educated patients that are part of the Prostate Cancer Lab. All had no or few TILs. What is the likelihood of that?

> [*Allen Morris comment: The chance of all not having brisk TILs, the biomarker predicting response to ICIs (immune checkpoint inhibitors) is ½ x ½ x ½ = ⅛, not quite the needed p value of .05 to show significance, but close*].

Background for all who are not pathologists: Pathologists grade TILs, only for melanoma, in a very rough semiquantitative fashion as: absent, non brisk, and brisk.
You're aware of that, right?

Andrew Rech
Yes.

Allen Morris
And you know that rough 3 scale grading of TILs has tremendous interobserver variability?

Andrew Rech
Yes.

> [*Allen Morris comments: This interobserver variability is, in part, because the requirement that one see cell death to call something a Cytotoxic T-cell subtype of TIL was lost in the John Wayne Cancer Institute literature, and no one corrected that oversight in the NCCN guidelines. Without the requirement of cell death, the lymphocytes could just as easily be Tregs, the (bad) immunosuppressive (cancer promoting) category of T-cells, the ones you don’t want in your tumor immune microenvironment. But apparently Tregs, or at least not a winning cadre of Tregs, are not present in the 60% of advanced melanoma patients who appear cured by double checkpoint blockade; so the need for pathologists to recognize cell death probably was not clinically important.*]

Andrew Rech 30:19
To your larger point about the twofold issues with the lack of diagnostics and prognostics around immunotherapy and in prostate cancer, I have nothing but agreement to add.

> [*Allen Morris translation: Both Dr. Rech and I agree that the tumor microenvironment concept is not just in its infancy, it is in a very early stage concerning bedside application, despite an avalanche of preclinical studies which Dr. Rech is steeped in and I know little about. Except for TILs (tumor infiltrating leukocytes) in melanoma, this infancy cannot be overstated. You will never see any prostate pathology report that mentions the tumor microenvironment (TME) now and in the intermediate future.*]

Allen Morris 30:32
There is the final substage in the final stage (4) of prostate cancer, as everyone knows, metastatic castrate resistant prostate cancer. The semantics of this suggests a very advanced state of cancer. But compared to other solid cancers such as lung, pancreatic, head and neck, squamous cell, colorectal, bladder, cervical; prostate cancer is not advanced with respect to

responsiveness to immune checkpoint inhibitors, as belied by a rich (hot) density of TILs (CTLs). CTL is the sentinel cell of the adaptive immune system poised to attack, but by immune checkpoint signaling, the “genomically advanced” cancer cell signals self, a friendly, so the CTLs stand down. Keytruda blocks those stand down orders, uncloaks the cancer as foreign/non-self, but only in about 10% of mCRPC patients. A figure so low, that in unselected cases, Keytruda failed in 3 phase 3 studies in prostate cancer as relayed to me by Brad. Once a clinically validated biomarker is developed selecting those mCRPC patients likely to be responsive to ICI (Keytruda, Opdivo, Yervoy, etc..) therapy, a phase 3 trial will succeed.

The immune checkpoint inhibitor breakthrough came about just recently, as everyone knows. A monumental breakthrough that resulted in the emergence of a fourth pillar of cancer therapy, immunotherapy; so important that a Nobel Prize was awarded in 2018.

Another brand new concept that resulted was the concept of **tissue agnosticism**. Keytruda in May 2017 was FDA approved for all adult and pediatric unresectable or metastatic MSI-high or Mismatch repair deficient (dMMR) solid tumors regardless of cell of origin (tissue/cell lineage), if one has progressed following 1st/2nd/etc treatment, and there are no satisfactory alternative options.

> [*Allen Morris translation: In other words, Keytruda is approved as a final hail mary for all solid tumors period. And why, in part, I put additional ICIs (Immune checkpoint inhibitors, e.g., PD-1/PDL-1, such as Keytruda/pembrolizumab, Opdivo/nivolumab) into Amit’s treatment plan; even though as a pathologist, I have no business weighing in on treatment, and I apologize to Amit for that.*]
>
> [*Allen Morris comments: To put this into historical perspective from 1850, the dawn of modern Virchow pathology, till 2017, cancers were all viewed, classified, risk stratified/prognosticated, and treated as to their cell of origin. One has Lung Cancer, Pancreatic Cancer, Colorectal Cancer, Prostate Cancer; not Keytruda responsive cancer. But in 2017, for the first time, a drug (Keytruda) was approved by virtue of molecular markers regardless of the cell of origin. The concept of tissue agnosticism was born in 2017. I believe all checkpoints will share this tissue agnostic capability as they appear to be a quintessential, and common for all cancers, immune escape mechanism.*
> *“Hey, I am self. I am waving the white flag at the checkpoint. T-cells, if you are armed/primed against my mutations, do not attack me.” This appears to be a universal escape mechanism of “molecularly advanced” cancers.”*
>
> *Immune escape: One of the hallmarks of Cancer as enumerated by Weinberg, in his “Hallmarks of Cancer”, the most cited paper in molecular biology. In addition to codifying the definition of what cancer is, Weinberg is the discoverer of the 1st oncogene (accelerator) RAS and the 1st tumor suppressor (brake) Rb. When is his Nobel Prize coming? It is long overdue. This whole “cancer is a genomic Darwinian disease” concept stems from the forgoing. Not smoking is the cause of lung cancer, soot is the cause of chimney sweep scrotal cancer; rather they are mutagens all acting indirectly through their capacity to damage the genome.*
>
> *A corollary of this, by virtue of an explosion of investigational drugs targeting molecular pathways, is the belief or wish, that all molecular mutation targeted therapy should be tissue agnostic. The Prostate Cancer Lab members, I believe, are completely on board with this hope and see companies like Guardant and Tempus as the repositories of untapped actionable information. But only a few genomic alterations have a significant track record such as decades of analytic (pathology/laboratory) validation, the first step, let alone clinical validation, in specific cell lineage cancers. First and foremost breast cancer, then in the last decade lung cancer.*]

