WEBVTT

NOTE duration:"01:12:26.9680000"

NOTE language:en-us

NOTE Confidence: 0.917187094688416

00:00:00.230 --> 00:00:12.600 Our next speaker will be Doctor Pardoel Doctor Portal comes to us from Johns Hopkins, where he is the director of the Bloomberg Kerrville Institute for cancer immunotherapy Alba law professor of oncology.

NOTE Confidence: 0.250898271799088

00:00:21.070 --> 00:00:21.850 OK.

NOTE Confidence: 0.898661732673645

00:00:23.150 --> 00:00:53.160 Great to be here for a number of reasons. A lot of friends and also actually my dad and his family grew up in New Haven and we used to come up here. Once a year to visit. His oldest sister my favorite app. So I have always had a lot of food available. So I always actually have this great feeling when I come to New Haven so.

NOTE Confidence: 0.934806406497955

00:00:53.160 --> 00:01:24.690 Following up on the wonderful presentations related to tumor microenvironment. What's going on in the tumor microenvironment. We've heard some very different perspectives on how to study and look at the tumor microenvironment and I'm going to give you a somewhat different form of perspective in.

NOTE Confidence: 0.927819967269897

00:01:24.690 --> 00:01:29.180 Looking at the tumor microenvironment related to.

NOTE Confidence: 0.933288395404816

00:01:29.780 --> 00:01:46.540 2 applying single cell analysis and also taking advantage of new approach that we've developed to look at T cell repertoire and Antigen specific T cell responses.

NOTE Confidence: 0.826555192470551

00:01:47.050 --> 00:02:02.190 So so certainly and I don't need to tell this audience block heater. The PD 1 pathway is been arguably.

NOTE Confidence: 0.902910351753235

00:02:02.780 --> 00:02:33.690 The centerpiece to the revolution in cancer immunotherapy. This is a list of I think it's gets added to every few months of the cancer types that have been for which one of 6 anti. PD one or anti PD. L1 antibodies have been approved the ones in.

NOTE Confidence: 0.909122407436371

00:02:33.690 --> 00:03:04.550 Orange are tumor types that have very impressive responsiveness. Although certainly not 100% the ones in yellow. The responsiveness certainly in patients that do respond. The responses tend to be much more durable, but I've also show that despite FDA approvals.

NOTE Confidence: 0.908477902412415

00:03:04.550 --> 00:03:35.280 There's certainly a long ways to go and we still have a lot to learn to understand T cell responsiveness and immune responsiveness in general to cancers and why cancer to spondin? Why cancers don't respond. Just an interesting slide. I decided to throw in we've gotten to know some of the folks in Bloomberg Intelligence.

NOTE Confidence: 0.0306479725986719

00:03:35.330 --> 00:03:36.130 Um.

NOTE Confidence: 0.923612296581268

00:03:36.680 --> 00:04:07.870 And so they actually provided me this slide, which is the revenues. They are interested in those things for targeted therapy versus immunotherapy. Obviously this is predicted but you can see that even a therapy is actually catching up, but I think this is going to have to end up being revised becaus more and more there are exciting.

NOTE Confidence: 0.93566769361496

00:04:07.870 --> 00:04:26.540 Results clinically through combinations of immunotherapy and targeted therapy. So I think those distinctions are actually becoming more and more irrelevant as this all really integrates together.

NOTE Confidence: 0.90744161605835

00:04:28.120 --> 00:04:59.810 There are actually formally 2 FDA approved either companion or complementary diagnostics. One is P. DL1 expression and David talked a lot and gave actually wonderful summary of where that stands and some of the challenges the 2nd is mismatch.

NOTE Confidence: 0.910872936248779

00:05:05.660 --> 00:05:35.770 Is mismatch repair deficiency an certainly people are looking very carefully at mutational burden has not yet made it to FDA approval. I think remains to be seen actually where that's going to go, although clearly the mismatch repair deficiency story tells you that you have extremely high tumor mutational burden that.

NOTE Confidence: 0.907703995704651

00:05:35.770 --> 00:06:06.220 Is a predictor for for response? What's interesting is that uh the simple notion that you have high mutational burden. You have there for a high immune response is going to be more gammon. Or she ran in

the tumor microenvironment by adaptive resistance that for going to be higher. P DL1 expression. It's actually quite surprising to me how non concordant.

