“Cancer Vaccines” (Lisa Butterfield) [#50]

Richard Anders and Brad Power
March 29, 2023

“In cancer vaccines people have been looking for targets – things that are different between the patient and the tumor. And there are a lot of targets. For a long time, these targets were sort of old timey things like PSMA and PSA, which are known to be overexpressed by a [prostate cancer] tumor, that we would try to target the immune system against. And the response has been okay, but not great.” – Lisa Butterfield, PhD

“Vaccines can do a lot. They can start a new immune response, they can boost an existing response, and they can make a broader immune response that recognizes lots of proteins.” – Lisa Butterfield, PhD

“Perhaps vaccines, even if they don’t fully work on their own, can initiate that response or boost it to then set the stage for a better response to something else.” – Lisa Butterfield, PhD

Meeting Summary

Advanced cancer patients see immunotherapies as offering one of the best paths to a durable response. Cancer vaccines have a lot of potential because they offer a possible treatment option to nearly every cancer patient. And immunotherapies offer the promise of durable responses -- they are 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.

Lisa Butterfield, PhD, Consultant in Immuno-Oncology, Adjunct Professor of Microbiology and Immunology, University of California San Francisco, is uniquely positioned to share the state of the art on cancer vaccines. Besides being a leading researcher, she brings the patient and caregiver perspective. Her husband had pancreatic cancer, and she was his patient advocate and caregiver. She experienced the extremely difficult challenges of trying to navigate this for him.

Her research is focused on cancer vaccines, immune profiling and cellular therapies for melanoma, hepatocellular cancer, and other tumor types. She was most recently Vice President, Research and Development at the Parker Institute for Cancer Immunotherapy, where she supported cell therapy initiatives and clinical trial biospecimen and biomarker projects.

Lisa was previously a tenured Professor of Medicine, Surgery, Immunology and Clinical and Translational Science at the University of Pittsburgh and Director of the Hillman Cancer Center Immunologic Monitoring and Cellular Products Laboratory; she earned a PhD in Biology from UCLA, followed by postdoctoral fellowships in Cellular Immunology and Cancer Gene Therapy also at UCLA.

“Cancer Vaccines” (Lisa Butterfield) [#50]

She was the first female President of the Society of Immunotherapy of Cancer (SITC) and a member of the SITC Executive Committee. She also previously led the Immunology Reference Lab for the ECOG-ACRIN NCI cooperative group and collaborated on biomarker studies in many clinical trials.

She has published over 185 peer-reviewed manuscripts, reviews and book chapters, and mentored over 20 students and postdocs. She currently chairs the FDA Cell, Tissue and Gene Therapy Advisory Committee, co-leads the SITC Women’s Leadership Institute and is a co-Editor of the SITC textbook “Cancer Immunotherapy: Principles and Practice” 1st and 2nd editions.

How does your immune system fight cancer?

The immune system has evolved to protect us from pathogens such as bacteria, viruses, and fungus. When an infection arises, the immune response is triggered, all of the infection is eliminated, and then the immune response goes back down. But a memory of the infection persists, and you get a healthy memory cell, so that if you see that pathogen again, it can react quickly. But that doesn't happen in cancer, because if the tumor doesn't completely go away early in the process, the immune cells become exhausted, and are just not as functional. So they may need to be reactivated, or even worse, the immune system never saw the tumor in the first place, because tumors can be clever, perhaps able to block recognition by the immune system. So there are a lot of hurdles: tumor evolution, tumor heterogeneity, different cells expressing different things and then this exhaustion phenomenon from the cells seeing the antigen too much.

How do immunotherapies work in cancer?

The idea is to try to find what's different about the tumor. Sometimes the tumor turns things back on that are generally turned off in people, sometimes it turns up the volume on things that are generally turned down. So if you can find these reactivated genes, you can perhaps get the immune system to recognize them and attack them. But the immune system doesn't usually recognize them without a vaccine because a successful tumor shields itself from the immune system by being a terrible antigen-presenting cell.

All of the cancer vaccines are focused on T cells. Decades of studies have shown us that the T cell is the one that kills the tumor. If you can get CD8 killer T cells and CD4 helper T cells to work together, it's even better. If you can add B cells and antibodies, that is a bonus. But all of these cancer vaccines are T-cell focused, which is why they're hard, because they have to be matched to you. An antibody is matched to a pathogen. If it's all the same pathogen, you get all the same antibodies.

What are the benefits of cancer vaccines?

“Cancer Vaccines” (Lisa Butterfield) [#50]

Vaccines can start a new immune response, boost an existing response, and make a broader immune response that recognizes lots of proteins.

How do cancer vaccines work?

Cancer vaccines look for targets – things that are different between the patient and the tumor. There are a lot of targets. For a long time, these targets were sort of old timey things like PSMA and PSA, which are known to be overexpressed by a [prostate cancer] tumor, that we would try to target the immune system against.

How successful have cancer vaccines been to-date?

The response has been okay, but not great. There’s one cancer vaccine approved, and it is in prostate cancer. 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. Some T cells were poked, but that doesn't really mean much to the tumor, and therefore, to the patient.

Who’s going to benefit?

People who already have the specific T cells in their circulation maybe just need a checkpoint blockade. But if you don't have those specific T cells, then a vaccine could reduce recurrence or mediate tumor progression. When you look at the side effects of cancer vaccines, they are minor compared to most other treatments.

When should a cancer vaccine be tried?

Cancer vaccines are still experimental. People want to get the standard of care first and something experimental later. The problem with that, for an immunotherapy vaccine, is you need your immune system to be able to respond. If you get chemotherapy, radiotherapy, steroids, or other standard treatments, your immune system is beat up. If you can, it would be better to get a cancer vaccine quickly (which can be done with RNA), and then wait a few weeks before getting the standard of care. If you’ve had standard treatments, try to reset your immune system as best as you can to be able to get the most from a cancer vaccine.

It looks like getting a cancer vaccine before a checkpoint blockade is better, but this needs to be tested.

How can you access a personalized cancer vaccine?

There's a cancer vaccine trial at UC San Diego run by Ezra Cohen and his partner Steve Schoenberger, which has been treating a number of solid tumors with low tumor mutation burden. The other clinician involved at UC San Diego is Aaron Miller. Another resources is Nina Bhardwaj at Mount Sinai, who helped design a cancer vaccine trial in prostate cancer.

“Cancer Vaccines” (Lisa Butterfield) [#50]

What’s new and exciting in research on cancer vaccines?

The exciting new cancer vaccine research is looking at combinations of cancer vaccines with other therapies. The hope is that cancer vaccines, even if they don’t fully work on their own, can initiate or boost a response to then set the stage for better response to something else. For example, a BioNTech mRNA vaccine study showed that a cancer vaccine plus checkpoint blockade is better. The study saw infiltration of the tumor with activated T cells. What’s great about RNA as a platform is that it’s fast. You sequence the tumor, tell the computer what sequences of RNA to make, and it does it, which is how we got a COVID vaccine so quickly.

“Cancer Vaccines” (Lisa Butterfield) [#50]

Meeting Notes

The information and opinions expressed on this website or platform, or during discussions and presentations (both verbal and written) are not intended as health care recommendations or medical advice by Prostate Cancer Lab, its principals, presenters, participants, or representatives for any medical treatment, product, or course of action. You should always consult a doctor about your specific situation before pursuing any health care program, treatment, product or other course of action that might affect your health.

Specifically, as a PhD research scientist, Lisa Butterfield never gives medical advice; she is trying to discuss immunotherapy and share data and publications for your personal interests and to answer questions about tumor immunology thinking in the field. She does not have the specific clinical expertise and background to make medical recommendations.

Discussion Outline

1. Introduction to Lisa and this discussion. (0:03)
2. Introduction of the data. (1:38)
3. Tumor evolution and tumor heterogeneity. (6:16)
4. What are the benefits of a vaccine? (11:22)
5. The Parker Institute’s PORTER trial in prostate cancer. (16:41)
6. Josh Brody. (22:28)
7. Vaccine as preventative vs. as treatment for disease. (26:32)
8. The complexity of the immunotherapy field. (31:46)
9. Questions about the mechanism of peptide-based responses. (38:12)
10. Virus vs. T-reg cells. (43:11)
11. Practicalities of getting access to personalized vaccines. (49:03)
12. What does RGCC do? (52:53)

SUMMARY KEYWORDS

vaccine, tumor, antigen, patients, peptide, t cells, cells, dendritic cells, immune response, mutations, trial, question, cancer, tumor cells, immune system, data, lisa, important, rna, ezra

SPEAKERS

Lisa Butterfield (69%), Rick Stanton (6%), Richard Anders (6%), Lauren Cohen (6%), Brad Power (5%), Gitte Pedersen (4%), Amit Gattani (3%), Russ Holyer (1%)

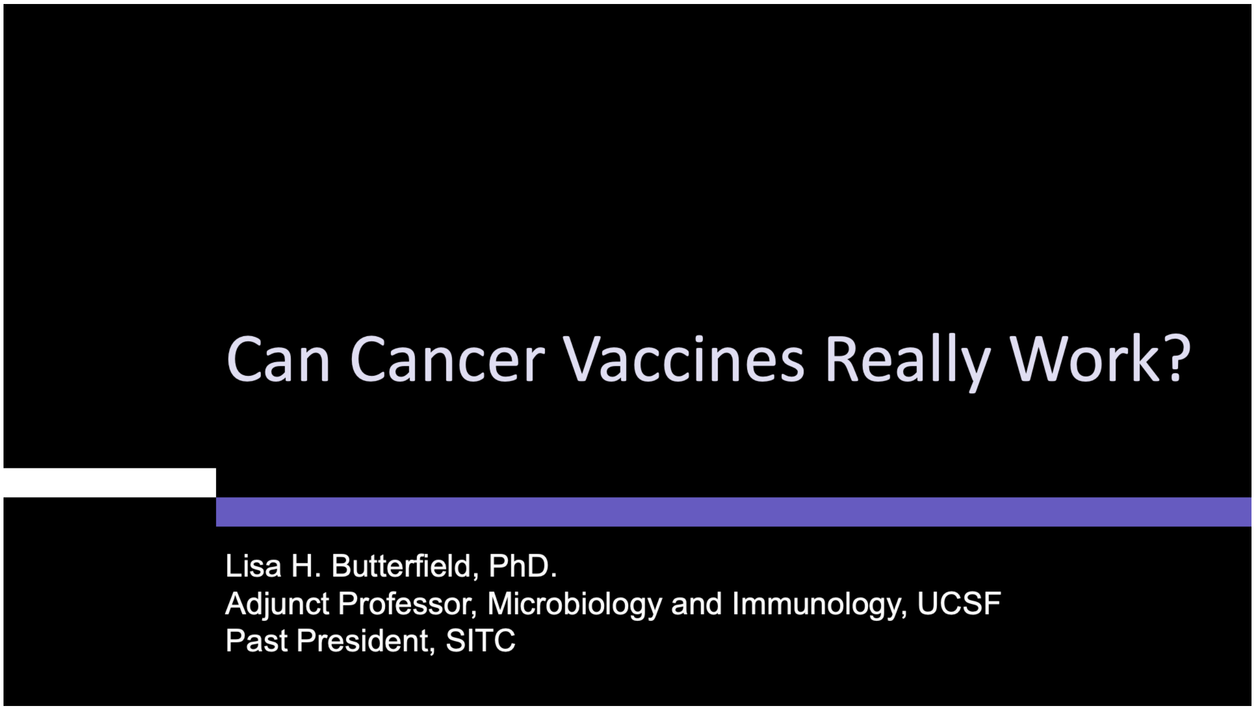
Brad Power

Lisa is a leading researcher in immunotherapies and immuno-oncology at UCSF. I got to know her when she and her colleagues at the Parker Institute were running trials to check the different algorithms for identifying peptides that would be put into personalized cancer vaccines. Lisa is no longer with the Parker Institute, but now is a consultant around personalized vaccines, which is what she'll talk about today.

Lisa Butterfield 1:38

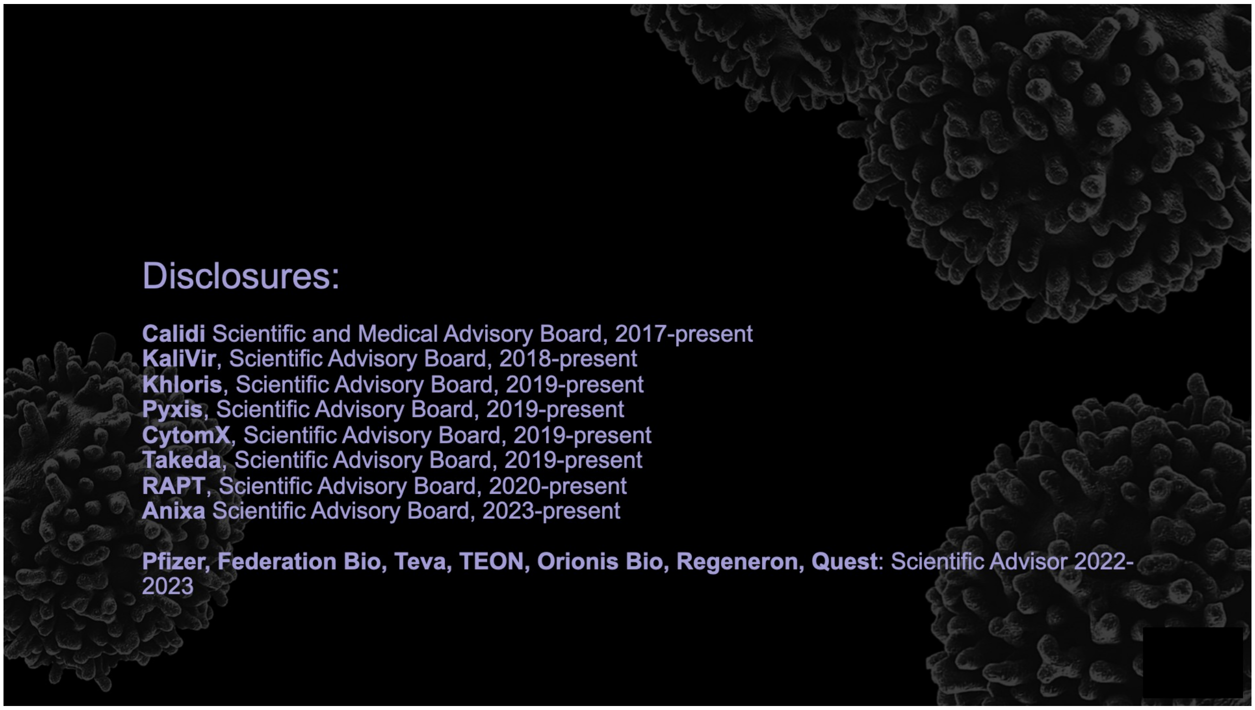
“Cancer Vaccines” (Lisa Butterfield) [#50]

As a PhD, professor type, I have 50 slides in my presentation, but I will try to go over them briefly and make this a much more interactive session.

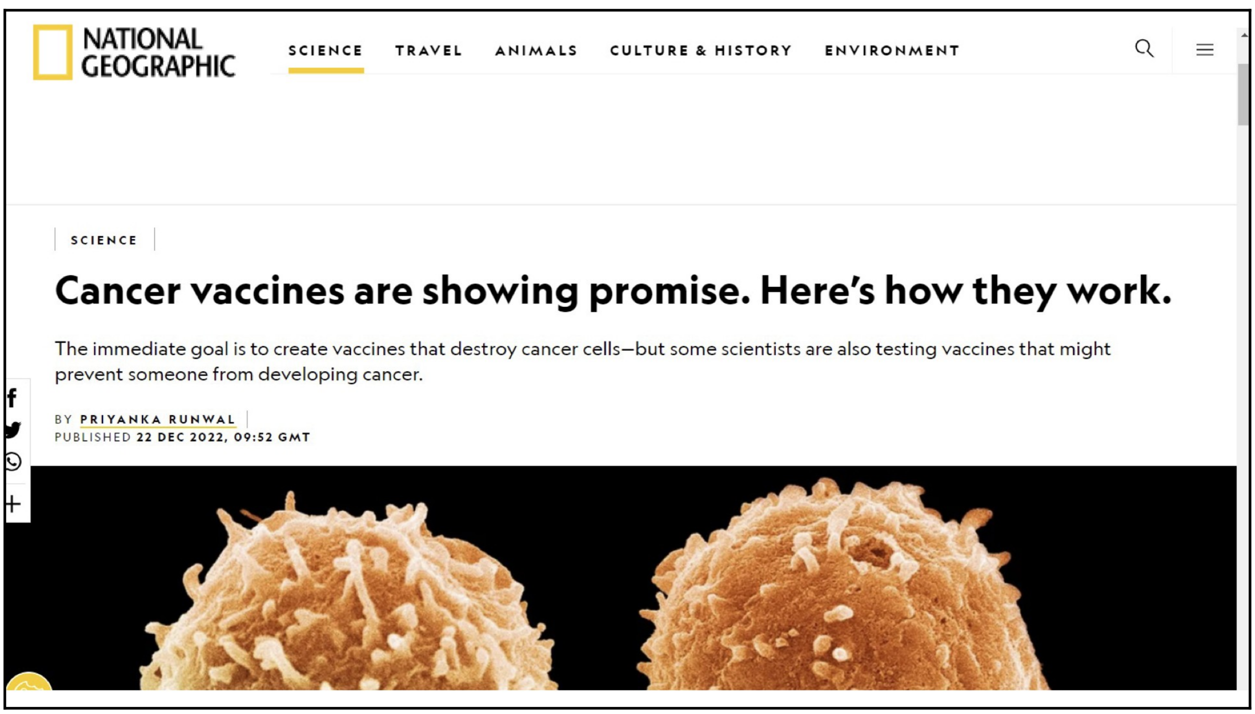


I've been thinking about cancer vaccines for almost 30 years. This is a topic near and dear to my heart. And now we're in another wave of excitement about them. Today I'll highlight some of that and go quickly through some of the data I have and then happy to talk. I have a number of disclosures.