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Concerning Immune Checkpoint Inhibitor biomarker validation, I see it in practice (at the bedside), including tumor (TPS) and combined scores with different cutpoints for PD-L1 expression.
Where do you think the research is as far as using PD-L1 as a biomarker for ICI use in prostate cancer?
Let me preface this, by Brad teaching me, that Keytruda has already failed three times in phase three studies concerning prostate cancer. Where is the validation process for PD-L1 expression in prostate cancer?

Andrew Rech 34:10
This is a whole rabbit hole. I'm not an expert in this. As you alluded to, this has been a very controversial topic about to what extent PD-L1 is or is not a biomarker. The larger point I would make, not being an expert in other tumor types, where things stand is, that's the best we have, which is really just not enough. We don't really have any way to distinguish.

Allen Morris 34:39
The roadmap exists which I will illustrate by first turning to HER2/neu, which along with ER (estrogen receptor) and PR (progesterone receptor) are the longest studied biomarkers in solid cancer oncology.

> [*Allen Morris background: Even though HER2/neu expression is not validated in prostate cancer, HER2/neu expression, compared to all other molecular markers that modern NGS is unearthing, is an incredibly well-established biomarker in breast cancer for greater than two decades, and has been validated in additional cancers including GE junction cancer circa 2012.*
> *HER2/neu analytic testing has gone through many cycles of optimization. And, if you believe in tissue agnosticism, maybe whatever was done to validate breast cancer and to validate GE junction cancer, will translate to other cancers like prostate cancer.*
> *When you go into the rabbit hole of validation for HER2/neu expression, the first approximation is that it is very nuanced and controversial, just like PD-L1, as Dr. Rech attests to; but when you dive deeper, there is virtually no difference at low power for what is graded as 0, 1+, 2+, or 3+ HER2/neu expression. Yes, at high power, there are several nuances as to where the cut point differences are for breast cancer compared to GE junction cancer. And admittedly it took many years of painstaking research by many groups to figure out these nuances. But otherwise, it is very translatable.*]

For both of these cancers, breast and GE junction, it is bedside/routine to test for HER2/neu expression as its clinical significance is established as a biomarker for successful trastuzumab (anti-HER2/neu) treatment.
Our pathology lab in Redding, California, routinely tests for HER2/neu. Yes, validation in prostate cancer has not happened yet. But the roadmap is clear and prostate cancer is biologically closer to breast cancer than GE junction cancer. It is just a matter of time before prostate cancer is validated for HER2/neu.

A similar scenario exists with the biomarker PD-L1 used for not only Keytruda, but also Opdivo efficacy. PD-L1 expression is validated as a biomarker for Keytruda therapy for many cancers including Non-small cell Lung cancer, cervical cancer, head and neck squamous cell carcinomas, triple negative breast cancer, and Esophageal and Gastroesophageal junction cancers, and PD-L1 is validated for Opdivo therapy in non-small cell lung cancer.

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For each cell lineage: PD-L1 expression is evaluated differently, based on the idiosyncrasies and nuances of the validation process.