NOTE Confidence: 0.933571338653564

00:06:06.220 --> 00:06:18.540 These can be there is clearly some concordance, but there's also a lot of non concordance, which tells us that there is a lot more that we have to understand and certainly tending to be.

NOTE Confidence: 0.917948007583618

00:06:19.050 --> 00:06:49.740 More be self focused in our lab, not to imply that there are the most important cells. It's just the cells that I actually know something about there are other factors variables that are very critical, including repertoire, Functional State of the T cell as you've been hearing. A lot about from Greg and then also my lawd cells and I haven't even listed fibroblasts in the stroma.

NOTE Confidence: 0.937649071216583

00:06:49.740 --> 00:07:20.210 And that doesn't even talk about signaling pathways. An metabolics so I think certainly if you look at the approved standard of care utilized biomarkers. They really represent a tiny percentage of what we need to understand who just looking at the micro environment itself.

NOTE Confidence: 0.929521381855011

00:07:20.300 --> 00:07:32.080 To really understand how we're going to guide future improvements in immunotherapy, which I think are still think we're just scratching the surface.

NOTE Confidence: 0.914186179637909

00:07:32.720 --> 00:08:03.340 So I'm going to talk about some of the analysis that we've been doing related to an initial clinical trial and ongoing work on the application of Anti. PD one lung cancer as neoadjuvant therapy so giving it before surgery surgery in Operable.

NOTE Confidence: 0.917370676994324

00:08:03.340 --> 00:08:33.350 Patients in lung cancer still roughly 60% of operated lung cancer patients relapse and the notion that we can do something different by giving anti PD one or other immunotherapy's up front is being tested now in 12 different cancer types. I think there is beginning to emerge.

NOTE Confidence: 0.924455285072327

00:08:33.350 --> 00:09:05.340 Some early evidence that this may be clinically beneficial. What's clear is that be cause. We get the respected tumor. After anti PD. One is given. It is a goldmine to be able to study. What is going on in responsive tumors versus non responsive tumors because you get so much material on therapy in particular for analysis like single cell transcriptome mix, etc.

NOTE Confidence: 0.928142428398132

00:09:05.340 --> 00:09:07.770 That's particularly useful.

NOTE Confidence: 0.921430945396423

00:09:08.730 --> 00:09:39.980 Now, when we began these studies in this was a collaboration between the lung cancer team at Johns Hopkins. The lung cancer team at Sloan Kettering supported by CRI and Stand Up To Cancer. The notion which was somewhat oversimplified was that you had T cells in the tumor that were tumor. Specific they were being blocked from recognizing.

NOTE Confidence: 0.913019955158234

00:09:39.980 --> 00:10:10.750 The tumor as you heard from David that's probably not that I think that's part of the case. But I would actually very much agree that may be very much less than half the case. But in any case, you block PD. One these cells proliferate, they somehow spill out into the circulation and then can leave the circulation traffic through that issue as activated T cells tend to do.

NOTE Confidence: 0.906792402267456

00:10:10.820 --> 00:10:28.320 Looking for their source of Antigen in in that issue and in the case of tumor specific T cells. Those are distant. Micro metastases, which are? What is responsible for relapse after surgery in this would potentially be a way to kill them?

NOTE Confidence: 0.900615632534027

00:10:29.010 --> 00:10:39.290 We think that that model is wrong through a number of studies and actually.

NOTE Confidence: 0.910675704479218

00:10:39.830 --> 00:11:10.340 One of the first studies, ironically was one that was actually 2 back to back papers from our group and leaping Chen's group when he was actually at Hopkins looking at the ability of PD one or P. DL1 blockade to mitigate tolerance generation among T cells.

NOTE Confidence: 0.889421463012695

00:11:10.530 --> 00:11:42.380 First encountering anagen in lymph nodes so in this particular model. I I had to pull this out of an old 'cause I couldn't find the original figures 'cause. It was 2007. You give just soluble aggregated ovalbumin peptide intravenously that's a classic methodology to induce tolerance.

NOTE Confidence: 0.911726355552673

00:11:43.090 --> 00:12:13.780 And when you look at what happens when you then try when you look at tetramers that pick up the ovalbumin peptide presented by H2K of be you get a little blip and this is actually looking in the

lymph node appan. The first immunization and then it goes right down to Essentia Lee virtually not detectable if you try to immunize again, you basically do nothing so this is classic.