“Cancer Vaccines” (Lisa Butterfield) [#50]



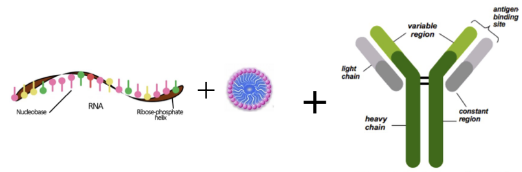
I’ve been on a number of scientific advisory boards, and I've done a lot of consulting, especially in the last year since the reorganization at the Parker Institute. And as of Monday [March 27th, 2023], I'm an employee of Merck. I knew we were in the third wave when National Geographic asked me to talk about cancer vaccines.



“Cancer Vaccines” (Lisa Butterfield) [#50]

Here was a little piece about cancer vaccines from right before the holidays last year. This is one of the reasons I decided to join Merck.

mRNA Vaccine Combinations



Adding mRNA-4157 ***reduced the risk of recurrence or death by 44%*** compared to Keytruda alone. The finding, which the partners hailed as the first demonstration of efficacy for a mRNA cancer treatment in a randomized clinical trial, lay the groundwork for a move into phase 3 and expansion into other cancers.

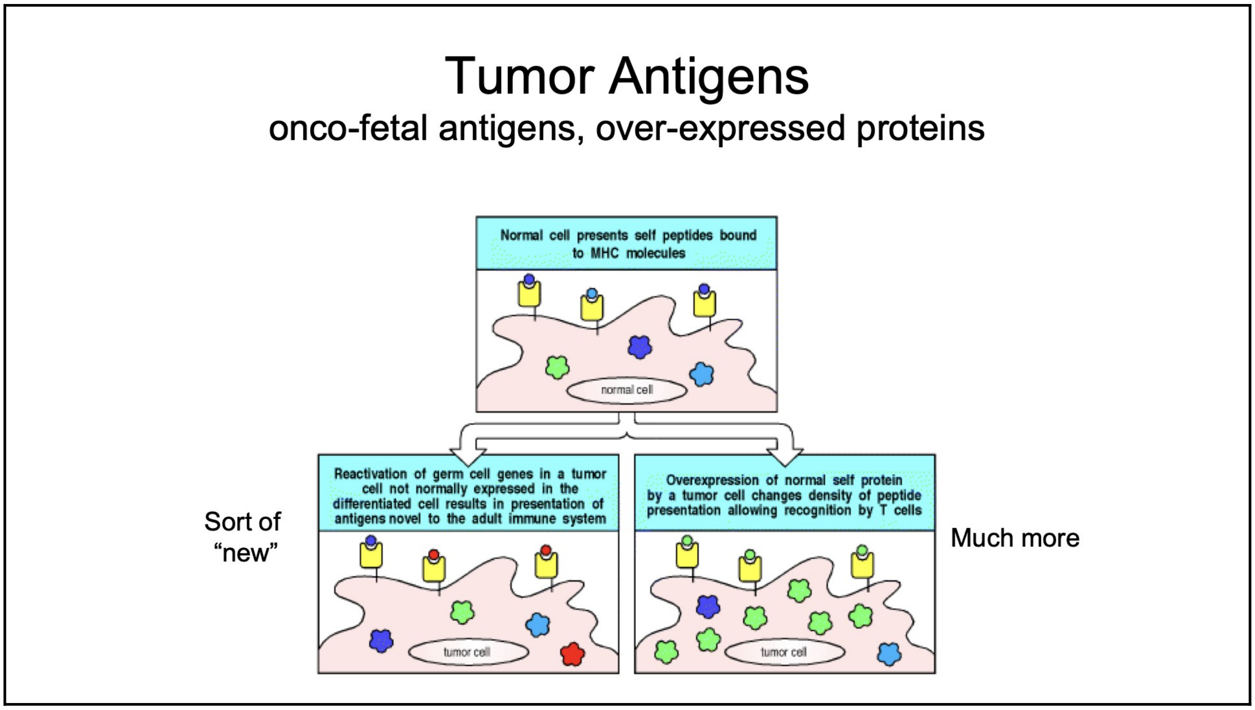
Moderna and Merck Announce…was Granted Breakthrough Therapy Designation by the FDA for Adjuvant Treatment of Patients With High-Risk Melanoma Following Complete Resection (**NCT03897881**)

KEYNOTE-942/mRNA-4157-P201 KEYNOTE-942 is an ongoing randomized, open-label Phase 2b trial that enrolled 157 patients with stage III/IV melanoma. Following complete surgical resection, patients were randomized to receive mRNA-4157/V940 (nine total doses of mRNA-4157) and KEYTRUDA (200 mg every three weeks up to 18 cycles [for approximately one year]) versus KEYTRUDA alone for approximately one year until disease recurrence or unacceptable toxicity. The primary endpoint is recurrence-free survival, and secondary endpoints include distant metastasis-free survival and safety.

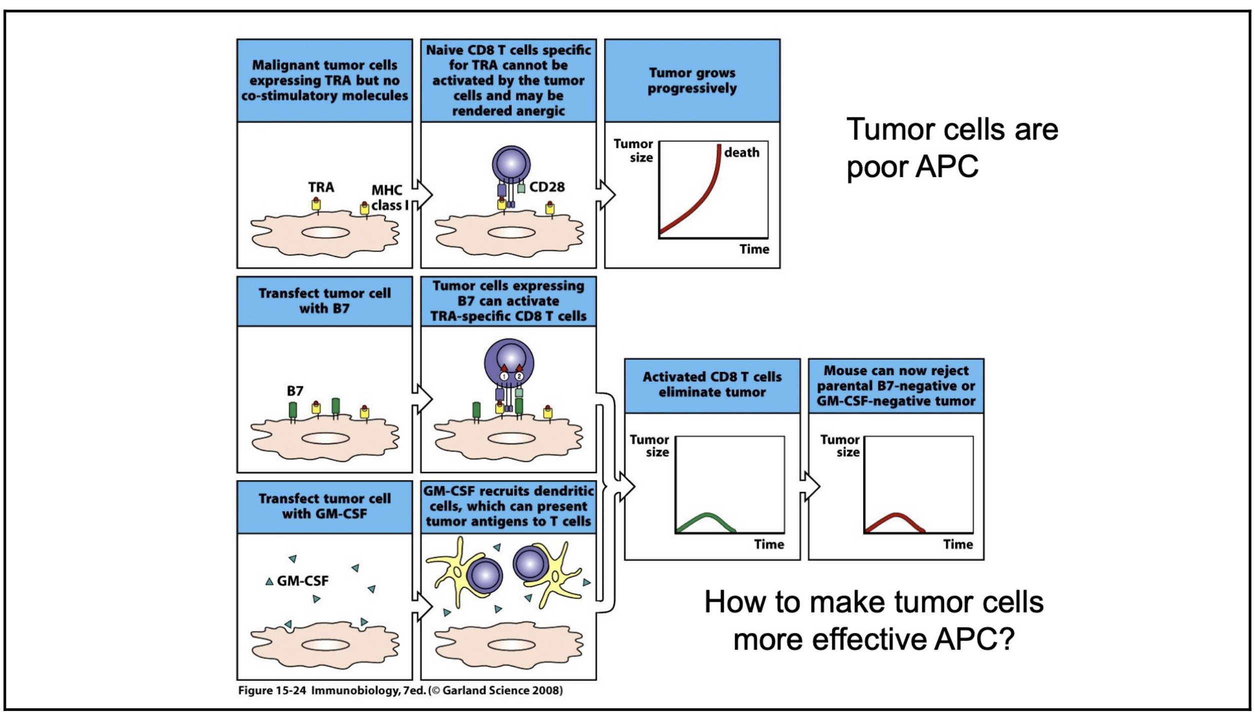
This is the public announcement about the Merck-Moderna collaboration. We are all fans of both the BioNTech and Moderna RNA COVID vaccines, and that's the platform that's been used, along with patient tumor sequencing information, to identify specific mutations, combine them with a nanoparticle, and then deliver that to patients with or without a PD-1 blockade [in this case, Keytruda]. The headline of those data – now granted an FDA breakthrough therapy designation – was that the vaccine reduced the risk of a tumor coming back in melanoma by 44%.

In cancer vaccines people have been looking for targets – things that are different between the patient and the tumor. And there are a lot of targets. For a long time, these targets were sort of old timey things like PSMA and PSA, which are known to be overexpressed by a [prostate cancer] tumor that we would try to target the immune system against. And the response has been okay, but not great.

"Cancer Vaccines" (Lisa Butterfield) [#50]

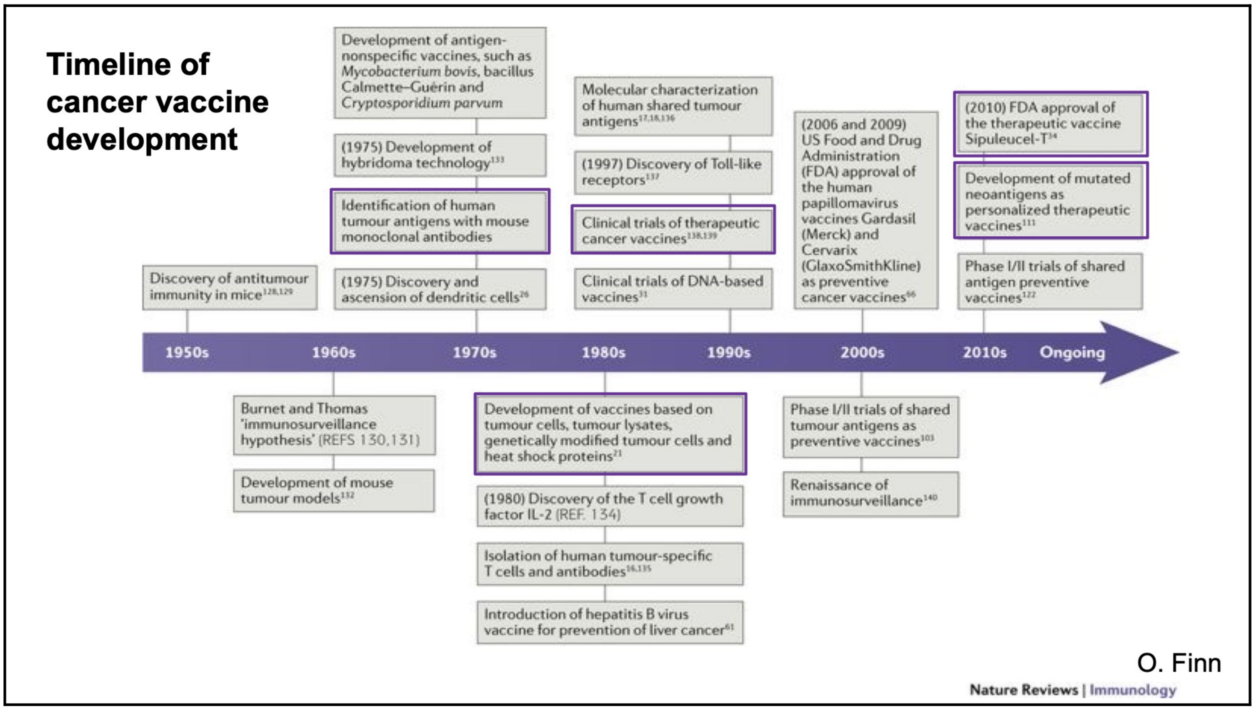


The idea is to try to find what's different about the tumor. Sometimes the tumor turns things back on that are generally turned off in people, sometimes it turns up the volume on things that are generally turned down. So if you can find these reactivated genes, you can perhaps get the immune system to recognize them and attack them. But the immune system doesn't usually recognize them without a vaccine because a successful tumor shields itself from the immune system by being a terrible antigen presenting cell.



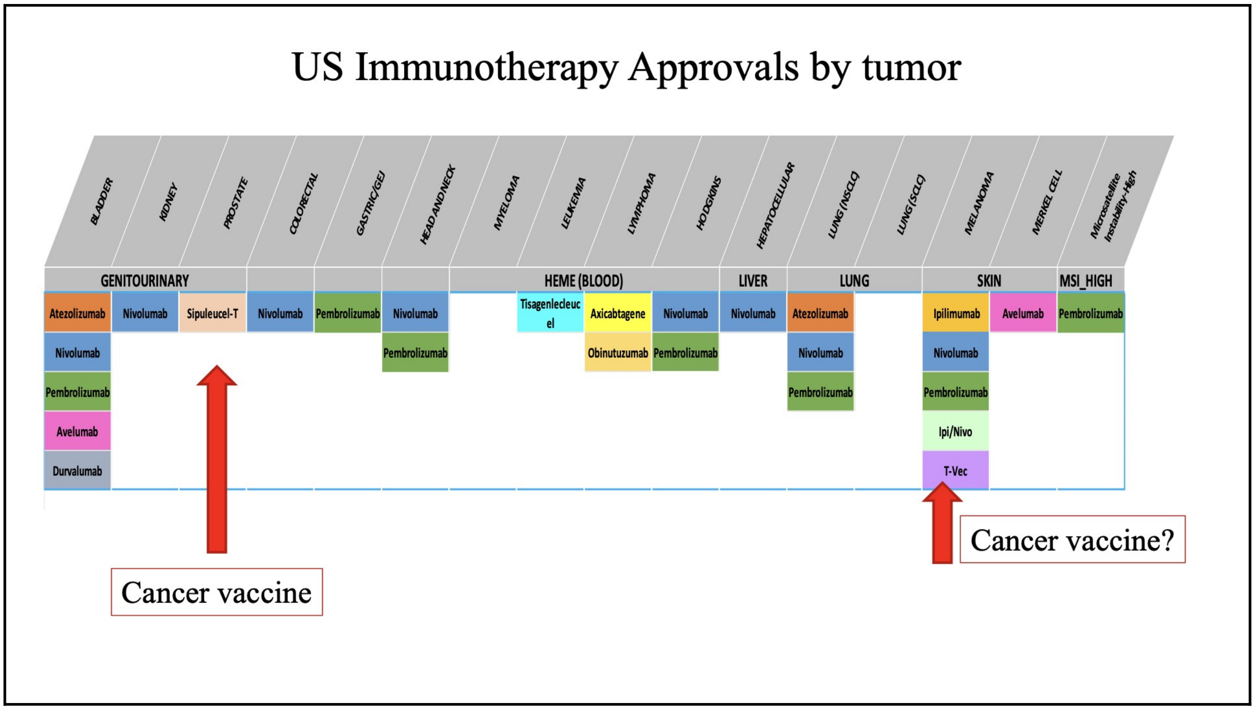
“Cancer Vaccines” (Lisa Butterfield) [#50]

This is mouse data, where we’ve tried to engineer tumors that activate an immune response by adding immune stimulatory molecules like B7 or GM-CSF. When my husband, who had pancreatic cancer, was in a clinical cancer vaccine trial, he received a GM-CSF-based vaccine.