> [*Allen Morris background: As an example of the idiosyncrasies of the validation process: Hot off the Press: Just in the last month, as an example of how these things can evolve, the College of American Pathologists just made a top down requirement that all tumor tissue must have the time of removal from the patient recorded, and the time the tissue is placed in fixative recorded with a standardized, partly arbitrary, requirement that the cold ischemic time cannot be greater than 6 hours. Because it is known that the greater the ischemic (ex vivo) time the greater the deterioration of molecules to be tested.*
>
> *If any of you relook at your pathology reports none of them will list when the tissue was removed from you and when the tissue was put into fixative except for breast cancer. But from now on, every cancer patient, including a prostate cancer patient, will have that on their pathology report required by CAP and then in lock step required for CLIA accreditation. Remarkably, what you will find is that the “adoption rate” for this, a pathology edict, will be measured in months and certainly less than one year. Prostate Cancer Lab members, start looking for that new entry on your forthcoming bedside pathology reports, and let me know what the adoption rate is? In other words, you, Prostate Cancer Lab members can be a part of accruing data for an adoption rate study.*
>
> *Pathology/Laboratory Medicine is an order of magnitude more rigorously regimented than any other field of medicine and inspected regularly. A cadre of inspectors descend on pathology departments with checklists, and prioritize the implementation of the new edicts. Case in point: Our pathology department has already adopted this cold ischemic time mandate, including having our client service department visit all 350 physicians in our community explaining why we placed new date and time checkbox requirements concerning ischemic time on our pathology requisition forms.*
>
> *You might ask, how could this be, that the very molecules that recent NGS is unearthing at Tempus and Guardant may already be underestimated by virtue of ischemic deterioration. How could this happen? Why was this never controlled before? If you do ask this, why did you not ask why temperature, atmospheric pressure, humidity, and ph are not recorded? After all, they are all important variables on the stability of molecules. Let alone gloves, humans are covered with RNAses, etc. There are just too many factors to be potentially optimized, and ultimately one eventually relies on the “proof is in the pudding”, clinical validation. But first, as Dr. Sartor taught us, you have to try to optimize analytic validation (I am impressed that a urologist would even know to mention the phrase: analytic validation); that is the horse first, before the cart.*
>
> *The analytic validation process is a foundational process in laboratory medicine/pathology. Indeed that is what laboratory doctors/pathologists do. It is not easy; it is nuanced; it takes a lot of time, and is not a one time process, but goes through continual updating/optimization; such as the above CAP top down ischemic time requirement. And even though for advanced prostate cancer, PD-L1 testing has not even emerged from analytic validation first round optimization; let alone clinical validation, it is just a matter of time. Keytruda and other ICIs (except for Keytruda 2017 by tissue agnosticism for the hail mary ultimate end stage, and Keytruda 2021 for MSI-H/MMR-d mCRPC patients, who have progressed through docetaxel and/or novel hormone therapy) are not FDA approved for PC.*

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> *Because of this, there is a need for a biomarker which can select for patients who will more likely respond to ICIs and the most likely candidate is the actual molecular synapse involved, PD-L1:PD-1.*
> *The below reference is a systematic review of over 100 research articles. So, painstaking research over many years has already been done. And more years will be required, but the roadmap is there, and this biomarker will eventually be validated for prostate cancer as it has been for many other cancers. Immune checkpoints appear to be a common “hot” tumor immune escape mechanism.*
> *https://doi.org/10.3390%2Fcells10113166*

Amit Gattani 36:24
I went through an immunotherapy trial. It was Keytruda, that Allen just mentioned, and I feel that I was on it for six months. So I understand the challenges with immunotherapy in this.

I'm curious about the state of the BiTE trials. I understand the toxicity related to CAR-T, and I know many of the CAR-T trials have been stopped or paused, at least for a while, because of toxicity. Can you comment on the toxicity with the BiTE trials or current unpublished success? What does the research community know about the BiTE trial? There was a Harpoon trial, and Amgen 509. Many of these BiTE trials are going on right now.

Andrew Rech 37:11
I don't know much beyond what I have read about it, the same things you've read. There's not a BiTE trial open at Penn. I'm really not able to comment on any unpublished work related to that.

Amit Gattani 37:40
What about the toxicity concerns and the BiTE trials? Any thoughts on that? Or any data on that?

Andrew Rech 37:50
What I'd say broadly about BiTE (bispecific T-cell engager) therapy and CAR (chimeric antigen receptor) T-cell therapy is that there are many attempts to figure out what pathways are. We can block therapeutically to limit CRS (cytokine release syndrome) and block some of these adverse events, in the best case without altering or suppressing the anti-tumor response. But in the broad spectrum of things, we're really at an early stage in understanding what those pathways are. The thing that is an active area of preclinical research now is understanding the extent to which that's different in the setting of prostate cancer, or any solid tumor for that matter, versus a hematologic malignancy, where there is more data about cytokine release syndrome. I don't think we know the answer to that yet. There are clinicians who run these trials that have their clinical experience in terms of using agents that we think are effective for limiting toxicity, but we're at the very early stages of understanding what's different, or if there are different approaches that are needed. It's a really difficult question and problem. It will take time to figure out the answer to that. Sorry, that's sort of a non answer, but that's the state of where things are at least in solid tumors to the best of my understanding.

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Robert Gurmankin 39:17
I know you deal with CAR T and overcoming barriers in CAR T-cell therapy, but in a more general sense, what can be done to improve the tumor microenvironment for immunotherapy? I literally just got my first Keytruda infusion yesterday, I have the biomarkers that hopefully will make it successful. The doctor I'm seeing at Penn is recruiting for a CAR T trial. And when I asked her, “What can I do to improve the situation where I am?” She really had nothing to offer. I asked about probiotics because there have been some things along those lines, of whether docetaxel improves the microenvironment. And it seems like nobody knows.