NOTE Confidence: 0.891905844211578

00:12:13.780 --> 00:12:40.340 Energic or exhausted teasel whatever terminology you want to use in contrast, if you block either. P DL1 or PD1. Not only do you get a much higher peak within the lymph node but you normalize out at a higher level and also you can re stimulate.

NOTE Confidence: 0.922690272331238

00:12:40.950 --> 00:13:02.870 So that shows 2 things one is that the PD 1 pathway. In addition to being important in that issue in the tumor also is playing a role in early T cell responses to Antigen and in particular, blocking that pathway.

NOTE Confidence: 0.907936632633209

00:13:03.410 --> 00:13:36.300 Can at the earliest stages partially reverse this energean I'll say that this? I prefer to use the term enerji in a case like this because this is not the classic exhaustion generated from chronic presence of antigen such as El CMV. But this is a case where you're looking at the first exposure to Antigen, but in the absence of appropriate coast simulatory signals.

NOTE Confidence: 0.871313214302063

00:13:36.300 --> 00:13:51.870 And a balance shifted to engagement of Co inhibitory signals like PD one. So you put that together and actually Max Krummel, Miriam rot have done some very elegant work.

NOTE Confidence: 0.905107319355011

00:13:52.570 --> 00:14:10.530 In image Ng tumor, draining lymph nodes and looking at the role of PD 1 pathway blockade there and so putting that altogether. Our current vision of how neoadjuvant therapy works.

NOTE Confidence: 0.904070973396301

00:14:11.500 --> 00:14:43.510 Is through potential blockade of the PD 1 pathway within the tumor draining lymph node where T cells normally recognizing tumor antigens presented by dendritic cells that pick them up in the tumor went to the draining lymph node is being blocked that now can partially break enerji tolerance.

NOTE Confidence: 0.914548993110657

00:14:43.510 --> 00:15:15.230 When the PD 1 pathway is blocked just as those experiments that we did, and leaping did that I showed you 12 years ago, those T cells and leave the draining lymph node and get into the circulation through the thoracic duct what we conventionally learned in medical school and then

from there. They actually circulate back to the tumor and into the tissue so when we see lymphocytes.

NOTE Confidence: 0.915128231048584

00:15:15.230 --> 00:15:34.460 That expand in number in after neoadjuvant anti PD one which was certainly one of the features this. In fact, we think now is really T cells. Following this pathway and trafficking back to the tumor.

NOTE Confidence: 0.926502168178558

00:15:35.300 --> 00:16:05.480 So, in that initial study that we did the results were really quite dramatic in that of 20 patients 9 patients or 45% had a pathologic major pathologic response defined as less than 10% of the viable cells in the tumor mass that the surgeon respects.

NOTE Confidence: 0.915618777275085

00:16:05.480 --> 00:16:27.360 Are actually viable tumor cells and Janice Top has done a lot of work in characterizing that more specifically a lot of characteristic fibrosis. Lots of lymphocytes necrotic tumor cells. It really does look different than neoadjuvant chemotherapy.

NOTE Confidence: 0.93641722202301

00:16:27.990 --> 00:17:01.140 But there are also certainly lots of patients that with anti. PD one alone don't really give you much of a pathologic response at all, so this gives us groups of patients that we can call pathologic responders or nonresponders. It's not the conventional radio graphic response. Although I would argue that this is actually a better measure of response becaus. The vast majority of these patients.

NOTE Confidence: 0.920120716094971

00:17:01.140 --> 00:17:33.040 Did not have a radiographic response and so until we start employing Anas imaging technology? When we see when we're looking at a tumor after therapy. We don't know how much of it really is tumor versus versus lymphocytes infiltrating in fact, one of these pathologic CRS so this is a patient in which the pathologist see no viable tumor.

NOTE Confidence: 0.928002059459686

00:17:33.040 --> 00:17:44.760 Was actually a patient in which the tumor on radiology? Slightly grew between initiation of anti PD? One therapy and 4 weeks later, which was the time of surgery.

NOTE Confidence: 0.033021617680788

00:17:45.320 --> 00:17:45.960 Um.

NOTE Confidence: 0.921005070209503

00:17:46.740 --> 00:18:16.890 So we're waiting this was a small group. There are a number of larger approval trials to see how pathologic response correlate

with ultimate outcome in terms of relapse free survival. But just for what it's worth. This cohort of patients has been doing very well. There have been 5 relapses four of the relapses are actually in.