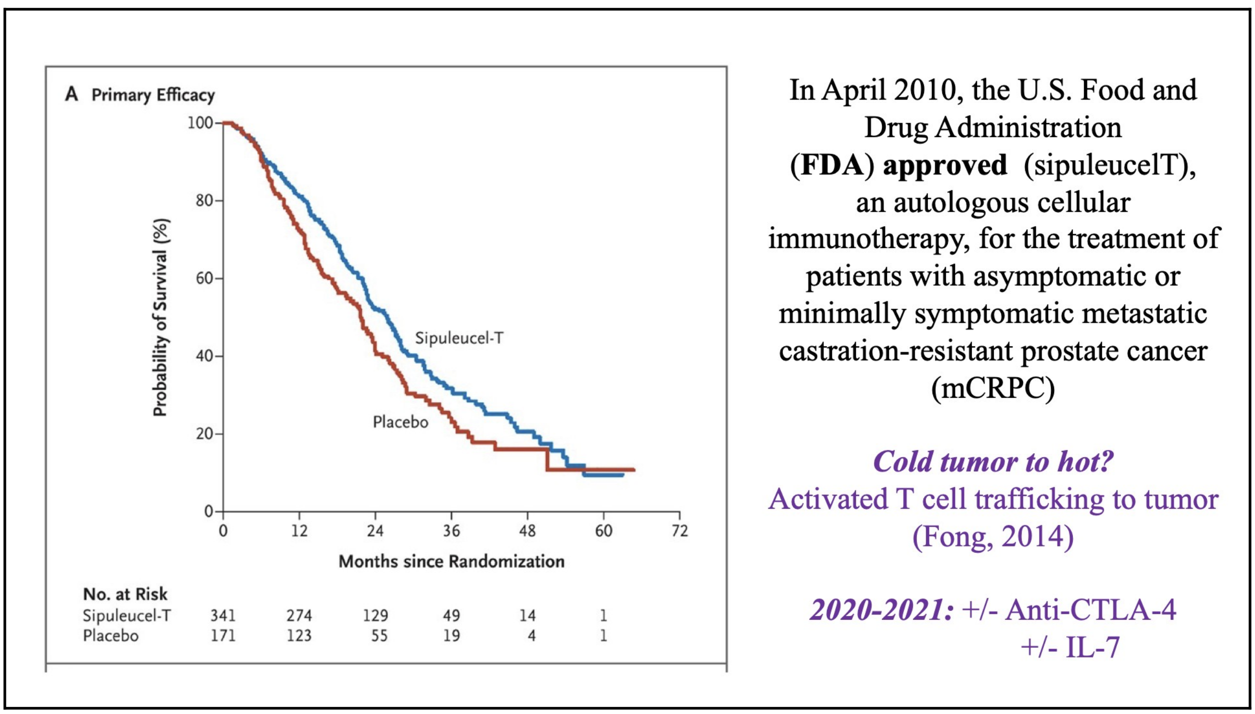


This slide shows the long history of cancer vaccines over many years, and in the cancer vaccine area. Once in a while you see something exciting. But not often.

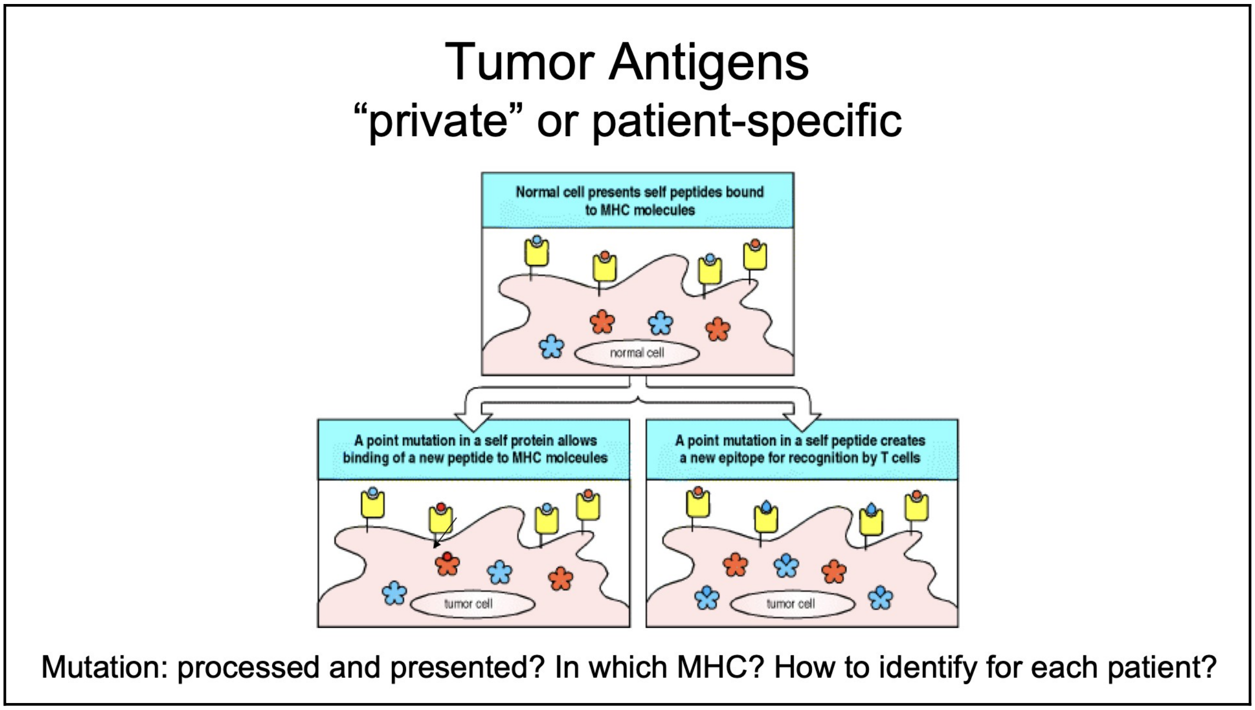
"Cancer Vaccines" (Lisa Butterfield) [#50]



There's one cancer vaccine approved, and it is in prostate cancer.



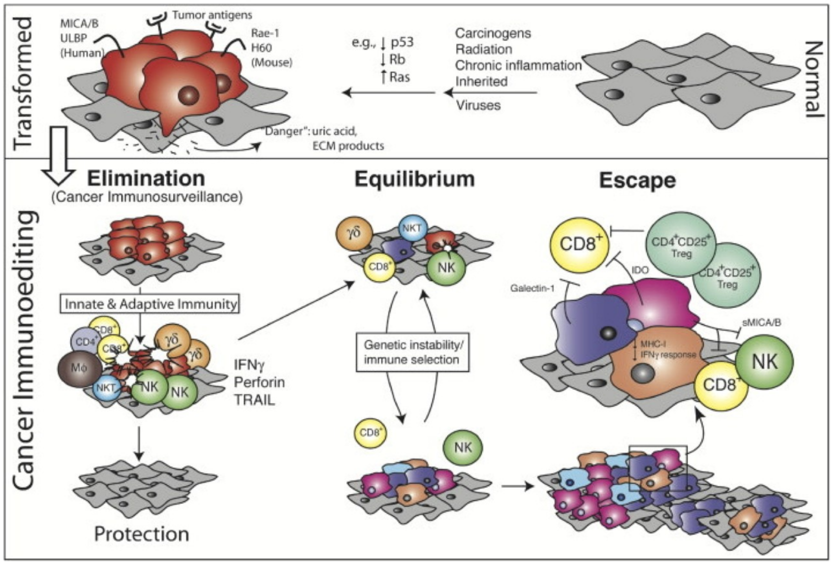
This is the Kaplan-Meier plot (a series of declining horizontal steps which, with a large enough sample size, approaches the true survival function for that population) of patients who got that experimental vaccine, targeting the shared antigen grown in GM-CSF. That's something we've known about a long time. Those familiar things do better. But it's not great for survival, but it is a shift away from chemotherapy, a little more time, but for the most part, non-toxic time.



Larry Fong, one of my immunotherapy colleagues at UCSF, keeps trying to figure out how to make this better. We’ve solved the technology hurdle of making a vaccine with a tumor-specific mutation (shown on the slide above as the little red flower-antigen). We can find those things in patients' cells, and show that antigen to the immune system. But the tumor keeps evolving, as you all know, and the immune system has probably been protecting us from early tumors for a long time.

“Cancer Vaccines” (Lisa Butterfield) [#50]

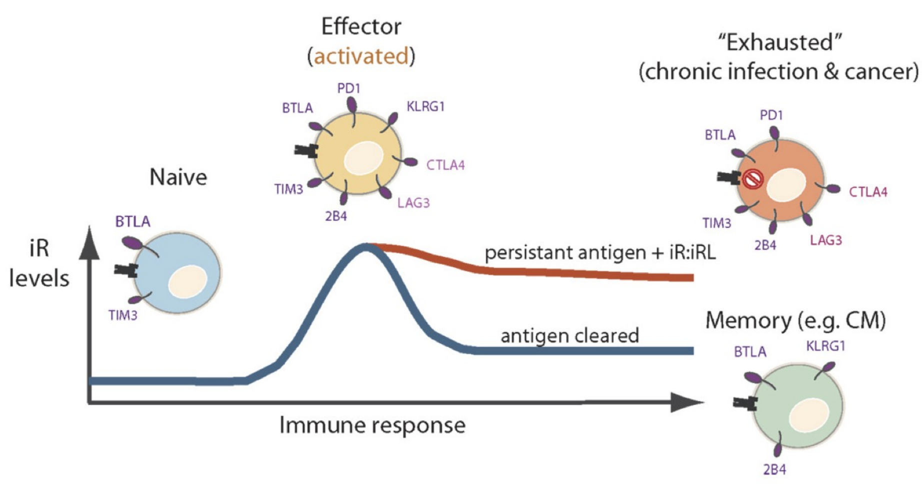
Three Phases of the Cancer Immuno-editing



Did we already get rid of the “easy” tumor cell targets?

Gavin P. Dunn , Lloyd J. Old , Robert D. Schreiber
The Immunobiology of Cancer Immunosurveillance and Immunoediting
Immunity, Volume 21, Issue 2, 2004, 137 - 148

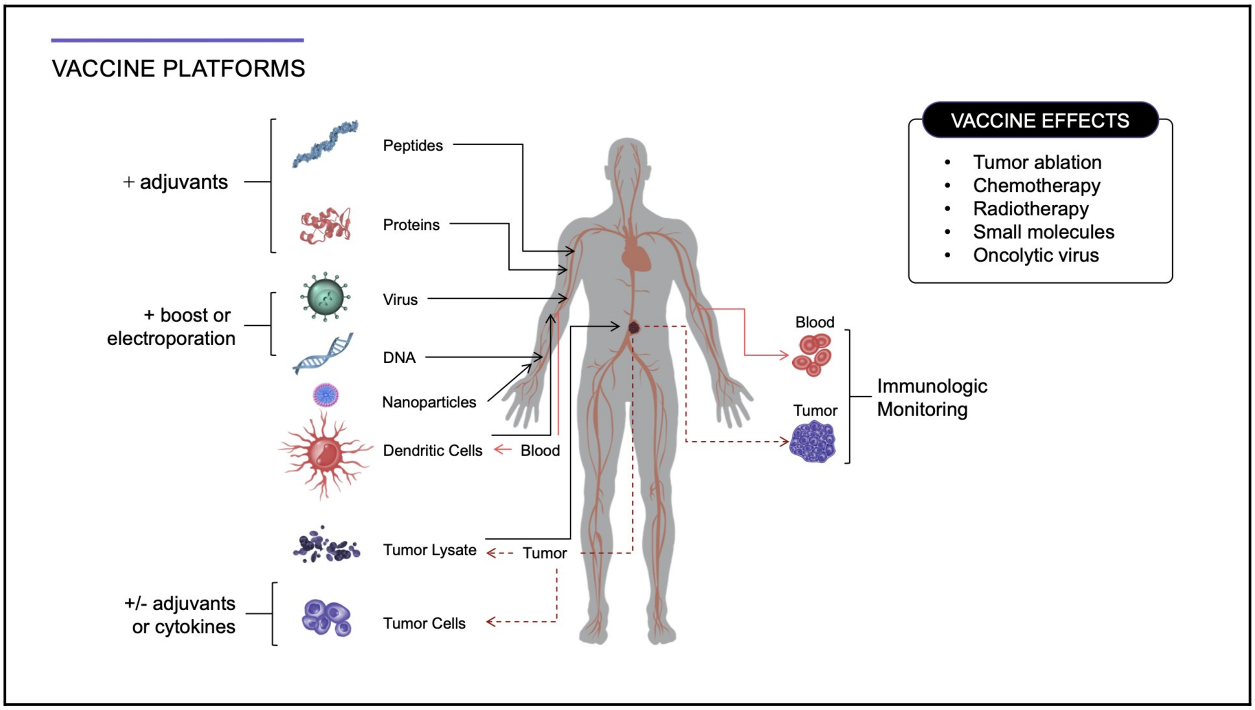
Once in a while they mutate and get away from the immune system, and while they can exist in equilibrium for a while, held back for a while, they may escape, perhaps by building immune suppressive niches around themselves so the killer T-cells can't see them anymore. There are multiple modes of immune suppression: myeloid derived suppressor cells, T regulatory cells (T-regs), lots of different other suppressive cells, suppressive metabolism, and with all that, the immune cells can’t get to the tumor anymore. There are other suppressive mechanisms as well.



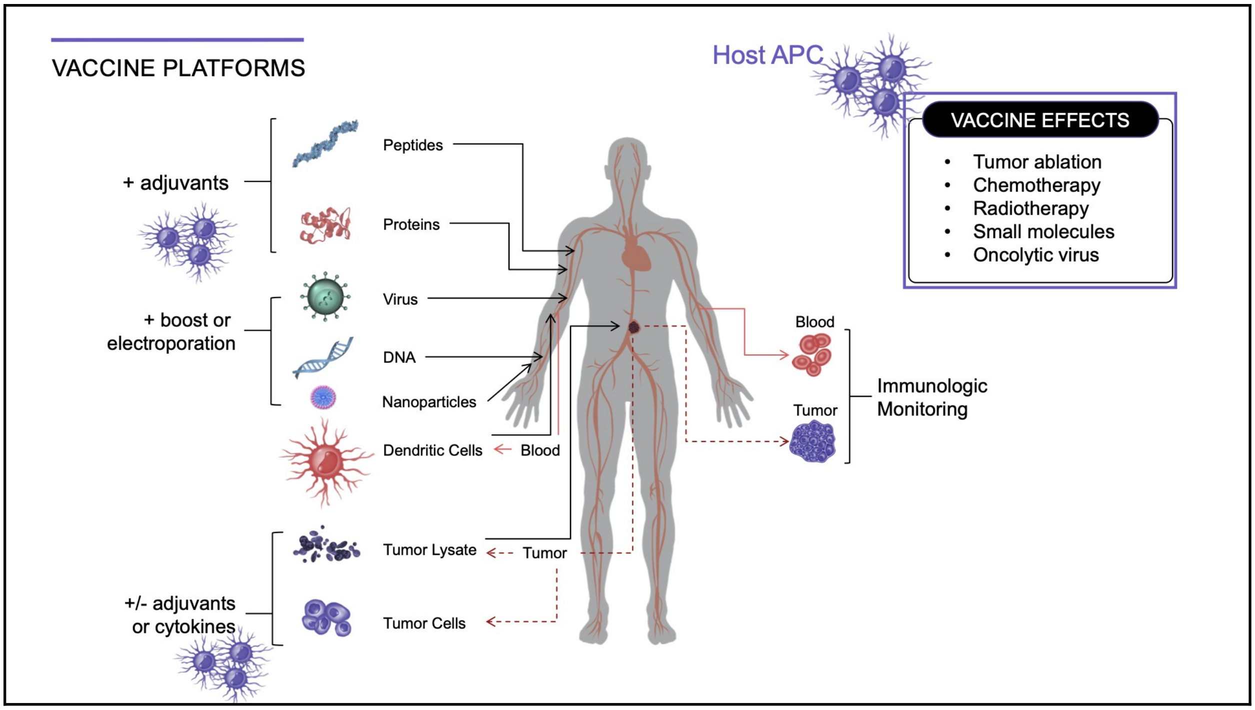
T Cell Exhaustion. Naïve cells express mainly BTLA and low levels of TIM3. Effector cells express a wider variety of **inhibitory receptors**. The levels of certain inhibitory receptors such as PD1, CTLA-4, LAG3, and TIM3 may peak at the effector phase. Thereafter, expression differs in chronically stimulated cells (“exhausted cells”) where inhibitory receptors are relatively maintained, as opposed to memory cells after clearance of an acute infection where inhibitory receptors are down-modulated.
Front. Immunol., 26 June 2015 Fuertes, Speiser

“Cancer Vaccines” (Lisa Butterfield) [#50]

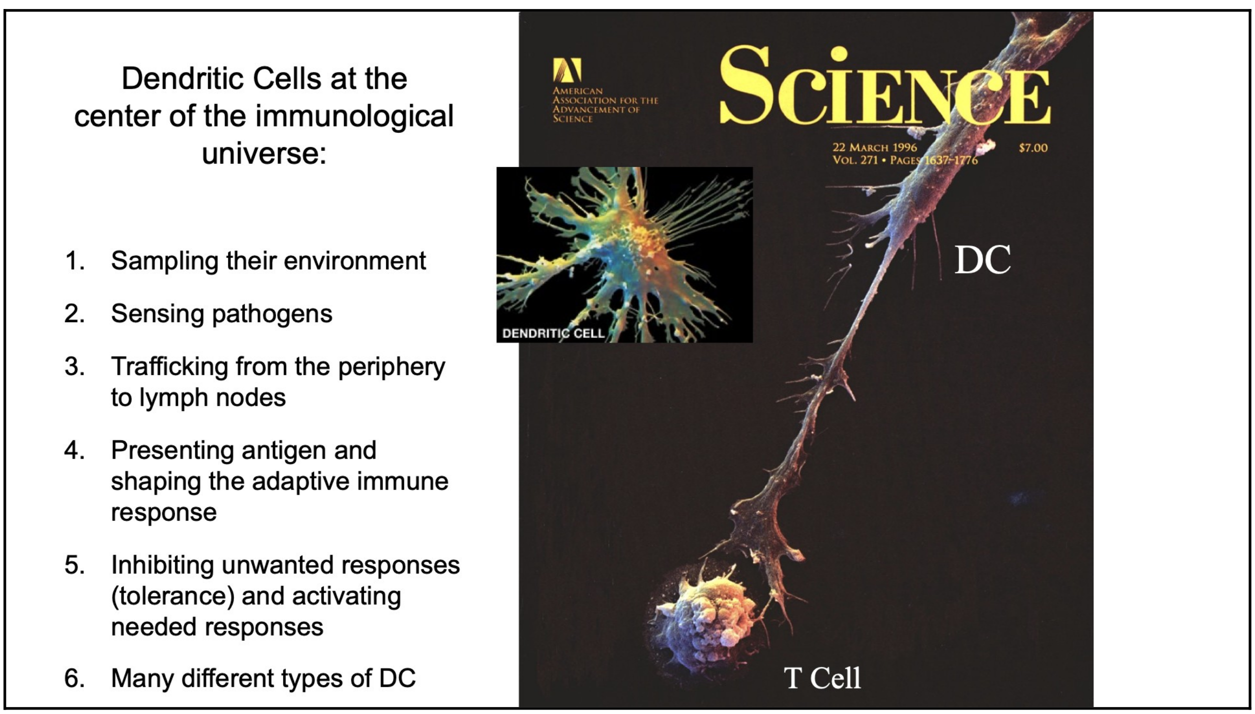
The immune system has evolved to protect us from pathogens such as bacteria, viruses, and fungus. When an infection arises, the immune response is triggered, all of the infection is eliminated, and then the immune response goes back down. But a memory of the infection persists, and you get a healthy memory cell, so that if you see that pathogen again, it can react quickly. But that doesn't happen in cancer, because if the tumor doesn't completely go away early in the process, the immune cells become exhausted, and are just not as functional. So they may need to be reactivated, or even worse, the immune system never saw the tumor in the first place, because tumors can be clever, perhaps able to block recognition by the immune system. So there are a lot of hurdles: tumor evolution, tumor heterogeneity, different cells expressing different things and then this exhaustion phenomenon from the cells seeing the antigen too much.