Andrew Rech 40:18
I think that answer is accurate. I mean, I share the same opinion. To the point that was being made about PD-L1/PD1 as an expression, as a diagnostic, the lesson from that in the immune checkpoint literature is that it is so hard to find a signal that is actually meaningful and clinically actionable with regard to the tumor microenvironment. From my perspective, as a basic researcher who studies patient tissue and tries to understand from a research context what's happening in the tumor microenvironment, as time goes on, our ability to assess different components is really rapidly increasing in terms of the resolution of studies, and what we're able to understand about how different immune components interact. It's a long road and a difficult one. But as our ability to actually understand the components that are important increases, the thinking is that you're able to translate some diagnostics out of that to better stratify patients, that there has been a tremendous difficulty for immune checkpoint blockade, and I'm sure it will be in prostate cancer as well. And we're behind other disease types and understanding that to the point that was made earlier, there have been very few studies in metastatic prostate cancer with regard to the tumor microenvironment, T-cell function, even the density of T-cells. From my perspective, that is the path forward in the long term. I don't have an answer beyond what your doctor told you. But in the long term, it's that deeper understanding about what specifically in prostate cancer are the major axis because we are starting to have the tools to block, inhibit, and engineer around immune suppressive pathways. We have to understand what they are, be able to even start to leverage those things. Picking your favorite immunosuppressive pathway, there's many that have been described in the prostate microenvironment, we need to understand in humans at a population level, where's the effect size that we can initiate trials against given pathways? And that comes down to the basic researcher. I know I sound like I am asking for more grant money, and I think that's the reality of it is you have to understand what's going on first.

Robert Gurmankin 42:25
She described the CAR T study that she's recruiting for, and…

Andrew Rech 42:31
I think I know who your doctor is. I think she's a colleague of mine.

Robert Gurmankin 42:34

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I spoke to the nurse. And it's scary. First of all, I didn't want to pull out T-cells right before we're trying to activate them. Then the whole idea of killing basically all of your T-cells to then re-implant the CAR T – that's a big reach to get people to go for that, I think, at this point.

Andrew Rech 43:14
For everyone, what Robert's referring to is the notion that we found this phenomenon, for reasons that maybe not worth going too deep into, that there is a benefit of removing or getting rid of some endogenous or normal T-cells before infusion that potentially helps proliferation of cells. So that's one of the steps that you're referring to, and, yeah, I agree with what you said.

Robert Gurmankin 43:43
Any kind of therapy that they have to put you in the hospital for several days raises a bit of a red flag, especially when you're talking phase one. Maybe somewhere down the line if I need it, and maybe things will get better.

Brad Power 44:06
Andrew, it's probably useful to set a baseline on Carl June and his June Lab, which is famous. Please give the one paragraph Wikipedia version on who he is and what that history is.

Andrew Rech 44:25
Penn has been involved in some of the CAR-T research from the beginning of the field and Carl contributed to the early efforts at FDA approval for hematologic malignancies for CAR T-cells directed against CD19 over the previous years. Since that time, he's shifted his effort along with many other labs at Penn towards trying to understand how to take what works incredibly well for blood cancers and figure out what needs to be modified to work in solid tumors. That's really a focus of his. He's been a tremendously important pioneer in the field, up to this point. His trainees, like me, are very focused on prostate cancer, pancreatic cancer, ovarian cancer, trying to understand what's different in these cases. Can CAR T-cell therapy work? What do we need to change? Etc.

Brian McCloskey 45:29
One of the objectives of the Cancer Patient Lab/Prostate Cancer Lab is to accelerate translational medicine. There's so much science out there that is available that is simply not brought to bear in our treatment decisions. As we're talking about the tumor microenvironment, it seems to me that understanding the TME is so critical. Spatial phenotyping, for example, would seem to be one of those tools that could help us to understand it. Yet getting access to it is really a challenge. I'd just love to get your thoughts on, Would it be helpful? How can patients in the Cancer Patient Lab/Prostate Cancer Lab get access to that type of technology?

Andrew Rech 46:26
That is my soapbox. It’s the 10-year vision I would to see occur.

I completely agree with what you said, obviously. You're preaching to the choir about this. It's in my personal area of interest. To get on my soapbox about it, we have a range of research

approaches we use in the lab that I'm interested in. I primarily work with spatial sequencing approaches. I'm a big believer in that approach, personally, but which are not available in the clinic, as you know, are not available to patients. How can patients get access? The difficulty comes down to interpretation of results. I mean, as a computational biologist in the lab, I work with 30 paired biopsies, let's say, from patients who got CAR T-cells, using these approaches, and it was really difficult to make any meaning out of these new things. We will get there eventually. But I think that a primary barrier is clinical actionability, we have to understand what the results can actually mean, in a couple of weeks or a month; whereas these things are just not engineered or designed or logistically able to fit in that timeframe and get out information that's helpful. It's a research approach, you're combing through the weeds for months.

Brian McCloskey 48:51
If more patients received spatial phenotyping, that could shed light on the differences across patients. It could give a better understanding of what's clinically actionable. More data is better.

Andrew Rech 49:23
I completely agree. We're doing that. We have two focuses on that area at Penn. Other groups around the country are also doing that in prostate cancer. We're using spatial biology approaches, in particular combined with other things, to try to understand some of the big axes like, “What's different in bone mets?” “What's different in black patients?” Those are two primary questions for us. We know there are massive differences immunologically. We at least hypothesize that on the basis of what's been published, but these have not been characterized in detail or what the relevance is for therapy. Those are the studies that we're doing. Now, of course, many of the studies have been done, but we have better technology now, that's been developed just in the past five years, spatial sequencing, single cell approaches, etc. That level of resolution may reveal insight that's more useful than what we have so far, counting lymphocytes, TILs in general, and CTL and Tregs in particular, are standing for a couple of things in tissue. It's very, very basic in the scheme of things.