NOTE Confidence: 0.903825402259827

00:18:16.890 --> 00:18:47.720 Nonresponders and interesting Lee Ann only one of the Relapsers was in a responder. Innopath responder patient. This is certainly not statistically significant just throwing it out there interesting. Lee, the relapse in the responder was Sala. Terry plural metastasis, which was treated about a year and a half ago with Artie and chemo.

NOTE Confidence: 0.915322721004486

00:18:47.720 --> 00:18:57.430 And has had no evidence of disease for the last year and a half. But certainly it's the larger studies that are going to tell us more so.

NOTE Confidence: 0.919915854930878

00:18:58.190 --> 00:19:30.440 So again the large amount of tumor that we get from surgery gives us a great opportunity to do single cell transcriptome mix and I'm going to also show you data from a platform that we've been using that 10X produces that combine single cell transcriptome mix and also T cell receptor sequencing. I should say that I now understand why the company is called 10X.

NOTE Confidence: 0.919479548931122

00:19:30.540 --> 00:20:01.570 Because um to use their system which is a great system. It costs 10X. What you actually have in your budget. But in any case, so one of the first things to look at so this is the dimensional reduction of the data is obviously a massive amount of data because you're essentially getting a whole genome transcriptome. IC profile from every cell originally people did use tiznit plots.

NOTE Confidence: 0.950574934482574

00:20:01.600 --> 00:20:23.820 Now really you map is a different dimensional reduction program, which is somewhat better in terms of the distances between 2 cells in 2 dimensions being more representative of their overall transcriptional connectivity or disconnectivity.

NOTE Confidence: 0.931322813034058

00:20:24.390 --> 00:20:54.440 I'm going to show you data from 6 of the patients were in the process of analyzing about 4 or 5 more patients. This is the merger of the 6 patients and these are the individual patients. These are the nonresponders. These are the responders and this is actually looking at PD. One expression in red scale. So the darker the red. The more PD. One is expressed in each of these individual cells an?

NOTE Confidence: 0.920739114284515

00:20:54.440 --> 00:21:25.030 When you look at the nonresponders verses of the responders. You can see similarities in the nonresponders an in the responders. But they're actually somewhat different both in their overall. You map, but also in their PD. One expression potentially suggesting that PD one expression in a responder may mean something different in PD one.

NOTE Confidence: 0.864178657531738

00:21:25.060 --> 00:21:29.200 Expression in a non responder.

NOTE Confidence: 0.919232130050659

00:21:29.840 --> 00:22:00.850 And indeed that's the case and so one can use an algorithm that looks at which jeans are most highly associated with PD. One expression and when you do that, you get a very different set of jeans. When you compare the responders of the nonresponders so this is a scale from 0 to one the more closely.

NOTE Confidence: 0.915850698947906

00:22:00.850 --> 00:22:30.300 A light or corresponding in particular, gene is with PD one. The higher the number. So so PD. One always gets 1 because it's perfectly correlated with itself. Obviously so now if you now when you start looking at the most highly PD. One associated jeans in the non path responders versus the pathologic responders. It's really quite striking.

NOTE Confidence: 0.910248517990112

00:22:30.920 --> 00:23:03.370 If you look at the Top 10 most highly PD, one associated jeans in the non responder. Seven of these are in fact, either classically associated with T cell exhaustion or have been shown in the literature to be to encode inhibit T cell inhibitory molecules so you can see number one most PD one associated gene in the nonresponders.

NOTE Confidence: 0.90601372718811

00:23:03.370 --> 00:23:35.000 Is in fax is in fact a talks 2nd? Is actually Tim 3? This is CD 39 if you look at the Top 10 in the responders you actually don't see any of these in fact, on this whole list. The only one that actually comes up is CTL I4 by the way. I forgot to mention also on this list coming in at #25.

NOTE Confidence: 0.908469974994659

00:23:35.020 --> 00:24:06.310 Is also lack 3 so this is a very different picture and essentially only in the nonresponders are the PD one associated jeans associated with T cell inhibition or exhaustion. There are some interesting jeans here, including PGK, one so actually metabolism. Does come up when we start looking at these signatures.