“Cancer Vaccines” (Lisa Butterfield) [#50]



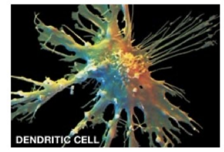
These suggest approaches for cancer vaccines, all of which are tried. I've focused on dendritic cell vaccines, because this is their job. Dendritic cells know exactly what to do when they see a tumor. They will take it up, process and present whatever they want to present, activate the cells, and they instruct the immune response. These are the beautiful giant cells on the cover of Science.



“Cancer Vaccines” (Lisa Butterfield) [#50]

We're more than two decades past that, more than 200 trials, …

DC Vaccines



- 200 DC trials since 1996
- 5 current phase III trials recruiting
- 5 current phase II trials of DC ***+ anti-PD-1***

Dendreon Sipuleucel T: **>**$80,000/patient; Pittsburgh: $6,500/pt.

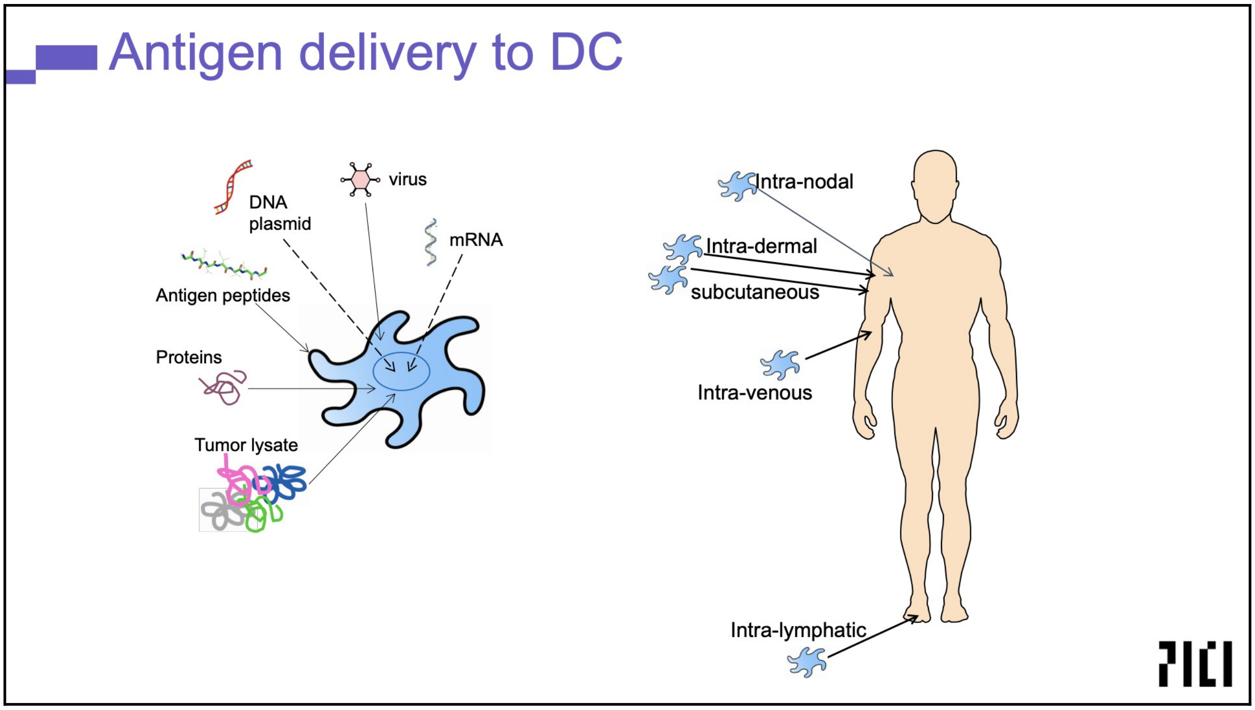
Historically, 5-10% CR+PR in late stage patients in some trials, 0% in other trials.

Recent DC vaccine studies (combinations, *author conclusions*):
1. Kongstad, Svane: **Cytotherapy 2017**: DC + chemo in 43 prostate cancer pt. (*safe and immunogenic*)
2. Schreibelt, De Vries: **CaRes 2016**: 14 stg. IV melanoma pt., CD1c+ isolated blood DC, 16 hour culture, + gp100 and tyrosinase. *4/14 pt. PFS 12-35 mo.*
3. Wilgenhof, Neyns: **JCO 2016**: 39 “adv. Melanoma” pt., mRNA: gp100, tyrosinase, MAGE-A3, MAGE-C2/DC + ipi. *“Encouraging” ORR, 8 CR+7 PR/39.*
4. Greene, Peoples: **CII 2016**: DC/tumor fusions + low dose IL-2 in 25 melanoma pt. *Benefit for some?*
5. Carreno, Linette: **Science 2015**: 3 stg. III melanoma pt., DC+ neoAg peptides, *some + immune responses (proof of principle).*
6. Chodon, Ribas: **CCR 2014**: DC + MART-1 ACT, 14 melanoma pt.*, objective responses, needs improvement for durability*
7. Ribas, Gomez-Navarro: **CCR 2009**: DC + anti-CTLA-4, 16 melanoma pt., *combo not better.*

Why DC Vaccines?

- Originally considered a stand-alone therapeutic approach to promote regression of tumors.
- After being proven “safe and immunogenic” over years, testing in earlier stage patients and in the prevention setting in high risk patients is being pursued.
- With the success of checkpoint blockade and data supporting the need for a pre-existing immune response in the tumor for checkpoint response, *vaccines may be critical to promote antitumor immunity in those who lack it spontaneously.*

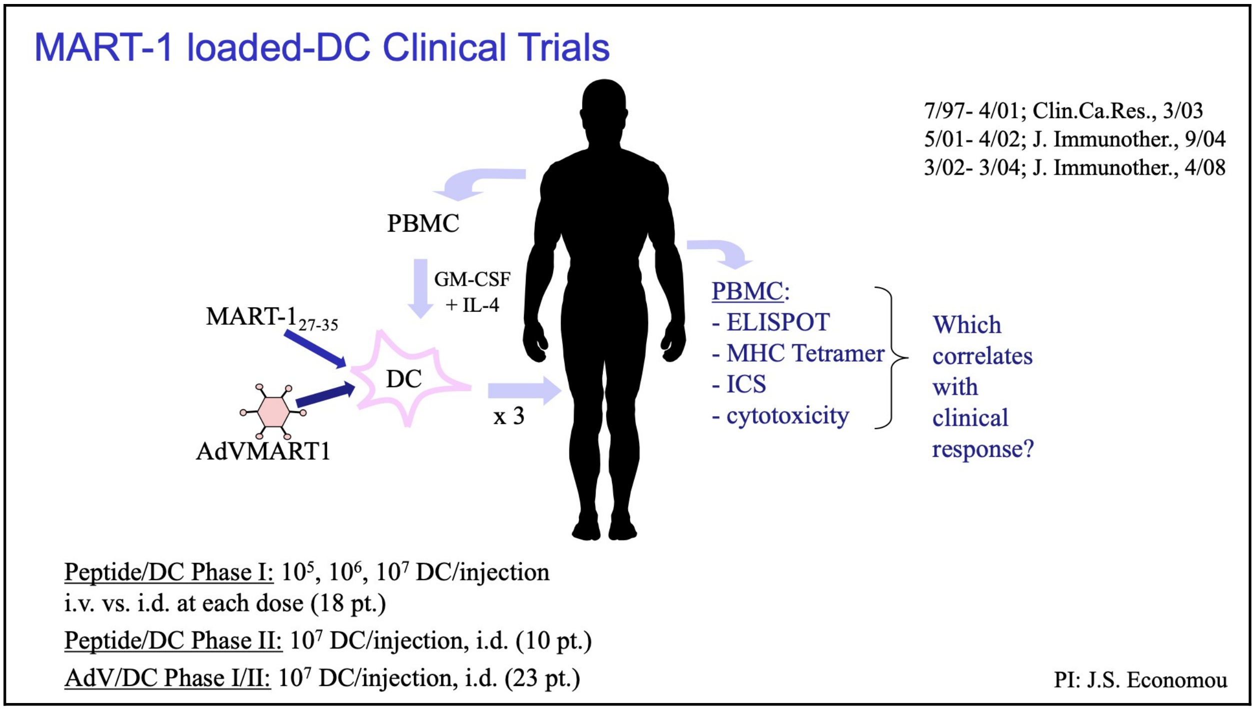
“Cancer Vaccines” (Lisa Butterfield) [#50]

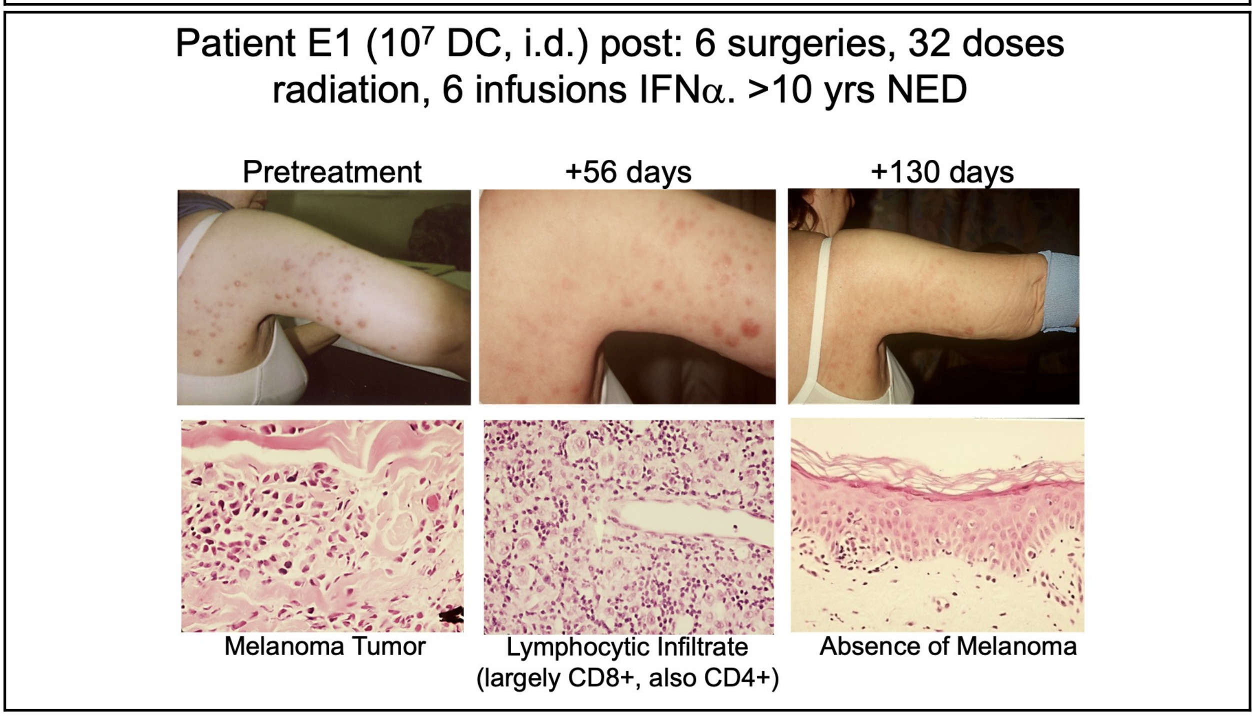


… some of them mine, reaching out to instruct a T cell exactly what to do. It's not expensive to make these cells. Once in a while they've been safe and immunogenic. That's like the worst thing to read in the results – “safe and immunogenic”. So we poked some T cells, but that doesn't really mean much to the tumor, and therefore, to the patient.

So we looked at combinations: what can we add to this? And that's the excitement over the Merck Moderna vaccine approach. BioNtech is doing things like this too. Maybe there's a way to add a vaccine to other therapies to help prevent recurrence. Dendritic cells have been used a lot.

“Cancer Vaccines” (Lisa Butterfield) [#50]

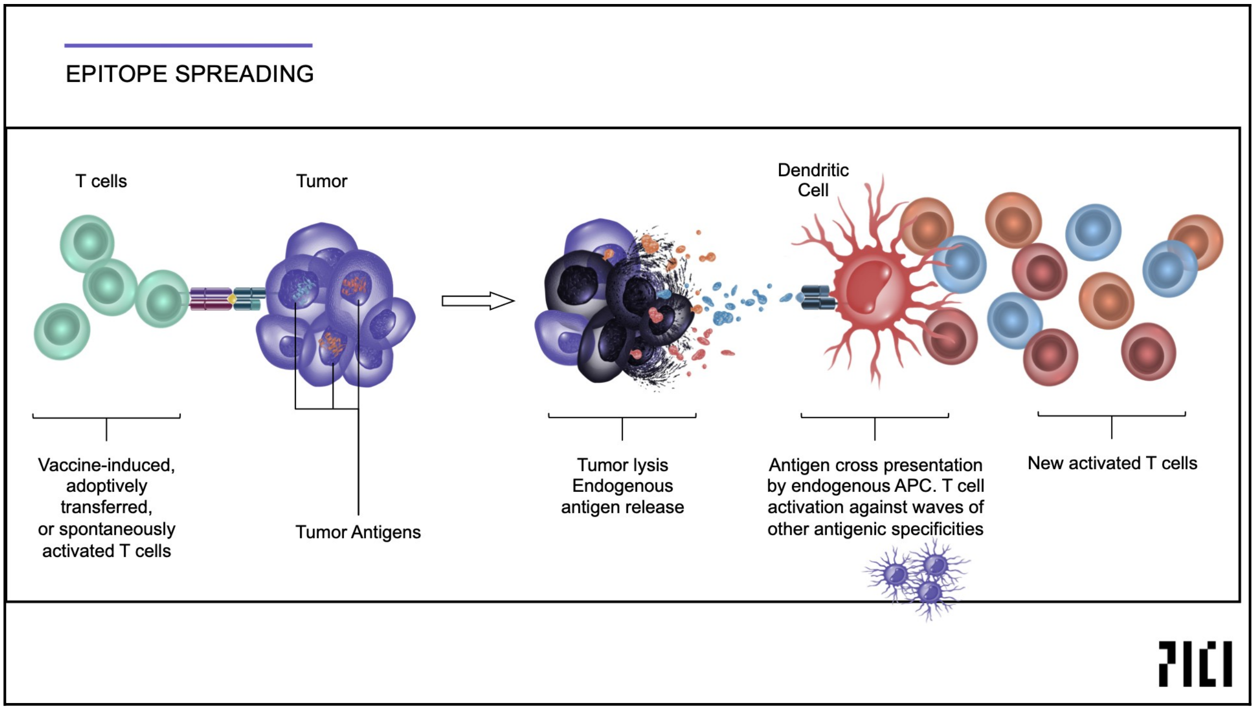




This is a patient who had had surgeries, interferon and radiation and still had a tumor happily growing. On the left you can see the tumor and all of these different metastases in her arm. It was a cold tumor with no immune cells in it. She got a vaccine and after 56 days, it’s a hot tumor, flooded with T-cells, mostly CD8 (killer cells), and then there's no tumor. Once you've seen this In a trial where you helped make that vaccine, and you worked with the physicians treating the patients, it's hard to go back.