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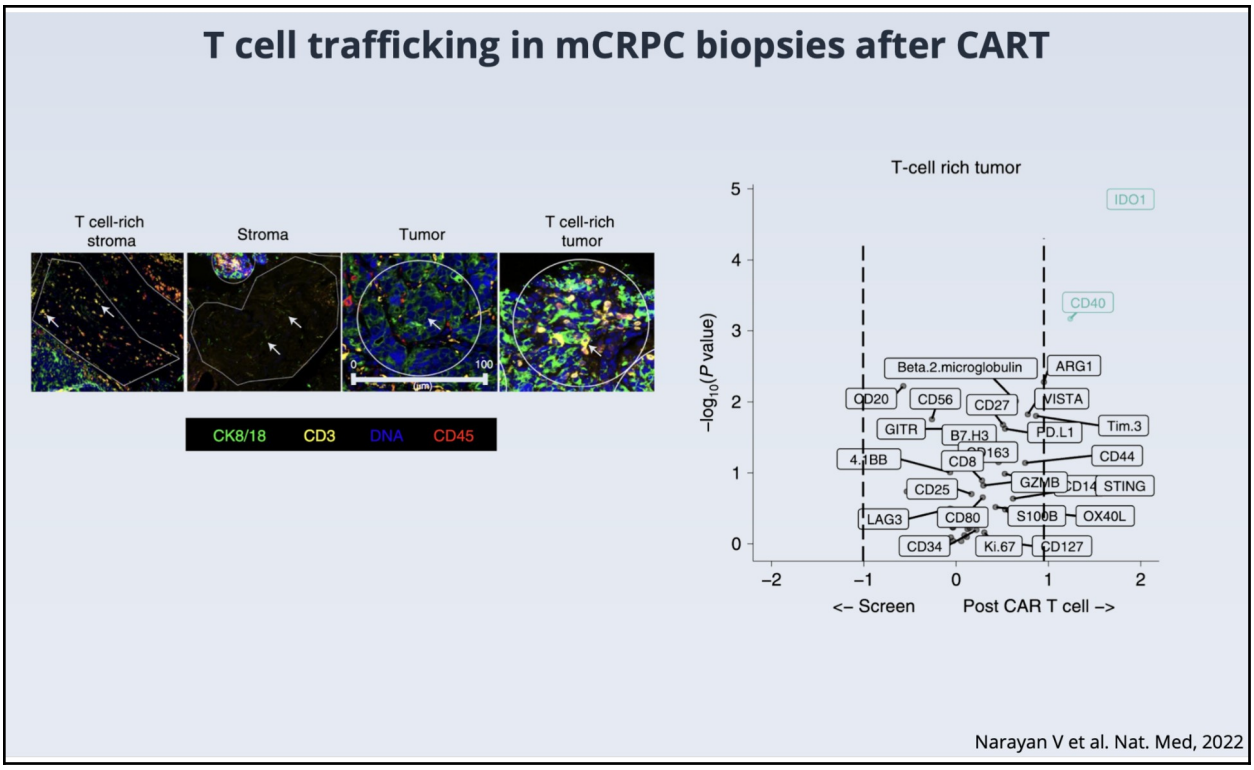

I just want to share some results from a paper from the Penn group that gives the direction with our unpublished work, where we want to head to understand what's going on in prostate tumors. This is from a recent paper that was published from Penn to the point that was being made about higher resolution approaches and spatial transcriptomics. What we've done here in this image is take these regions of stroma surrounding tumors that are rich in T-cells, which are these yellow dots, or stroma, that is does not have any T-cells, or regions of tumor, which stain with this antibody here in green organ regions of T-cells, tumor and T-cells were like these, these are the big axis we care about what is different about tumor where you can get T-cell infiltration versus not. What we're able to do is take these biopsies and divide up tumors like this before and after CAR T-cell infusion, and look in the environment in a T-cell rich tumor, for instance, and see what pathways or proteins are up or downregulated. One of the findings we've published is an upregulation of this suppressive protein IDO1, which I won't talk in detail about. (Indoleamine 2, 3-dioxygenase 1 is a rate-limiting metabolic enzyme that converts the essential amino acid tryptophan into downstream catabolites known as kynurenines.) But this is an example of what we're now doing in a higher throughput, higher resolution way. We want to want the limited number of tumor tumors from Phase 1 trials and understand as much as we can in as high a resolution as we can, to start with. The pie in the sky dream is that if you learn something at the level of five patients, you can go to a cohort and then eventually that becomes something which has clinical utility down the line. That's where we are with understanding the microenvironment and prostate cancer. It's working in these small, small numbers of samples in our Phase 1 trials to discover what the important differences are.