NOTE Confidence: 0.945305526256561

00:24:06.310 --> 00:24:07.570 In more detail.

NOTE Confidence: 0.0343124270439148

00:24:08.090 --> 00:24:08.790 Um.

NOTE Confidence: 0.909049332141876

00:24:09.530 --> 00:24:40.170 There are indeed more T regs as determined by Fox P3 in the nonresponders than in the responders in the U map basically the T regs represent this Peninsula coming off the overall CD 4 population. So basically the bottom half of the you map. Turns out to be the CD 8 cells and the Top half.

NOTE Confidence: 0.916288435459137

00:24:40.170 --> 00:25:10.350 Of the you map turns out to be CD 4 cells and the tier egg. Cells are this Peninsula here, but not only do you see about 3 to 4 times more tier egg cells in the nonresponders but there is also this non responder specific subcluster that you actually don't see in the responders at all. Now there's been a couple of ways that we've analyzed this.

NOTE Confidence: 0.909047961235046

00:25:10.350 --> 00:25:40.880 Just Stena cow she is the person who's done all the great work on this on the wet bend side and then hung kaiji and xiang have been doing the bioinformatics but one of the things that JA did was she went back and pulled out about 78 jeans that from the literature had been associated with.

NOTE Confidence: 0.913383007049561

00:25:40.880 --> 00:26:11.110 T Rex, an A lot of these jeans. Indeed, when you look at the you map plots of the single cell analysis are more highly expressed in T regs. But she was specifically looking for jeans that were expressed at reasonable levels in the nonresponders, but we're not expressed in the T regs of the responders and there were two that sort of jumped out.

NOTE Confidence: 0.90327125787735

00:26:11.110 --> 00:26:41.550 One is actually Garp and you can see so these are the nonresponders and these are the responders interesting. Lee this is an amalgamation of single cell analysis from lung cancer resections that didn't receive neoadjuvant therapy actually interesting. Lee these are untreated they in many ways tend to look like the nonresponders and.

NOTE Confidence: 0.885940551757813

00:26:41.550 --> 00:27:12.760 Look different than the responder's in the same way that the nonresponders so Garp UC sprinkled quite significantly throughout the T regs and largely is the T Reg Group. Whereas there only about 3 cells among all the tier eggs in the responders. The Torghar positive. The other one is EB I3, an interesting Lee Greg when we

NOTE Confidence: 0.90178394317627

00:27:12.760 --> 00:27:42.850 Looked for P-35 or P28. We didn't see it in the single cell analysis single cell analysis doesn't go all that deep so we're actually think this week. Arbor is actually doing QRT PCR to look at at the sorted T Rex, but these are two of the jeans that stood out as being selected for the.

NOTE Confidence: 0.889430642127991

00:27:42.850 --> 00:28:14.140 For the nonresponders within the T Rex just to remind people that EB I3 originally identified as one of the two subunits of the aisle 12 family. I'll 27 but Dario's lab and actually Greg was involved in looking at.

NOTE Confidence: 0.863240897655487

00:28:14.140 --> 00:28:28.130 In identifying the receptors for second EB I3 containing cytokinin, which pairs with Kyle Twelves P-35.

NOTE Confidence: 0.887066185474396

00:28:29.060 --> 00:28:59.310 Garv is a very interesting cell surface receptor, which is highly expressed. Auntie regs and turns out to actually bind the latent form of or TGF beta latency associated protein which binds TGF beta on the surface of the T Rex L inactive form and it's only released.

NOTE Confidence: 0.874837875366211

00:28:59.310 --> 00:29:30.820 When when you have the binding to certain integrants without Garp basically tier egg cells can't release bio active TGF beta and actually a recent paper from Z. Hailes lab with knockouts, indeed showed that knockouts have higher levels of tumor immunity because Garp sustains the function.

NOTE Confidence: 0.910731852054596

00:29:30.820 --> 00:29:45.830 An accumulation of Regulatory T cells that's probably because TGF beta is not only inhibitory to target cells, but it also feeds back in and autocrine fashion on T Reg expansion.

NOTE Confidence: 0.916806578636169

00:29:47.890 --> 00:30:18.080 Another way we can look at differences and T Reg cells between responders and Nonresponders is to sort of look at the whole populations. In particular of interest to compare? What are differences in gene expression globally between the T Rex that you see in the responder versus specifically this non responder T Reg cluster.