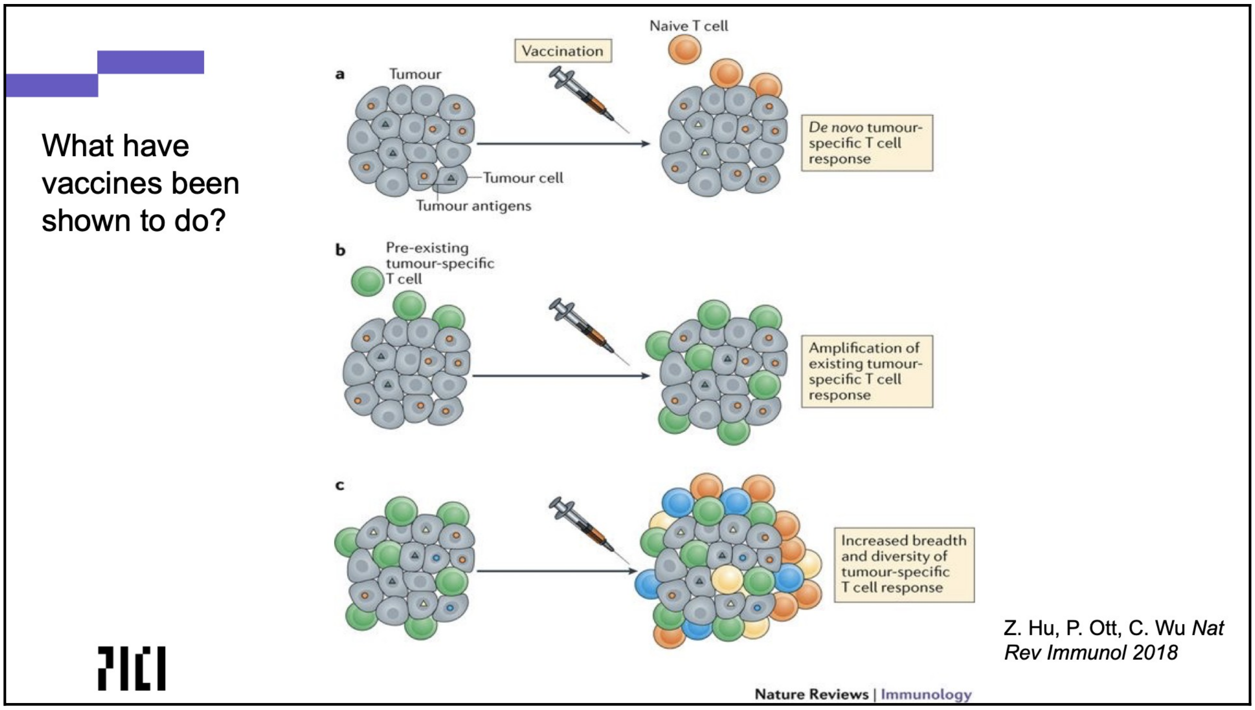
“Cancer Vaccines” (Lisa Butterfield) [#50]

Let me skip a bit to things we're doing now. You activate T cells with either a vaccine, or a bag of T cells specifically adapted to parts of the tumor that express targets. If all goes well, …

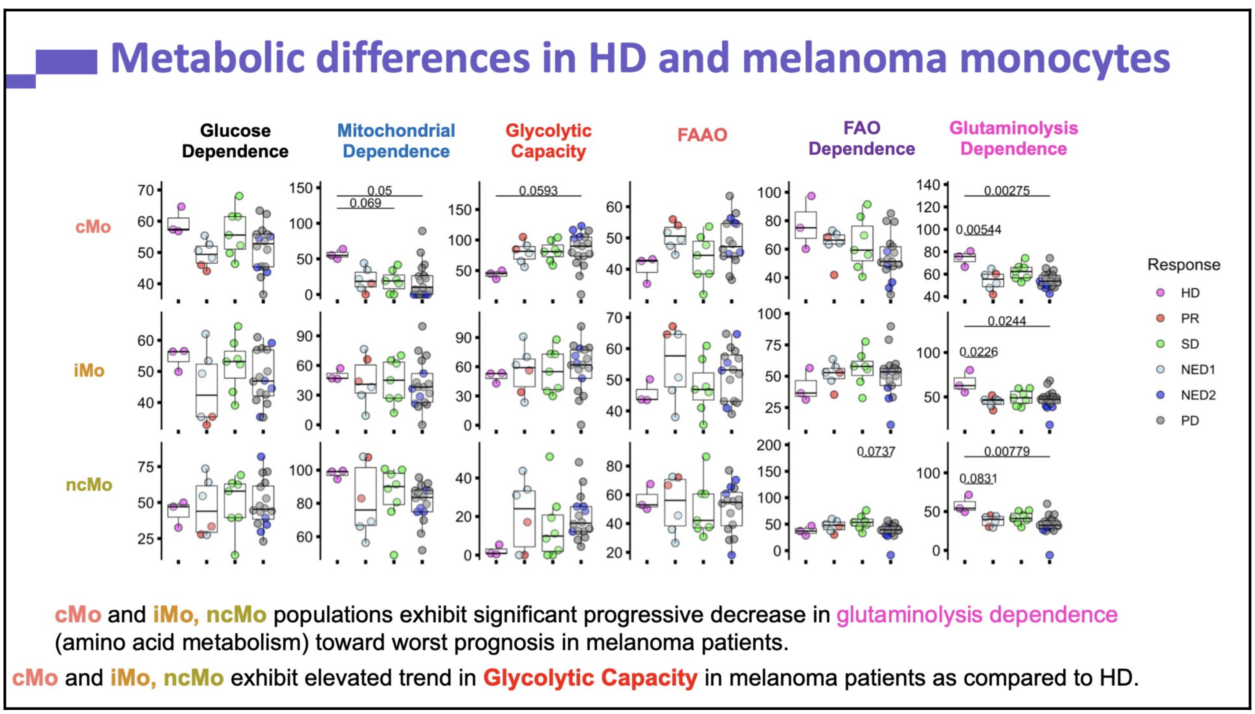


the tumor breaks open and releases more of its tumor proteins, in addition to the ones you started with. The body’s internal mechanism now has more targets to find and attack by generating new cycles of T cells, and you essentially develop autoimmunity recognizing more antigens. If this phenomenon does not occur, you can't fight the tumor because it is so heterogenous that the original targets you constructed in the vaccine might not exist in all the cells of the tumor, or might be lost as the tumor evolves. So this is the mechanistic key: you have to have post-antigen presenting cells. The myeloid and dendritic cells in the patient have to be good enough to be able to support this phenomenon. And you have to have T cells which can be activated. This is something that was looked at in our study a long time ago, and is looked at in a lot of other studies now. That's a key mechanism.

“Cancer Vaccines” (Lisa Butterfield) [#50]

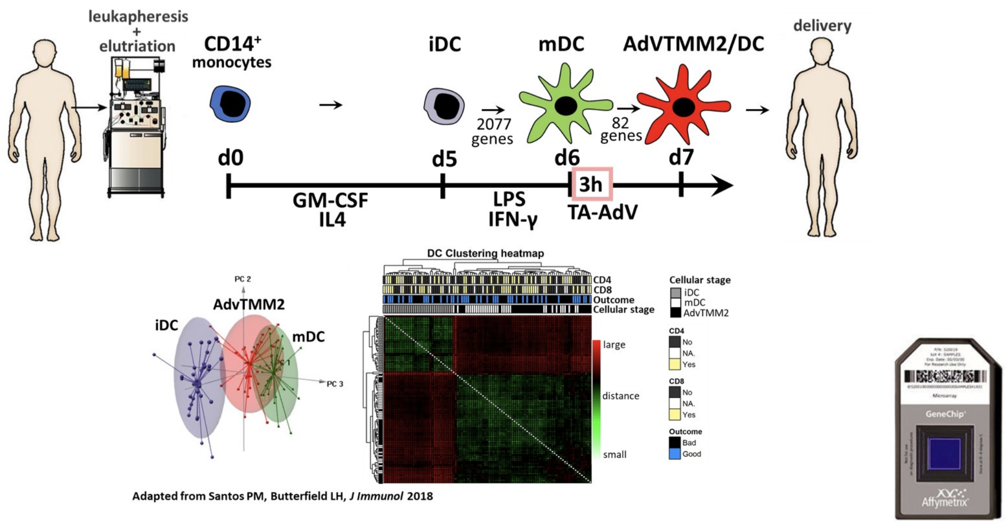


Vaccines can do a lot. They can start a new immune response, they can boost an existing response, and they can make a broader immune response that recognizes lots of proteins. We found metabolism was important.



We've looked at dendritic cells …

DC Vaccine Profiling



Maurer, Adamik et al., *CIR 2020*

DC Potency

Safety:

Sterility, endotoxin, mycoplasma

Identity:

Morphology

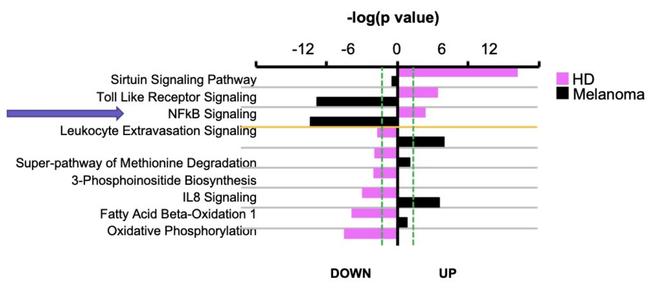
Phenotype (HLA-ABC, HLA-DR, CD80, CD86, CD40, CD83, CCR7)

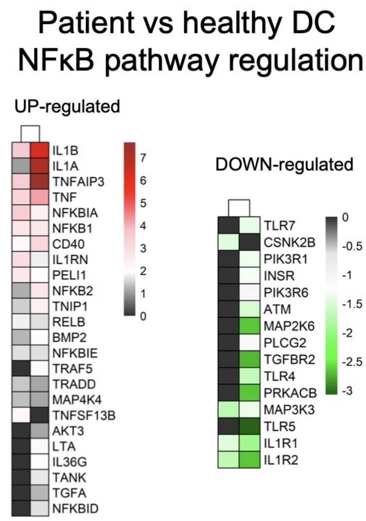
Potency:

IL-12p70 heterodimer?

Trafficking?

NFκB Signaling is Predicted to Be Dysregulated in Melanoma Patient mDC





Maurer, *CIR*, 2020

DC Vaccine Analysis

Antigens Matter: AFP antigen induces metabolic suppression in DC with downstream impact on T cells. Amount of antigen expressed in a DC doesn't matter.

Checkpoint Molecules Matter: High CTLA-4 and PD-1 gene expression networks in lymphocytes correlated with inferior clinical outcomes.

Sequence of combination: *vaccine first, then checkpoint*?

Costimulation Matters: Patient DC vaccines have significant signaling defects (including NFkB) that negatively impact costimulation (including ICOSL).

Metabolism Matters: DC metabolism is impacted by tumors and tumor-derived factors, and defects in metabolic pathways are correlates of *in vivo* antitumor immunity and cancer vaccination clinical outcomes.

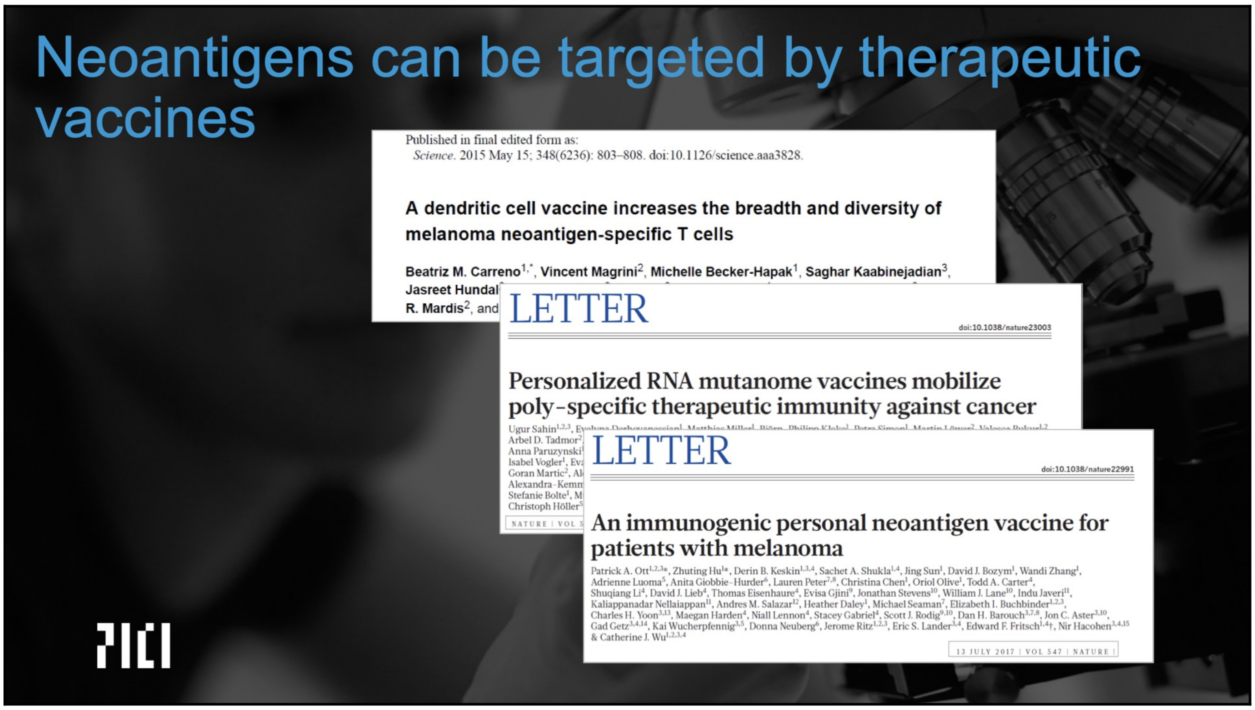
… and we found things that were wrong and right about patient cells. Here are slides …

Tumor Mutations

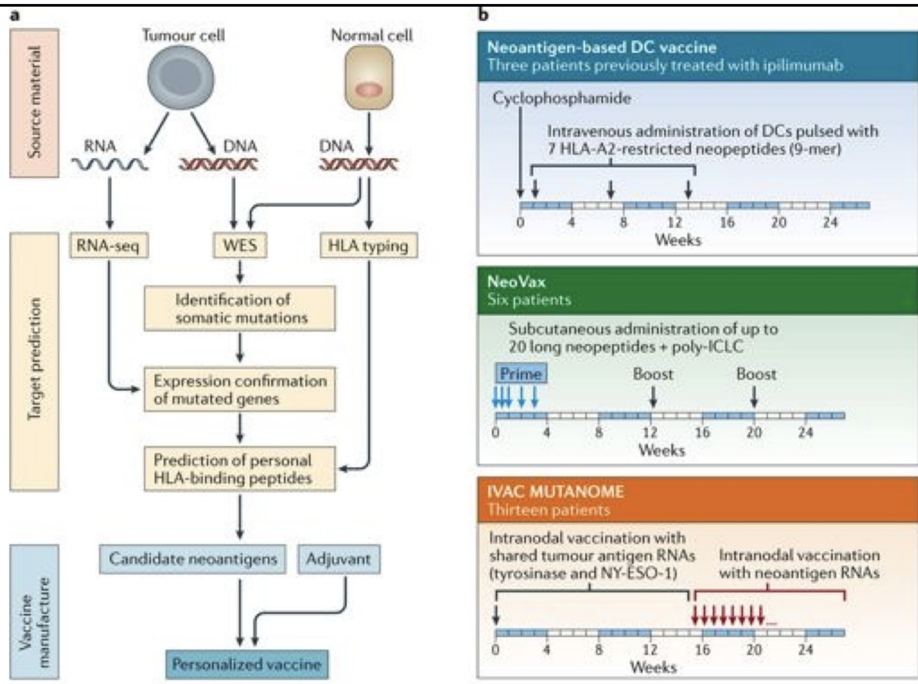
Malignant transformation of cells depends on accumulation of DNA damage.

The immune system frequently responds to the neoantigens that arise as a consequence of this DNA damage.

Recognition of neoantigens appears an important driver of the clinical activity of both T cell checkpoint blockade and adoptive T cell therapy as cancer immunotherapies.