Ricardo Salgado 53:32

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I understand when you get immunotherapy, you will want to be as little protective of the cancer cells as possible. There are for instance, Tregs (regulatory T-cells), M2 macrophages (infiltration of macrophages in and around the tumor nest represents one of the most crucial hallmarks during tumor progression. The mutual interactions with tumor cells and the stromal microenvironment contribute to phenotypically polarization of tumor associated macrophages. Macrophages consist of at least two subgroups, M1 and M2. M1 macrophages are tumor-resistant. Contrastingly, M2 macrophages are endowed with a repertoire of tumor-promoting capabilities involving immuno-suppression, angiogenesis and neovascularization, as well as stromal activation and remodeling.), and MDSCs (Myeloid-derived suppressor cells, immature myeloid cells that are characterized by the ability to suppress immune responses and expand during cancer, infection, and inflammatory diseases.). Are there new approaches where you can, let's say, unleash checkpoint inhibitors, but also help the tumor microenvironment with some of these? Bring down the Tregs, or on the M2 macrophages maybe try to polarize them to M1 macrophages, or some other complementary things to make it more effective?

Andrew Rech 54:33
Yeah, there are, and it's a two part challenge. The first part is understanding. You just hit a bunch of the top things that are hypothesized to be important for limiting T-cell responses, and we have to rank them. We don't have the research done yet to understand in the largest group of patients, what are the key suppressive pathways, what are the most important to inhibit first, and then the second part of that is actually engineering therapy to do it. I mean, in this recent study from Penn using as an example, this CAR T-cell had an extra protein which blocked a common suppressive pathway in the microenvironment. That was discovered in prior studies and in preclinical models to be important. The idea is marrying the understanding with a new ability over the past few years to actually engineer cells in the context of CAR T-cell therapy to deal with some of those pathways. But to the point that was made earlier, you need the core studies in parallel to understand what is the most actionable to begin with, and that's on the back of new technology availability and the studies being done.

Ricardo Salgado 55:36
The challenge with that is that it takes forever for those things to happen. Versus, for instance, right now, Allopurinol, which is used for gout, can inhibit the inflammasome. (Inflammasomes are cytosolic multiprotein oligomers of the innate immune system responsible for the activation of inflammatory responses.) For example, capecitabine can deplete MDSC. There are things or repurposed stuff out there like sorafenib, where you can polarize M2 to M1, but people don't know how to utilize these. It's not going to be perfect, which is a challenge.

Gitte Pedersen 56:15
A couple of comments. We are analyzing all RNAs in tumor tissue. But we also started in liquid tumors in reaching for B- and T-cells and doing RNA sequencing on those. It's still early days for that in liquid tumors. But what we have seen in solid tumors is we can see all the hallmarks of inflamed or non-inflamed tumors. Whether that's CAR T on another treatment that uses the immune system as a way to treat the cancer. There are many overlooked targets that could be explored. When I look at the pipeline, it's like everybody's knocking on the same doors. I think

that there is a possibility that we end up with really individualized treatments for prostate cancer in particular. I believe they express MAGE, MHC II, which is a tumor antigen, actually a whole class of uniquely expressed tumor antigens that are not expressed in all other tissue, which is the important part. (The Melanoma Antigen Gene family has garnered growing interest as biomarkers in cancer and targets of immunotherapies because a subset of these >40 human proteins are classified as cancer-testis antigens, which have restricted expression to the testis. MHC Class II molecules are a class of major histocompatibility complex molecules normally found only on antigen-presenting cells.) What do you think of those groups or classes of targets?

Andrew Rech 58:19
I can answer in a couple of parts. Broadly in solid tumors and prostate as well, it is extremely difficult to find good antigens. This is a major barrier for BiTE (bispecific T-cell engager) therapy and for CAR T-cell therapy. I'm actually not familiar with literature in prostate cancer. I know of several studies that have looked exhaustively for tumor antigens in prostate cancer, and that was the basis for the current therapies. STEAP1 (Six transmembrane epithelial antigen of the prostate), for instance, has emerged as a potential target just in some recent work in Nature Cancer, published a couple of weeks ago. There’s a really nice paper and slide about that that I didn't get to, but the people are doing the work with single cell RNA and other approaches, and then going back for the validation to identify new targets. But it's a fundamentally difficult problem.

Allen Morris 59:36
I think Brad is protecting you from me because clearly I have diarrhea of the mouth. I've been thinking about this stuff for the last 40 years. That's one of my excuses.

Andrew Rech 59:47
PD-L1 has not been around for 40 years so that is not true.

Allen Morris 59:51
The stuff I was referring to are CTLs (cytotoxic T lymphocytes) in the tumor microenvironment. The awareness of TILs (tumor infiltrating lymphocytes) has been around since the beginning of the modern medicine era. In fact, the link between inflammation and cancer development was first reported by Rudolf Virchow, the father of pathology, in 1864, when he made an observation that inflammatory cells frequently “infiltrate” solid tumors; the first mention of tumor infiltrating lymphocytes (TILs).

> *[https://sci-hub.st/10.1016/s0140-6736(00)04046-0*
> *Inflammation and cancer: Back to Virchow, 2001. Lancet.]*

And pathologists have noticed the cytotoxic subset of TILs called CTLs, just as I did in the 1980s.