NOTE Confidence: 0.927690923213959

00:30:18.080 --> 00:30:52.510 That you don't see in the responders and there are many differences, but one of the pretty amazing. Things that jumped out at us when comparing C versus A is this very, very high expression of multiple core

stress induced proteins in the non responder specific T. Reg population and so this raises the question of is this something that selective to the T Rex Cells.

NOTE Confidence: 0.91387403011322

00:30:52.540 --> 00:31:22.550 Or is this a more general phenomenon. An lo and behold, it is indeed more general so this is looking at HSP A1A and this is looking at HSP A1B. HSP A1A is actually HSP 70. You can see just by the red color that there are many more cells and much higher level.

NOTE Confidence: 0.93022096157074

00:31:22.550 --> 00:31:52.900 In the nonresponders this is the integration of the nonresponders. This is the integration of the responders and basically I could show you virtually these kinds of plots for virtually all of the core stress induced proteins. An they would essentially look the same and this is just breaking it out into the individual nonresponders responders. Nonresponders responders to show you that this difference is not just driven by one patient.

NOTE Confidence: 0.892359256744385

00:31:52.900 --> 00:32:26.050 But is really quite consistent so stress comes up and were beginning to try to Deconvolute. What kinds of stress. And in fact, hypoxic stress as well as reactive oxygen stress oxidative stress seemed to certainly be coming up and all hearken back to gregs.

NOTE Confidence: 0.941415905952454

00:32:26.050 --> 00:32:59.160 Presentation on this and in fact, if you just simply do a correlation coefficient or correlation plot between percent residual tumor and percent of the T cells that Express' HSPA, one above standard threshold. This is the normal associated long and there's really not much of a correlation coefficient. But even with this relatively small number of patients.

NOTE Confidence: 0.922092139720917

00:32:59.290 --> 00:33:29.580 The Association within the tumor infiltrating lymphocytes gives a correlation coefficient of .88 with a P value of .0037. So this is really was unexpected. But very striking correlation with non responsiveness. I'll just show you 1 interesting protein downstream of the core stress.

NOTE Confidence: 0.92809921503067

00:33:29.580 --> 00:34:00.140 Pathway, which is called Cesar in one, which we see much more highly expressed in CD4 cells in the non responder very little expression in the CD 4 cells in responder's an interesting. Lee we found a paper published in 2017 basically showing that it's regulation of the Earth Junk P38 map kinase activation complex in fact, Inhibits.

NOTE Confidence: 0.882848262786865

00:34:00.140 --> 00:34:23.760 Immunity in aged T cells so we think that these stress T cells really are not working as well. Presumably that may be part of

why these patients don't respond be cause. They are exposed to stress.

NOTE Confidence: 0.924006819725037

00:34:24.380 --> 00:34:55.490 One can do now, a clustering within the single cell analysis. I won't go into the methodology's. The particular methodology's that are bioinformatics, folks like is called FINA graph. There are a number of ways to do it and these different colors represent the different clusters. This is all of the responders nonresponders and also untreated.

NOTE Confidence: 0.934492886066437

00:34:55.490 --> 00:35:29.080 Together, but one can now look at these different clusters and look at their proportions in responders versus nonresponders versus untreated and what you see is that there are some major differences in some of the clusters and I'll simply point out cluster 8 and cluster 17, so cluster 8. The red bars are the responders so these are much more these cells are much more highly represented in the responders.

NOTE Confidence: 0.897115051746368

00:35:29.080 --> 00:35:59.750 Then in the nonresponders and as I mentioned the untreated's tend to track with the non response. Actually, this is the non responder. In contrast, cluster 17 is these are again within the CD. Eights is expressed represents of quite a high proportion of T cells in both the nonresponders also in the untreated.

NOTE Confidence: 0.905080080032349

00:35:59.750 --> 00:36:30.150 Anne is virtually absent in the responders So what are driving these clusters so in the responder cluster cluster 8 you see a number of markers of activation including mcy Class 2 expression, but also the most highly upregulated is one of the granzymes grandson.

NOTE Confidence: 0.923936486244202

00:36:30.550 --> 00:37:02.770 In the nonresponders you see a lot of the stress proteins. Other interesting molecules, but this other highly upregulated molecule are for a one we wouldn't have known what to make of it until relatively recently when Shandong published a paper using a genome wide analysis identifying identifying NR 4A. One is a key mediator of T cell dysfunction.