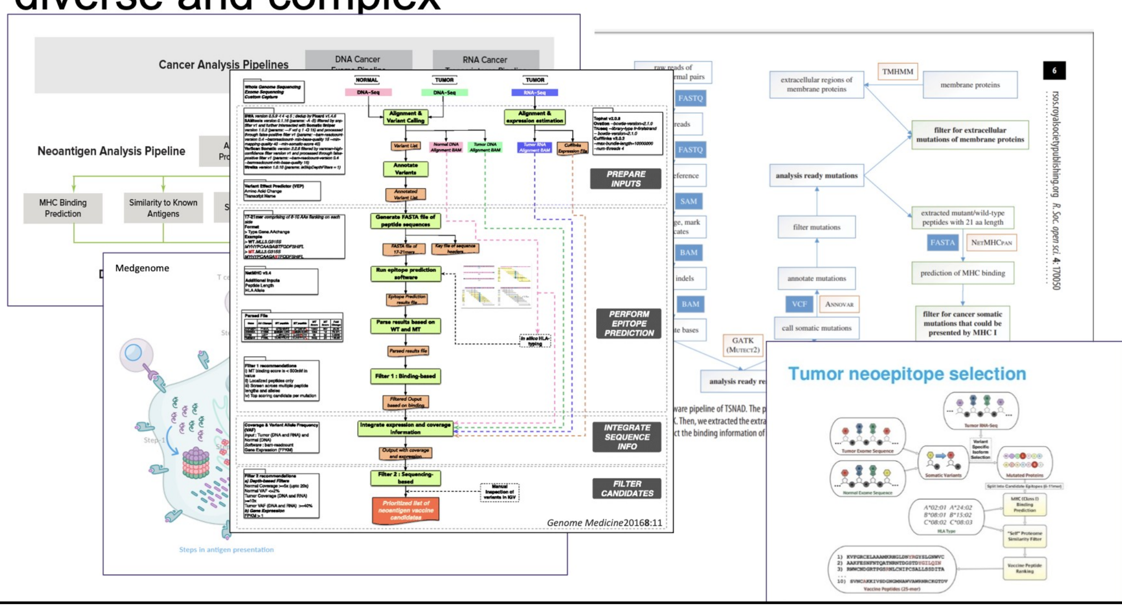


“Cancer Vaccines” (Lisa Butterfield) [#50]

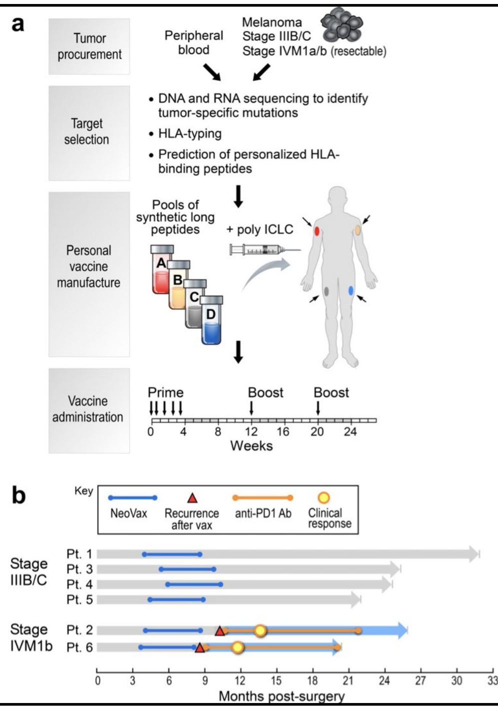


Z. Hu, P. Ott, C. Wu *Nat Rev Immunol* 2018

Neoepitope pipelines are becoming more common, diverse and complex



“Cancer Vaccines” (Lisa Butterfield) [#50]



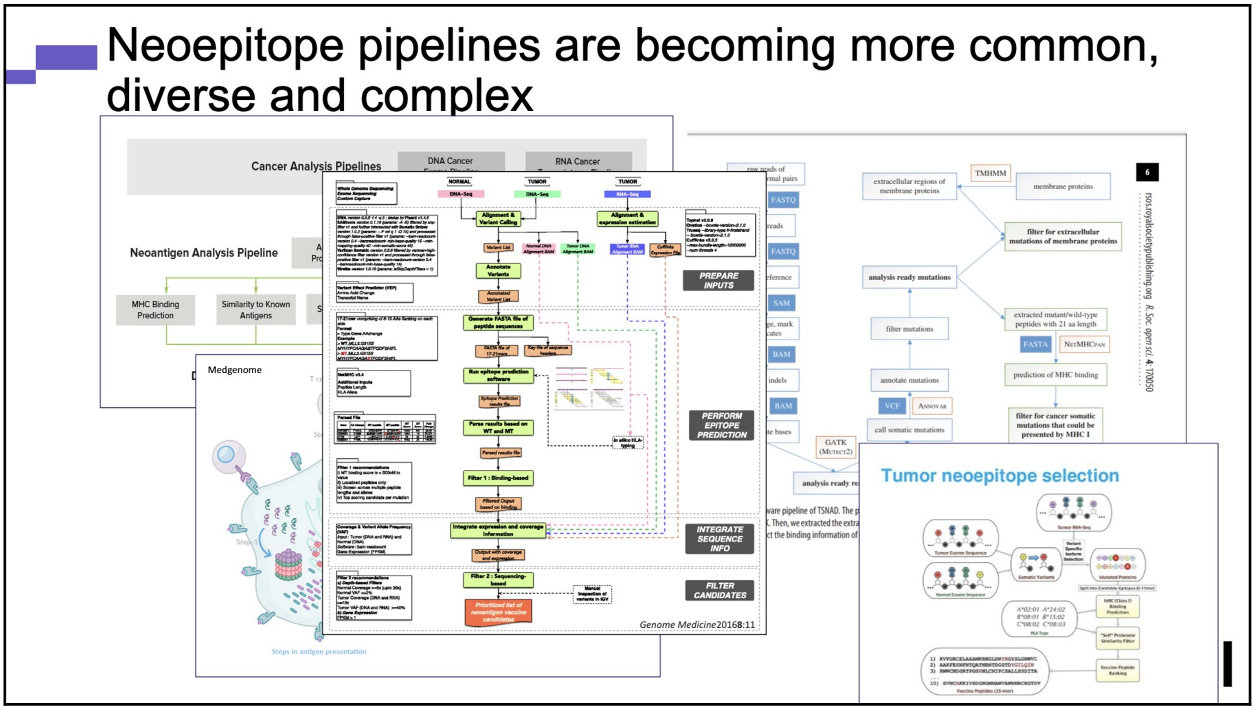
Generation of a personal, multi-peptide neoantigen vaccine for patients with high-risk melanoma

A. Somatic mutations were identified by WES of melanoma and germline DNA and their expression confirmed by tumor RNA-sequencing. Immunizing peptides were selected based on HLA binding predictions. Each patient received up to 20 long peptides in 4 pools.

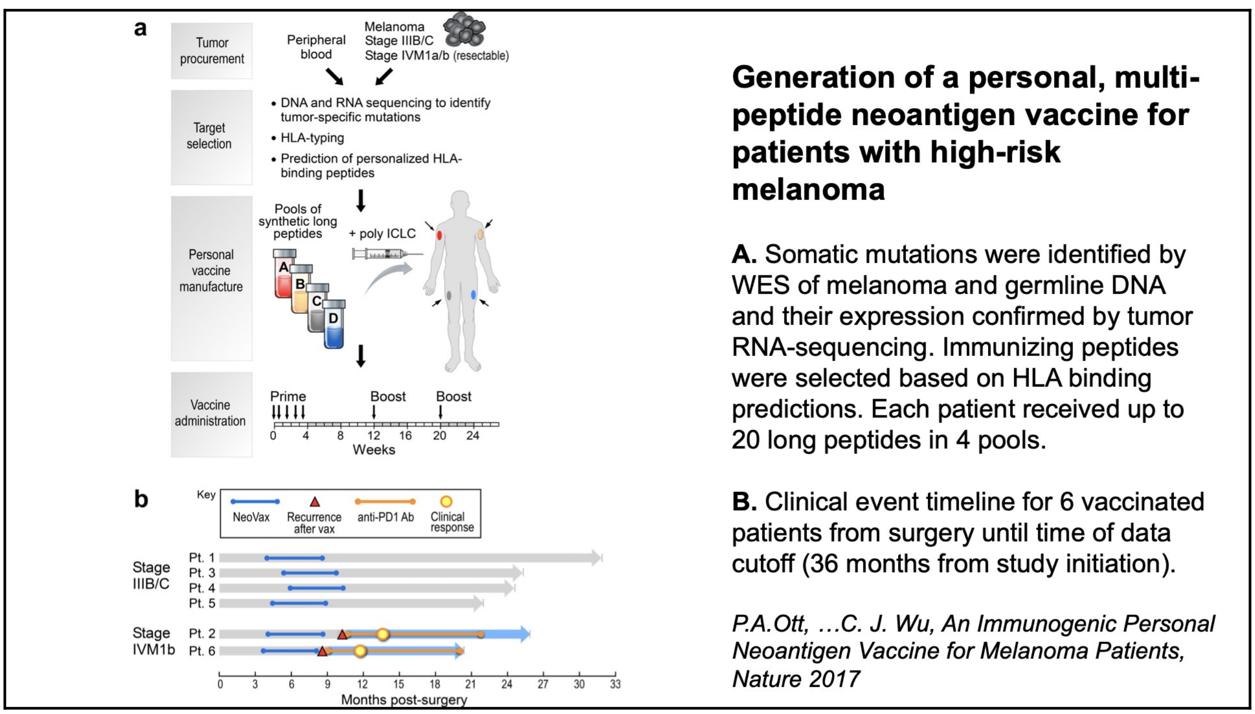
B. Clinical event timeline for 6 vaccinated patients from surgery until time of data cutoff (36 months from study initiation).

P.A.Ott, …C. J. Wu, An Immunogenic Personal Neoantigen Vaccine for Melanoma Patients, Nature 2017

… about tumor mutations involving neoantigens. As these tumor cells grow and divide, often they are genetically unstable, especially when things such as UV exposure in melanoma, smoking exposure in head and neck or lung cancer, fill your cells with mutations which can progress into tumor cells that are full of mutations because of those external hits on your DNA. Other tumors may have more or less of a mutational burden. And even within a cancer, one tumor may have more mutations than another. But the hint we got was, the more mutations, the better the immune response and the more likely there is a T cell response and the more likely you can respond to checkpoint blockade. Perhaps vaccines, even if they don’t fully work on their own, can initiate that response or boost it to then set the stage for a better response to something else. So these ideas really set the field on fire.



This is how you identify the neoantigens as we do them now, by asking, “What does the tumor cell make? What is the tumor cell’s DNA? What is the normal blood cell DNA?” You find out the patient's HLA type, you find out what is specific to the tumor that is made by the tumor, and then down here at the end, you can use that information to create a patient-specific vaccine.



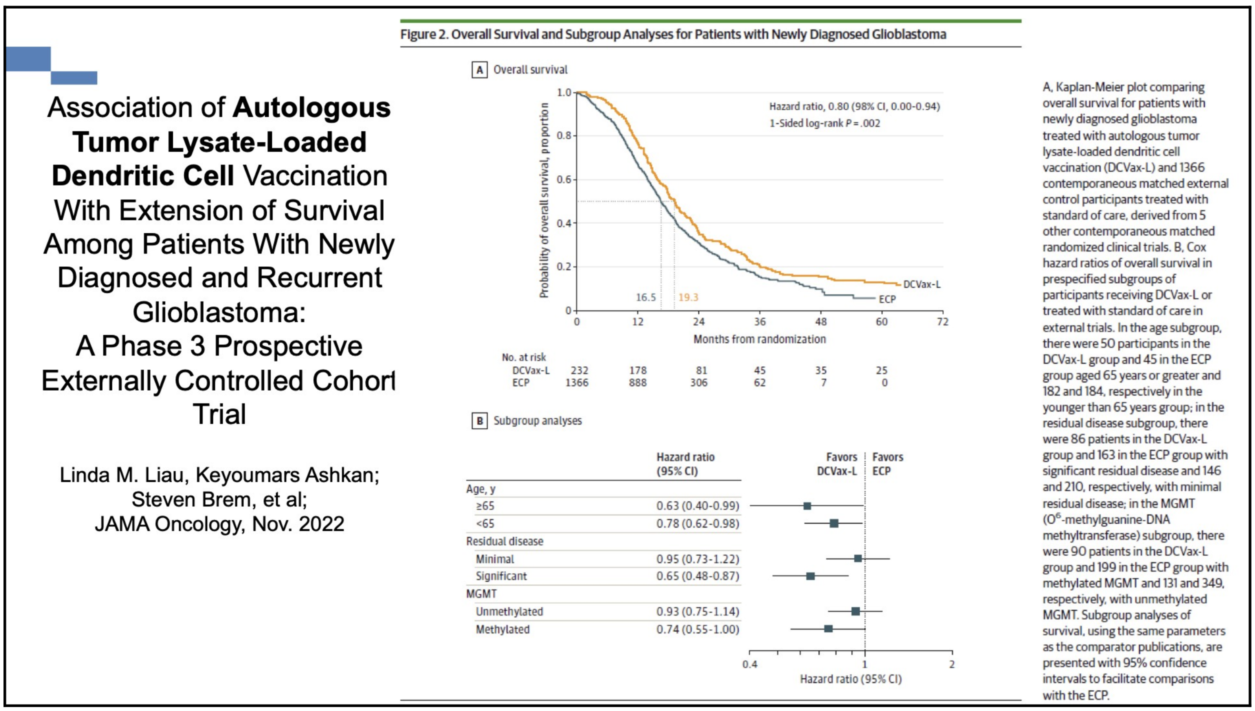
“Cancer Vaccines” (Lisa Butterfield) [#50]

This is the Tesla project that Brad mentioned. This was a very exciting way to improve the pipeline when I joined the Parker Institute over four years ago. The tools to figure out neoantigens and each person’s bioinformatics tools were different. Everyone had their own thing. Every company’s tools were proprietary. No one wanted to share their secret sauce. The Tesla project compared various products and found some were better than others, and told the field, “Here are five things that we think are important as you consider mutations to be neo-antigens.”

While companies each think they’ve found their secret sauce which they're going to continue to use no matter what, this is at least something to be considered more academically than commercially.

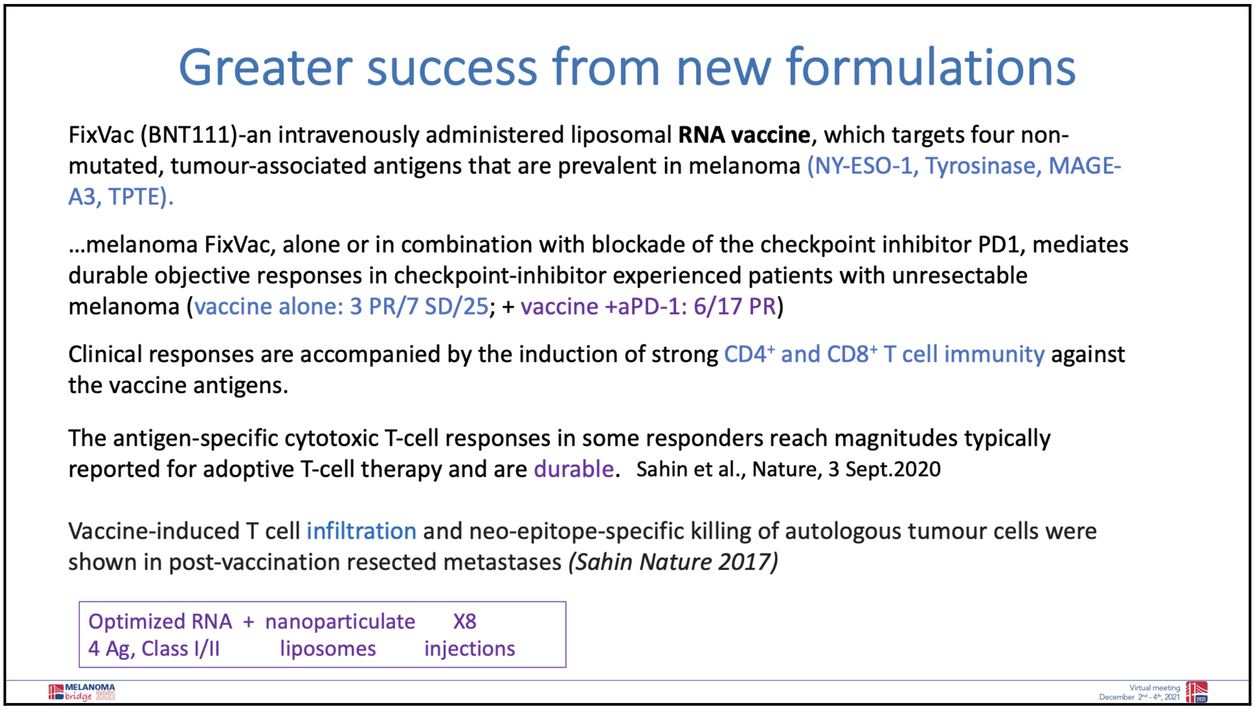
Lisa Butterfield 14:11

So here's another recent study which came out just four or five month ago, showing a cancer vaccine in a brain tumor.