> *[Allen Morris: I have been thinking about the immune system's role in immune surveillance of cancer, including enumerating what TILs are, since my days as a pathologist at the John Wayne Cancer Institute where the first major push for a*

melanoma vaccine was made, unsuccessfully. Though I admit I was not aware of Checkpoints then, since the concept of a checkpoint only began in 1989, 34 years ago when the gene for programmed death-1 (PD-1) was discovered. I became acutely aware of the PD-1:PD-L1 synapse in 2014, when Merck drug representatives descended on every pathology department in the country to “spread the word” with educational lunches, the Keytruda FDA approval breakthrough in melanoma.

Think about it for a moment: 25 years for the PD-1:Keytruda nexus. This is an example of how long it takes from the discovery of a molecule by a molecular biologist until a breakthrough treatment is bedside for a clinician like Dr. Sartor to standard of care use and his patient to benefit.

I was acutely aware of the PD-1:PD-L1 synapse in 2014. Our oncologists demanded that the pathology department reflexively send melanoma specimens for BRAF-V600E mutation analysis.
Background: The 2014 FDA indication included a patient inclusion requirement of + BRAF-V600E mutation after treatment with a BRAF inhibitor. Remember experimental treatments are ethically initially tested on end stage patients.
We had to push back on the oncologists. Pathologists could not know whether a particular melanoma patient had been treated with a BRAF inhibitor, so that if we reflexively tested all melanoma patients for BRAF-V600E, we would be committing Medicare fraud.
Background: Medicare requires that pathology departments only do medically necessary tests, otherwise the pathology department is committing Medicare fraud. Because of this stress, I completely remember when I became aware of Checkpoint inhibitors - it is now almost a decade ago.]

Andrew Rech 1:00:00
Are you familiar with Jerome Galon’s work in colon cancer and his immunoscore? You should read about that. I think he's probably done the nicest work counting TILs in colon cancer. He has beautiful results.

Allen Morris 1:00:12
No, I have not heard of Dr. Jerome Galon. Thank you for the reference.
(2020. https://doi.org/10.1158/1078-0432.CCR-18-1851)

[Allen Morris comments: Galon’s work includes subtyping TILs as CTLs by virtue of their identification as co-expressors of CD3 and CD8. His work does appear to be a major contribution. Thank you, Dr. Rech, for turning me onto this. Galon’s work indicates that the quantitation of TILs by his immunoscore method outperforms significantly all other known colon cancer risk factors for risk stratification; attesting to the absolute importance of TIL/CTLs in the Tumor Microenvironment, at least for a hot tumor such as colon cancer. I believe his analytic method will be translatable to all hot tumors and anticipate it or a similar more optimized method will make it into clinical practice some day.

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> *“Galon showed the prognostic value of Immunoscore for stage II-IV CRC patients, as well as the predictive value of Immunoscore in stage III colon cancer patients; predicting and stratifying patients who will benefit from adjuvant chemotherapy. Moreover, the Immunoscore allows the classification of tumors into hot and cold categories, and could consequently guide clinical decision for the choice of treatment [ICI].”*
> *But I ask the PCL audience, how long do you think this will take to reach the bedside for hot tumors?*
>
> *Concerning mCRPC patients, I suspect a Galon-type immunoscore will only have significance in the 10% of the most advanced mCRPC who are hot. In all other stages of prostate cancer, it will have no practical importance, as prostate cancer does not have significant numbers of TILs. How do I know? I have seen 10s of thousands of prostate cancer biopsies; admittedly mostly in the initial biopsy stage. I have never seen lymphocytes surrounding an individual prostate cancer cell showing cell death except maybe in a completely undifferentiated subtype of Gleason pattern 5. I ask any pathologist out there in the connected world, if they have? And Brian provided me with a TIL case series study of 3 advanced PC patients.*
>
> *Dr. Galon’s research has not reached the level of clinical practice; so I suspect the vast majority of pathologists would not know about his research. Bedside practicing pathologists have so many new things to absorb from all the fields of medicine, not just oncology, as we are “the doctor’s doctor”; that most do not have the time to explore pre-clinical research or even phase 1 or 2 research.]*

Bob Gurmankin asked a question: What can be done to improve the tumor microenvironment for immunotherapy?”
And you've already answered that question: that there are a lot of molecular candidates in the TME which could be targeted; but research right now has not answered which candidates are the dominant ones which should be prioritized and that, in part, is what your research is aimed at figuring out.

The Prostate Cancer Lab/Cancer Patient Lab, by virtue of being part of the collective brain, has tapped into cancer vaccine researchers, such as Keith Knutson. I happened to catch a glimpse of part of his proprietary formulas for a personalized neoepitope vaccine. And the vaccine formula included, believe it or not, something targeting the tumor microenvironment (TME). The personalized neoepitope vaccine included low dose cyclophosphamide. Cyclophosphamide, I thought, was a good choice since this chemotherapy drug has a long vetted history of use, safety, and efficacy. And the presumed mechanism of action is to remove Tregs, the bad tumor promoting T lymphocytes.

Do you have a comment concerning cyclophosphamide’s presumed mechanism of action: knocking out the Tregs; the immunosuppressive (tumor promoting) T-cells. Do you have any comment using cyclophosphamide to target the tumor microenvironment in this way?

Andrew Rech 1:02:04
I'm not familiar with the primary paper you're talking about.