NOTE Confidence: 0.898904979228973

00:37:02.790 --> 00:37:34.720 A couple months after that, Angela row similarly identified in RFA, one and talks as being major mediators of T cell dysfunction, so one actually begins to see in looking at sort of real tumors post therapy patterns that begin to teach us. At least at the T cell level? What are differences associated with response?

NOTE Confidence: 0.829988539218903

00:37:34.720 --> 00:37:39.570 Or non response now one of the.

NOTE Confidence: 0.925017356872559

00:37:40.460 --> 00:38:10.950 Things that I've always been concerned about with regard to all of these single cell papers on tumor. Infiltrating lymphocytes is that there is a lot to suggest that vast majority of tumor inflate infiltrating lymphocytes are not specific for the tumor. There just sort of passing through an I was actually interested to see an amazing turtle.

NOTE Confidence: 0.89416778087616

00:38:10.950 --> 00:38:41.820 Horse paper from Singapore group that try to identify with tetramers among till tumor. Neo Antigen specific T cells, an in 40 patients they tested 1100. Neo Antigen tetramers and were able to actually see a signal with two I don't want there were a lot of names on that paper.

NOTE Confidence: 0.891857445240021

00:38:41.940 --> 00:38:51.910 And I would not have been wanted to be one of the post. Docs that was working on some of the negative tetramers, but

NOTE Confidence: 0.951186716556549

00:38:52.880 --> 00:39:07.260 But I think that's really an issue and so I'm going to present an alternative approach to be being able to really specifically look at Mutation Associated Neo Antigen specific T cells.

NOTE Confidence: 0.889804780483246

00:39:07.760 --> 00:39:39.630 We call them manner, so just to remind people about how diversity is generated and T cell receptors as well as in Munich Globulins. Really, the business and after VDJ recombination and N region diversity generation between the V and the D and the D end of the J is essentially this region here.

NOTE Confidence: 0.923077404499054

00:39:39.630 --> 00:39:48.020 Which contains all the diversity information and that's the so called Cdr 3?

NOTE Confidence: 0.934278964996338

00:39:48.840 --> 00:40:20.240 And that at the nucleotide level. There are 10 to the 8th different. Cdr threes that these 2 mechanisms can generate per chain, which means that if you were to take all of the naive T cells in your body and sequence, the Alpha and beta chains. You would not ever come up with the same sequence twice, but obviously you do see lots of sequence is repeated.

NOTE Confidence: 0.912900626659393

00:40:20.240 --> 00:40:51.390 And those by definition represent T cells that have seen their antigen. Anna have expanded that in fact expansion of T cells is the single commonality in T cells when they recognize antigens. So verti cell becomes activated to an effect or sell it. Obviously expands but they'll be a

smaller expansion button expansion. Nonetheless, even if that T cell is on the way to Enerji.

NOTE Confidence: 0.906315922737122

00:40:51.390 --> 00:41:21.300 For exhaustion then I'll like him back to those papers. I showed you from 2007, where even in the Energic T cells. There was that blip that one can see so that's essentially what deep sequencing of the Cdr 3 regions. We call TCR seek this was pioneered by a company called adaptive. There are also too expensive so we now have an in-house version.

NOTE Confidence: 0.918855011463165

00:41:21.530 --> 00:41:54.270 But the beauty of this is not only do you get these pictures of clonal size but you also each Cdr 3 Essentia Lee represents an endogenous bar code for that T cell clone that you can use to follow it in various tissues time points in the blood, etc, etc. So taking advantage of this Kelly Smith Anne Frank Russo came up with a nifty as they called manifest.

NOTE Confidence: 0.930811166763306

00:41:54.390 --> 00:42:13.150 Which Sam stands for mutation associated Neo Antigen functional expansion of specific T cells Needless to say we came up with it because it's got a catchy acronym. It's actually relatively straightforward. It's much more sensitive and specific than Anneli spot.

NOTE Confidence: 0.900353312492371

00:42:13.660 --> 00:42:45.130 Fairly simple steps you whole exome sequence you put your mutations through a conventional MHC binding algorithm. We use variant of net. MHC pan for you, then take your Top predicted peptides. Anybody who's done that realizes that this is highly imperfect, but it gives you sort of the Top possibilities.