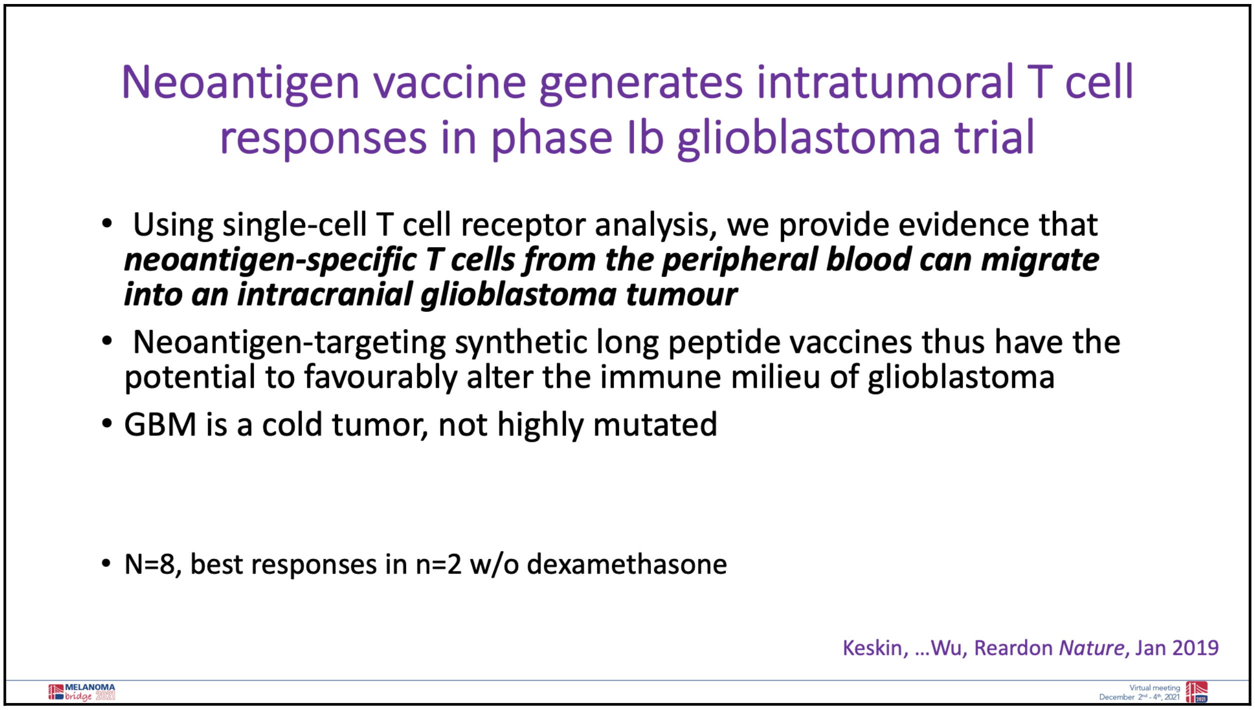


And not a hot brain tumor. It looks like the sipuleucel T curve, but here are brain tumor patients who got a vaccine, made not from neoantigens, but from their own tumor lysate (which would probably include any neoantigens they might have). It's not a randomized study, but they did have an externally controlled data set, and they did better than the control arm.

“Cancer Vaccines” (Lisa Butterfield) [#50]



Here is published data from a BioNTech mRNA vaccine showing that vaccine plus checkpoint blockade really is better. There are three partial responders from vaccine alone, and six partial responders when you add checkpoint blockade. So that's exciting. The study saw infiltration of the tumor with activated T cells. What’s great about RNA as a platform is that it’s fast. You just sequence the tumor, tell the computer what sequences of RNA to make, and it does that. Which is how we got a COVID vaccine so quickly. Now we know that neoantigen-targeted T cells can get into a brain tumor.



But if the patient receives steroids (which is standard of care and immune suppressive) the patients didn't respond anymore. So in this very small data set of eight patients, the best responses are the patients that didn't have the steroid dexamethasone.

The other reason I joined the Parker Institute was they were running centralized clinical trials with multiple arms. In breast cancer this was pioneered in the I-Spy trial, where they just kept enrolling new arms. For the Parker Institute an arm might be 15 patients, and if the results were good, the arm gets expanded, and if it doesn't, while you're enrolling to other arms, then that arm's finished [i.e., an adaptive trial]. We learn what we can learn from deep immune profiling, and move on to the next agent. And these were all novel combinations.

“Cancer Vaccines” (Lisa Butterfield) [#50]

Parker Inst. Platform trial: PORTER

HYPOTHESIS: Anti-tumor immunity can be induced in typically non-immunogenic tumors such as **mCRPC** through combination therapy. Sipuleucel-T, a cellular immunotherapy, is the only approved immunotherapy for **mCRPC** and provides modest clinical impact.

The PORTER B regimen includes step(s) to improve trafficking of APCs and potentially aid the induction and maintenance of an anti-tumor response.
Radiation (SBRT) combined with
FLT3L (CDX-214) and
poly ICLC
are administered to induce immunogenic cell death, recruit/expand antigen-presenting cells (DCs) and provide a DC activation/maturation signal through TLR3 agonism respectively. To avoid tumor-mediated immune suppression, Nivolumab (α-PD-1 antibody) is included in this combination treatment regimen.

PICI

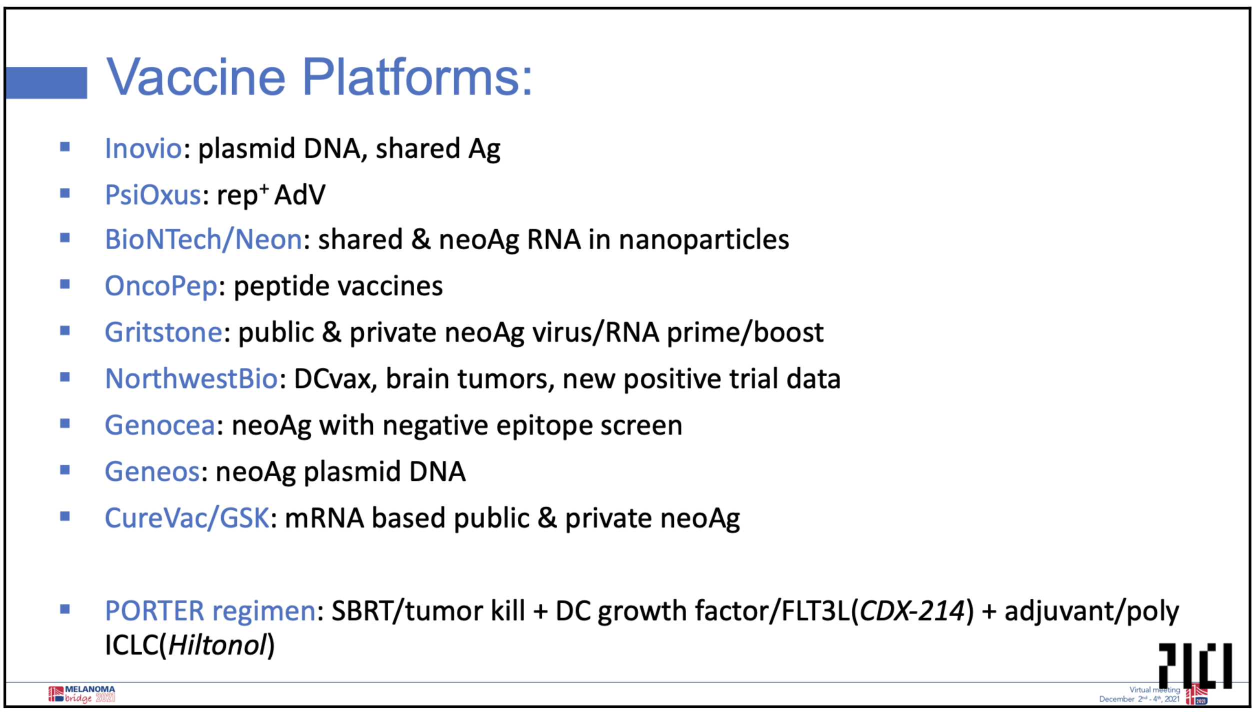
Summary of PORTER findings

- Evidence of many expected pharmacological effects of therapeutic components in the periphery: monocyte/DC enrichment (CDX-214), DC maturation (poly ICLC)
- Demonstration of some clinical responses - most notably in patients with soft tissue disease using current criteria/definition of clinical response
- No safety concerns/limitations (SAEs)
- Low TMB in patient tumors, as expected
- Preliminary analysis demonstrates immune cell infiltration into tumor that increases following treatment in radiographic responder

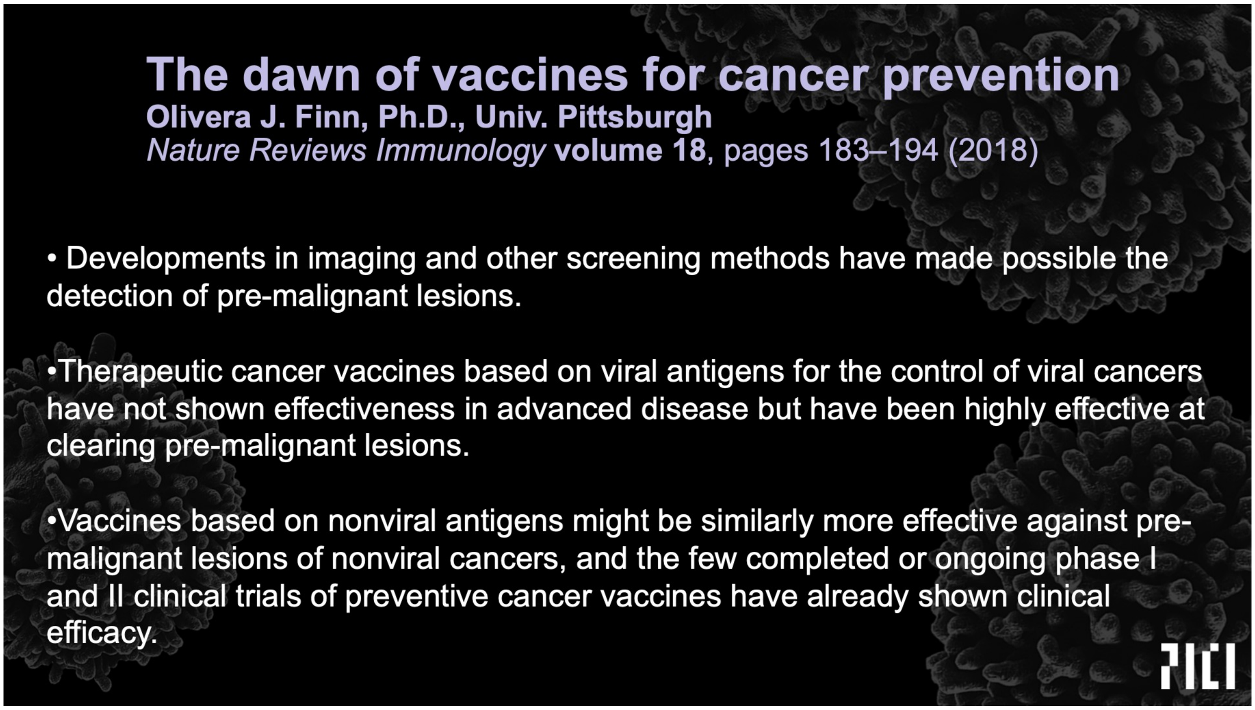
The PORTER trial (everything was named after musicians except Tesla), was in prostate cancer. It was a three arm trial, and the best arm was the vaccine arm. This was a trial involving metastatic castration-resistant prostate cancer. The vaccine was formed after radiation to kill tumor cells and release antigens, followed by administration of FLT3 ligand to generate dendritic cells from dendritic cell precursors resident in the patient’s bone marrow, and then push them to mature to T-cell activating dendritic cells with the immune activator poly-ICLC. The strategy in short was: release antigens, push the bone marrow to make more dendritic cell precursors, and then push them to being the type of dendritic cell useful in a vaccine – one which can take up antigens and activate T-cells. And then, finally, add a PD-1 checkpoint blockade with nivolumab. The quick summary (the publication is in the works) is that:

1. In the first trial where all these drugs were combined, the drugs did what they were supposed to do.
2. There were some clinical responses and PSA decreases, especially in patients who had soft tissue disease (i.e., presumably, not bone metastases)
3. It was all safe.
4. The successful patients were – not by chance – all patients with a high tumor mutation burden (TMB), but rather typical of a prostate cancer which is ordinarily lower TMB. We did see immune cell infiltration into the tumor that increased, and some of those cold tumors became hot.
5. We were excited about prostate cancer as a first try at this particular combination.

I left the Parker Institute just as there was a big reorganization, and they decided not to do centrally run trials and immune profiling anymore. Instead it was all pushed out to the top immunotherapy academic labs doing this sort of work. So the Parker Institute supports all that work, but externally, so there's no core group that actually runs these things anymore. A lot of companies are also involved in this



NorthwestBio developed the dendritic cell brain cancer vaccine I discussed. Genocea (which folded last year) had neoantigen vaccines. They had a screen to select the peptides which would be most effective in generating an immune response. Obviously their work was not positive enough for the company to continue. Moderna and BioNTech have been really leading the RNA vaccine world.



Cancer prevention is a new direction and this is the year that my former colleague, Olivera Finn, from my 15 years in Pittsburgh, is starting to release her data. She did vaccines in pancreatitis (a possible precursor for pancreatic cancer), high-risk smokers prior to the onset of lung cancer, and colorectal cancer polyps before they became colorectal cancer. Those trials should read out this year.

Lisa Butterfield 22:28

Someone in the chat asked if I knew Josh Brody. I do. He has an insight to vaccines at Mount Sinai. He's seen more in blood tumors than solid tumors. But he's seen some very exciting data. You should have Josh come.

Gitte Pedersen 23:01

What you're doing with the in situ vaccination is very, very exciting. Josh Brody has published the data set for metastatic breast cancer patients. One of the patients was metastatic for 14 years and is nowNED (no evidence of disease). Still hanging in there completely tumor free. That was an interesting data point. Obviously, it does not necessarily happen for everyone. But when it does, you go back and try to figure out, “What was it that we did right here?” And, “Can we do it for more people?”

Lisa Butterfield 23:58

Part of our struggle is who's going to benefit from a vaccine. Not all vaccines are equal. Some are better than others, but there are a lot of solid platforms. They're going to activate T cells specific to the tumor. A good platform can be RNA with some sort of particle or dendritic cells. DNA is tough. You have to do a lot to make DNA immunogenic and really activate an immune response, for example in that FLT3 ligand plus poly ICLC. We've seen a lot with shared antigens like PSMA and PSA and neoantigens. One of the approaches that I think Immatix has used is to give the shared antigens first, so the patient has something to get the immune response going. And then while you're working on the neoantigens, and then come in with the neoantigens.

We're just finally starting to see neoantigen vaccines alone promote tumor regression. That's been an important hurdle because it was just that and checkpoint reduces recurrence, unless it was all the checkpoint. Now, vaccines alone, neoantigens, and it's past that hurdle.

Who's going to benefit? People who don't already have the T cells specific in their circulation, maybe they just need a checkpoint, but if you don't, then a vaccine is probably something that certainly won't hurt. It could benefit and reduce recurrence or even mediate tumor progression.

Gitte Pedersen 25:55

When you go in and analyze the side effect profile of these vaccines, it's so minor compared to all the other treatments that cancer patients typically have access to. Even the targeted therapies come with their own set of issues. I'm super excited about the whole field taking off.

Amit Gattani 26:32

I am trying to understand the difference between regular treatment with a medicine versus a vaccine. When we typically think of a vaccine, it's preventive, before the disease occurs. For example, in flu season, we take a vaccine or shot. It's going to help. I'm an advanced prostate cancer patient. One of my biggest worries is I for my 19-year-old son. What's the risk profile for him to get this disease early in his life? Is the vaccine targeted towards that? Or is a vaccine a treatment for me? And if it is a treatment for me, when is the cycle targeting when I'm in remission and future prevention, or when I'm actively with high disease burden? I’m trying to place where vaccines fit in the grand scheme of things.

Lisa Butterfield 27:41

Most classic vaccines that you refer to are against infectious disease and are indeed preventative. The way they are preventative is they generally activate B cells to produce antibodies, and then that acts as a block to infection in the first place. Almost all of these things against infectious disease are basically a protein, or an inactivated virus, and they're trying to make an antibody to block infecting any cells in the first place.