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Allen Morris 1:02:07
It is not a paper. It is at the bedside. It's actually been used in personalized neoepitope vaccines.

Andrew Rech 1:02:13
I'm aware of cyclophosphamide. I'm aware of the general research topic. I think that that's actually probably the largest area of combinatorial investigative research for CAR T-cells in solid tumors is modifying the many suppressive factors. I can think of probably two dozen approaches that are in sophisticated preclinical models, or approaching or or actively enrolling in phase one trials across solid tumors. The 30,000 foot view is we don't know what the important pathways are, or if there is even one such pathway. We've talked about Tregs and many things today. And, you know, there isn’t a silver bullet. That's probably in part because of heterogeneity in tumors in terms of what is important to those patients.

> *[Allen Morris translation: I think Dr. Rech’s answer is that the choice of low dose cyclophosphamide as an immune adjuvant, presumably targeting Tregs in the Tumor Microenvironment, concerning Knutson’s vaccine secret formula design, is just a shot in the dark.]*

Allen Morris 1:03:06
Concerning your last slide: I want to compliment you because I think your route is the route to figure out the tumor microenvironment. And I want to point out to everybody, CK 8 and 18 are cytokeratins, structural proteins, and in particular bad TME markers. Andrew is looking at T-cells indicated by CD3, all lymphocytes as a denominator indicated by CD45, and CK8 and 18, altered bad stromal markers in his spatial tissue view; the latter the pathologist’s view, under the microscope.

The reason I'm going down this route is that it concerns a prostate cancer medical curiosity. Background: there is one and only one cancer of all cancers that goes from stage 1 to stage 2, by virtue of a physical examination finding. That physical examination is the digital rectal exam (DRE). The urologist with his finger is trying to detect something hard. My question to Andrew is: have you ever pondered the question of why prostate cancer is the only cancer of all human cancers that goes from stage 1 to stage 2 by virtue of going from a soft state to a hard state. The 1st huge leap in cancer stage, the first step in solid cancer progression? After all, there are only 4 stages.

Andrew Rech 1:04:40
There are many solid carcinomas that can progress quickly.

Allen Morris 1:04:45
It is a question unique to prostate cancer. And prostate cancer does not progress quickly. In fact, prostate cancer is the prototype of a slow moving cancer.

> [*Allen Morris editorial: A slow moving cancer should be the best cancer to study to figure out how a cancer physically progresses (moves up in stages). This initial step from soft to hard is an incredibly early oncogenic event, and I submit, the step from*

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premalignancy to malignancy. You cannot catch lung cancer or pancreatic cancer in this step, or for that matter any other cancer except Prostate Cancer.
One clue is the very word cancer. Cancer means crab or scirrhous/skiros, the latter, a latin/greek word for hard. Latin: scirrhous, from Greek skyros, hard tumor: first known use 1563.
This is how a woman, by self examination, discovers cancer; because unlike her soft breast, cancer is hard. The hard part is actually not the cancer cells, but the Tumor Microenvironment (TME). And that hard exam, I believe, in part is molecularly underpinned by the altered CK 8 and 18 stromal extracellular skeletal matrix that Andrew is exploring spatially. (Sidenote: Many of these altered cellular-stromal cytokeratins are routinely/bedside available. For example, I test breast cancer for CK5/6 routinely, which if + fits the basal, most advanced, molecular phenotype. Note: breast cancer is about two decades ahead in research; though that gap is narrowing.
So, I was trying to give Andrew a huge clue which actually is in his slide and he is already exploring.

Background: Molecular biologists believe in a concept of stemness which includes mesenchymalization called EMT (Epithelial-Mesenchymal Transition). EMT, molecular biologists believe, is the basis of invasion (a sine qua non of cancer) and the basis of movement (a sine qua non of organ polarization), embryonic differentiation into our bilaterally symmetric organized around the notochord, multiorgan self. EMT, I believe, is the most sentinel physical event begetting movement/invasion, marking the premalignant to malignant tumor progression. EMT, I believe, is the molecular underpinning of what is transforming prostate cancer from Stage 1 to Stage 2; this first stage progression in solid cancers. This is profound and can only be studied in vivo in prostate cancer. No other cancer can be caught in this transition; not even breast cancer, though admittedly maybe papillary thyroid cancer can be via the newly recognized state: NIFTP.]

This hard exam upstaging of only one cancer, prostate cancer, segues into another prostate cancer medical curiosity which I'll ask you about since you're a pathologist. Do you believe Gleason 3 pattern is malignant or pre malignant?
(Dr.Rech answers: I don't know.)

This whole concept, including the two prostate curiosities:

a. *Unique Stage 1 to 2 progression by virtue of a positive digital rectal exam*
b. *Gleason 3 uniquely invading through the basement membrane but still premalignant*

I am discovering by asking Dr. Sartor and now Dr. Rech, appears to draw blank stares and perhaps even derision. I was wondering if the PCL thinkers can google any of this to see if this yellow brick road has already been enumerated? Especially since there are 1 million+ biomedical researchers out there which would suggest, if there is any credence to the above, that someone else must have connected the dots.
Or if not, what in the literature might argue against what I am proposing?

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I welcome an audience to explain, if you are interested.]