“Cancer Vaccines” (Lisa Butterfield) [#50]

All of the cancer vaccines are focused on T cells. Decades of studies have shown us that the T cell is the one that kills the tumor. If you can get CD8 killer T cells and CD4 helper T cells to work together, it's even better. If you can add B cells and antibodies, that is a bonus. But all of these cancer vaccines are T-cell focused, which is why they're hard, because they have to be matched to you. An antibody is matched to a pathogen. If it's all the same pathogen, you get all the same antibodies.

Olivera Finn [see above] is the one asking if it makes sense to vaccinate in that pre-malignant state when you've got a little inflammation. You're high risk for cancer. Should you get the vaccine? We don't know yet because it looks like from our early studies, even the pre-malignant state impacts your immune system. This ties into your question about “when”. The vaccines are still experimental. People want to get the standard of care, and then wait and get something experimental later. The problem with that, when it's an immunotherapy vaccine, is you need your dendritic cells, your T cells, to still be able to work and respond. If you've had chemotherapy, radiotherapy, or a bunch of other things in late stage disease, your immune system is beat up. The clinical trial will have a 30-day washout from your last therapy to try to reset the system. That's tough.

In my study we had people who got checkpoint blockade as standard of care before they got the vaccine, and some had checkpoint blockade after. Getting the checkpoint blockade first wasn't better, getting checkpoint blockade at the same time in a test tube wasn't better, getting vaccine first thing, getting checkpoint, you get this huge boost in your T cells afterwards. We need to test whether vaccines first, then checkpoint earlier is better. So that's tough. You have to have a vaccine that's fast enough. RNA is fast enough. So if a patient comes in, you can give them something now, and you know, ask them to wait three weeks or six weeks before the standard of care, but get the vaccine early. The way the trials are trying to be designed is to test how to clear or reset the immune system – no steroids on board, no chemotherapy on board.

In those advanced prostate cancer patients I talked about, one of the ways to reset is come in with and make fresh dendritic cells. Let's push new dendritic cells out with the growth factor from the bone marrow that's mature them with this poly ICLC. But they're not beat up old dendritic cells; they're fresh new dendritic cells, and that hopefully can reset the immune response.

So those are all the parameters when you think about when to get it and what to avoid before the vaccine. Earlier is better is what the data suggest, and trying to reset your immune system as best as you can to be able to respond and get the most out of it.

Amit Gattani 31:41

There's a lot involved. It seems like in so many years of work only one vaccine has been approved, if I understood your presentation correctly. There's still a lot in this field to be done and understood compared to other drugs where the approval rate has been pretty high in that period of time.

“Cancer Vaccines” (Lisa Butterfield) [#50]

Lisa Butterfield 32:17

The complexity is that everyone's immune system is different. Everyone's ability to respond is different and renew antigens. You just added another level of personalization. When you do an academic trial, especially if they're cells, you run out of money at patient 10. And so you've got 10 diverse people, 10 diverse responses, and it doesn't lay the groundwork for something where it's a drug, I'm going to 100 patients, 200 patients, we're going to see it or we're not, and then it gets approved, because it's better than anything by 10 weeks. So it's a different benchmark and the immune system has just been more complicated, and more expensive.

Gitte Pedersen 33:03

Add to that, that it is super complicated because you want to turn your own immune system against your own tumor cells. It's a balancing act, because you can't go too far, then you kill the patient. You have to do the immune therapy earlier in the number of treatments that you get, because once you've been through one, two, three, or four chemotherapy treatments, your immune system is no longer functioning the way it was three or four treatments ago. If you are considering immunotherapy, you used to look into clinical trials, because not a lot of them have been approved yet. But I'm very optimistic. We are right there where we are finding the different volumes to push with the number of different drugs in the right sequence.

Rick Stanton 34:37

Has any advanced prostate cancer patient been helped to date by a personalized vaccine? And if so, what lab did it, and where can we get it? This is, as you know, an interested audience.

Lisa Butterfield 34:58

I should have been ready for that one. There's a trial at UC San Diego run by Ezra Cohen, who is the rare medical oncologist and researcher, and his partner is my friend and colleague, PhD Steve Schoenberger, who is working on the predictive algorithm. Ezra and Steve have been treating a number of solid tumors with low tumor mutation burden. I'd have to look back at the slide to see if there was a prostate cancer patient there, and if so, what the response was, but that cancer comprises the biggest diversity of tough-to-treat, low tumor mutation burden, solid tumors I've seen. So I would ask that question to Ezra, or the other clinician involved – Aaron Miller, at UC San Diego.

One of my other favorite vaccine colleagues is Nina Bhardwaj at Mount Sinai, who helped design the PORTER trial. That was her arm with the radiation plus the CD 40, FLT3-Ligand, plus the poly ICLC, and the PD-1 blockade nivolumab. I would reach out to see what she's doing in prostate cancer, and what she's seen beyond the data I just showed you, which were suggestive, although that trial’s not open anymore, and what might she be doing after that?

Most of the other places I'm thinking about are all starting with the high tumor mutation burden cancers such as melanoma and lung. So those are the two I would start with for prostate cancer.

Rick Stanton 37:08

That’s wonderful. Yeah, I worked with Ezra and Steven while I was at Human Longevity. Is Roger Perlmutter still running around at Merck?

Lisa Butterfield 37:24

I don't know. I'm at the South San Francisco site, and I've been in the office one day so far, and it was all orientation. My next day in the office is going to be tomorrow. I don't think so, at least not where my desk is.

Rick Stanton 37:42

Well, that's encouraging to all of us, just to let you know, because Ezra and Steven are super powerful guys. And they're fairly accessible.

Richard Anders 38:12

I have a couple of questions. The first is an understanding check about mechanism and kinetics. I don't know what the dynamic course of a T cell response is through the dendritic cells, but in conventional vaccines, the ones that seemed to have the best longest responses are live virus vaccines, where there's effectively an exceptionally long depot of epitopes on which the immune system vaccine can focus. In a cancer vaccine, I don't know what the longevity of a peptide versus an mRNA-created protein in an RNA-vaccine, but my understanding is the RNA in an mRNA vaccine does not last very long, and the proteins created wash out of the system pretty quickly. The questions are: do you know of any kind of processes which potentiate the amount of epitopes that are available to create the best T-cell response? What is the amount of time needed for an optimal T-cell response? And finally, would you suggest that if you could make a peptide vaccine as quickly and easily as you could make an mRNA vaccine that peptides might be the better approach, or is there no reason to believe that? A little bit of a riff on those questions would be really helpful.

Lisa Butterfield 39:40

A patient on my peptide/dendritic cell trial accidentally came back in seven days instead of fourteen and gave us blood where we already had a T-cell response. If you've got a peptide already presented by the patient's cells – think of a cold or the flu, where you’re sick for three days, but don't know it, and then you start to ramp up with symptoms, and you clear in about a week. For T cells, from seeing the pathogen to being an active T-cell response, seven days is the magic number. That’s confirmed by my n-of-1 patient. I’ve also had a lot of patients measured after 14 days, and almost everyone had responded by then. With a peptide vaccine, the peptide is already ready to go on a cell, but with an RNA vacccine you need a little more time for the RNA to get taken up by cells and start being translated to protein. So I'd give that process 24 to 48 hours. At that point you're right where you were with a dendritic cell vaccine: it's on a dendritic cell, it's processed and presented, it's being shown to T cells. So that’s seven days for the T-cell response plus one or two for that initial step to get from the RNA to the peptide on dendritic cells. It’s fast. Of course DNA vaccines take longer because there’s an extra step.

Patients who get more vaccine doses – eight doses for example do better than patients who get three, although patients can respond from three. We are all guessing on how many vaccination doses. What people are trying to do, because of the transient nature of a peptide, it can fall off the cell in hours. We engineered our dendritic cells with a viral vector which keeps making the entire antigen so it is taken up by the dendritic cell and keeps making the antigen and putting it on the surface of the cell. That turned out not to be better than just using the peptide alone. And it was more expensive. Why wasn’t it better? I don't know. It should have been better.

So that's the amount of time to initiate a vaccine. How many vaccinations do you want? It looks like BioNTech and Moderna have settled at what, eight or nine? They call it priming, it's like three, and then a month and another one, and then a month and then annually. There are good theoretical immune system reasons to do that. It makes sense. It's all non-toxic, so why not? But do you still want to give the same antigen? We've already moved from the COVID to the double [bivalent] COVID vaccine, which might be better. I don't think there's any point in continuously going after the same antigen. The key is to have effective epitope spreading, and we don't know how to induce that. Some patients do it, some don't. But that's what we have to study. And that will then dictate if you've had the spreading event, boost the spreading event epitopes, don't keep hammering the same basic epitope over and over unless the tumor magically keeps expressing it. It probably doesn't. To your question, the quick response is either platform, multiple vaccinations makes sense. It's non-toxic. So why not? We don't know how many vaccinations. We don't know how long to keep it up. We don't know when to switch to the new antigen. All we can do is keep drawing blood and keep doing the assays and keep trying to figure out what you need and when you need it. Did I get close?

Richard Anders 43:12

That's a great answer. So the virus vaccine didn't actually have any benefit? Is it possible that there was an immune response to the virus? Did you check that the virus was continuing to produce epitopes that were being presented to be sure that wasn't the issue?

Lisa Butterfield 43:28

We got T-cell and antibody responses to the virus component. It didn't make any difference. Some patients had it at baseline; some didn't. That didn't make any difference. What it did is allow us to treat patients who weren't HLA-A2 which is the tissue typing for about 50% of

Caucasians. When there’s a small peptide or T-cell receptor trial, they're the usual people allowed to enroll because the therapy must be created for a specific HLA type and this one is very common. By engineering the whole protein and letting each person’s own cells do the work of presenting it within the structure of that person’s own HLA, we could vaccinate anybody. While the platform didn’t produce a better response, it did enable us to broaden the candidates for our clinical trial.

Richard Anders 44:06

You probably answered my question, but I want to be sure that I said it properly. It’s possible that the virus could have been neutralized because if it was not presenting antigen for long, then, similar to a peptide vaccine, it was a very short-lived antigen presentation.

Lisa Butterfield 44:44

Yes. In this setting, it wasn't free virus circulating in the patient. The virus was used to infect the cells in the test tube before we gave it to the patients, to protect against that happening. That was to protect against that. The dendritic cells only last a week or two anyway. We gave three dendritic cell vaccinations. The genetically engineered virus, instead of the peptide, didn't make a difference in terms of the magnitude of the immune response. We were able to get a broader immune response on the front end. That seemed to help. But I've actually spent the last six years trying to sort through all of the things we did and figure out what was important and what wasn't. But that’s ongoing research. It seems like once you were successfully vaccinated against those antigens, you're successfully vaccinated. We didn’t need long-lived depots of virus. Just a quick burst would do.

Richard Anders 45:35

In the Genocea burnout, were they trying to make some laboratory-based version of selecting the best epitopes not to trigger T-Regs? Do you think that’s a promising approach?

Lisa Butterfield 46:15

They were trying to eliminate peptides that could activate regulatory inhibitory cells and only include peptides that would activate the killer T cells. We don't have the right pipeline yet. I don't know that. I saw the way they screened to do that. I was aware of that stuff, too. It's not something I ever wanted to run with or incorporate into my own studies. There were issues around data interpretation and immune system behavior. We immunologists are always doing a lot of hand waving because we don't know how it all works and because it's complicated. Their secret sauce wasn't competitive enough. And there were other fish in the sea. If that's their secret sauce, and it isn't a big breakthrough, then that's it for their pipeline. Let's look at somebody else's pipeline. That's my guess. If I was a venture capitalist, why keep putting money into it? Put money into somebody else's platform.

Richard Anders 47:19

When you say “their platform,” do you mean the hypothesis that T-regs are valuable, and not having T-Reg epitopes is meaningful?

Lisa Butterfield 47:31

I don't know. I don't know how well they did it. I didn't see compelling T-reg data. But I used to run this immune monitoring corporation in Pittsburgh, and I would stick my nose into everybody's trial that was going on, and I saw a lot of mouse data saying T-regs are incredibly important in cancer. And when you have an immune profile, T-regs may or may not be important. Now myeloid-derived suppressor cells are harder to measure, but are unequivocally a huge hurdle in developing any vaccine. The T-Regs issue was how suppressive they were, not how many there were. We did a study in melanoma. We measured it over time and more was good. And sometimes more was worse. Why would it be good? Because that's counter regulation: you've activated immune response, then your T-regs go up after, but the amount of them didn't matter. How suppressive they were is what mattered. They were just a natural part of an immune response that came up. We don't have a good way to inhibit them. We can deplete them for like a week, and then they come back, sometimes they come back more. So that's not something I've ever focused on, because I just don't have the data, and that's the important thing. The myeloid suppressive cells are absolutely super important and need to be addressed. That might have played into it with Genocea and the T-Reg focus. Perhaps it’s very important in a mouse, and not quite as important in a human.

Rick Stanton 49:05

I will definitely reach out to Ezra Cohen and Steven Schoenberg. I used to work with them.

Lisa Butterfield 49:33

And you should reach out to Nina Bhardwaj at Mount Sinai as well.

Russ Holyer 49:37

One of my future plans has something to do with this. It seems like it might play very well into high androgen DNA damage. I have a future potential therapy that I discussed yesterday with my medical oncologist that might be up this alley.

Lisa Butterfield 50:13

Here is my academic UCSF email address: lisa.butterfield@ucsf.edu for follow-up questions that we didn’t get to.

Brad Power 50:31

If you wanted to get a personalized vaccine, you would want whole genome sequencing. Ideally, it sounds like you need some RNA sequencing. One of the hackathons we ran was for Kasey Altman, who went through Ezra Cohen’s personalized vaccine pipeline. She didn't have enough variants of significance to have enough peptides to make a vaccine. Could you talk about handicapping the different pipelines, which was the results of the Tesla trial, where you found that none of these algorithms are really knocking it out of the park? If you wanted to get access, what would you need to bring to the table, and could you handicap some of the issues you've seen in the practicalities of the process?

Lisa Butterfield 51:29

If your pipeline is liberal, and you don't validate the peptides, you're willing to guess in your trial. You probably have a lot of mutations. You probably have a lot of things to try. What Ezra Cohen and Steven Schoenberger are doing is validating the peptides. They have a different screen, they see different things, and then they test them. You need enough to make those peptides. They have to get your blood for T cells. They do an in vitro culture to make sure that you have the T cells to be boosted by that vaccine. That's a more stringent screen. If they think you're not going to respond, then they won't enroll you. You won't pass their criteria. Everyone's going to have different criteria. The good news is, if you're going that route, you have T cells that are very likely to respond, but you might not. Sometimes peptides are too hydrophobic. The HLA2 molecule has very deep pockets. Depending on the amino acid sequence of the peptide, it may not synthesize. It may be a little ball of oil, and it can't be made by the machine. There are also synthesis problems. And the predictions are going to vary, as are who passes enrollment criteria, and who doesn't.

Rick Stanton 53:07

I have a question about RGCC. They are looking for circulating tumor cells, isolating them with flow cytometry, and then querying for responsiveness to existing drugs. Do you have any comments?

Lisa Butterfield 53:59

Circulating tumor cells is tough. I've seen those. They're a little sketchy looking, but it's validated. It's limited. Circulating tumor DNA could be a source of getting your tumor sequence and identifying mutations, identifying neoantigens, following what's going on. That's finally quantitative. The measurements actually mean something. That is a platform worth looking at further.

Lauren Cohen 54:38

“Cancer Vaccines” (Lisa Butterfield) [#50]

I am not a scientific or clinical advisor, but I can speak about RGCC in general terms. We will be coming to the group within a month or two to do a presentation. We will have a scientific representative who will be able to answer your scientific questions.

RGCC is utilizing personalized medicine and is truly personalized. A lot of personalized medicine is a misnomer. It's personalized to the cancer or the cancer mutation, as opposed to the person themselves, or how the cancer cells are actually interacting with that individual's physiology.

There's a lot of information that can be extracted from the exploration of the CTCs, the DNA and the RNA, that can truly personalize your cancer care. There's a CTC count that you can utilize as a measure for the volume of cancer in your system. The tumors shed cells, so there are circulating tumor cells. There are also what we call stemness markers, which help to determine the activity level of those cells. Are the cells active and have a propensity for proliferation, recurrence and metastasis, or are they less active? We also do genetic expression profiling so that we can see the genetic and physiological expressions that are happening within your body specifically, so that there are additional treatment options, that would be say a little bit more indirect, and where there are cytotoxic-type treatments which go after the cancer cells directly. Then there are physiological processes where some of these genetic expressions are upregulated or downregulated, and contribute to cancer development or cancer growth. So there are different types of supplementation treatment options that can assist in either up- or down-regulating those same expressions so that they're not as supportive of cancer growth. Within the profiles we also do chemo sensitivity testing for a little more than 50 chemo chemotherapeutic agents along with 53 natural substances. There is the opportunity to see which treatment options will or will not be effective, so that we're not doing that one size fits all shot in the dark and hoping that things will or will not work.