NOTE Confidence: 0.905739426612854

00:42:45.130 --> 00:43:15.420 He then just synthesize those peptides. You do a one step simulation. But instead of analyzing cytokine production. As you would with a Nelly spot. You analyze using TCR seek clonal expansion and the beauty of this in terms of specificity is that if you're testing 50 peptides and you see a given clone expanding peptide 7 then.

NOTE Confidence: 0.919188320636749

00:43:15.420 --> 00:43:45.510 For that clone peptide one through 6 and peptide 8 through 50 are your negative controls so we see a lot of non specific expansion of various clones and you see these in multiple wells, but only when you see it in one well. We have a statistical algorithm that we used to determine the specificity, but only then do we call it specific?

NOTE Confidence: 0.917769908905029

00:43:47.440 --> 00:44:18.570 So with this and again a lot more sensitive. We've been picking up more and more T cell responses against oncogenic mutations. If you go to the literature. You read papers from folks like Steve Rosenberg from Tom Schumacher Rosenberg has one or 2 cases where he's found it but you would think that these were actually very, very rare responses against thyaga genic mutations.

NOTE Confidence: 0.931818425655365

00:44:18.570 --> 00:44:49.660 It turns out there are actually much more frequent than had been a previously appreciated when you use a more sensitive asset so this is an example in one of the lung cancer patients very interesting, Lee where we picked up a positive these are 3 different T cell clones. These are 2 different T cell clones above the background against A10 Marana Ninemire, both incorporating this particular patient.

NOTE Confidence: 0.900020718574524

00:44:49.660 --> 00:45:19.670 Uncle genic B RAF Mutation N, 581 I which in lung cancer. Turns out that only half of the B RAF mutations. RV 600, E so this was one of the others that clusters way will be proud of the fact that I'm becoming more and more of a lung cancer doctor. I almost sound like I know what I'm talking about, but don't ask me too. Many detailed questions. 'cause then I'll refer you to Julie or Patrick but in any case.

NOTE Confidence: 0.915111780166626

00:45:19.670 --> 00:45:38.100 We're we're seeing this more and more and I think that's very interesting in terms of the thinking about the ability to generate T cell receptors that you can use for adoptive transfer that are specific for common oncogenic mutations.

NOTE Confidence: 0.916428983211517

00:45:38.670 --> 00:46:09.920 So what one can do with this assay then is actually very cool. Given this single cell platform that allows you to simultaneously. For every cell not only do the transcriptome, but also look at the Cdr threes for both the Alpha and the beta chain of that T cell So what you can do is you can take your.

NOTE Confidence: 0.897809743881226

00:46:09.920 --> 00:46:41.030 Manifest validated T cell clones and again using this bar code and look at the subset of till that you know are specific for neo antigens in the tumor and sort of pull those out from the Sea of other T cells. And this is useful as I mentioned for potentially finding and cloning out T cell.

NOTE Confidence: 0.909749090671539

00:46:41.030 --> 00:46:55.750 Receptors for specific aquagenic drivers, but also discovering jeans associated with dysfunction versus reactivation IE in the responders after checkpoint blockade.

NOTE Confidence: 0.92831963300705

00:46:56.520 --> 00:47:27.810 So this is an example of a complete responder patient from the actually from England Journal study that was published last year. This is an example of 3 clones. It actually turns out that these 2IN frame. TCR betas are from the same T cell. Clone we found out that actually happens in about 1% of T cells that you actually have 2IN frame betas.

NOTE Confidence: 0.863823592662811

00:47:28.100 --> 00:47:58.760 And you even more frequently you can actually find 2IN frame Alphas. But so this is really 2T cell clones present in quite high frequency pre anti. PD one treatment in this adjutant patient interesting. Lee went down in the tumor appan reception. This recent paper talking about clonal replacement.

NOTE Confidence: 0.924624145030975

00:47:58.760 --> 00:48:29.090 I'm not so sure it's not just clonal dilution, but interesting Lee in the tumor draining lymph node. These clones are present at quite high levels and that's at least concordant with the notion that there is stimulation of tumor. Neoantigens specific clones. That's going on. Not in the tumor. But in fact, in the tumor draining lymph node and if you look in the peripheral blood.

NOTE Confidence: 0.935633540153503

00:48:29.090 --> 00:49:00.260 And we see this commonly between 2 and 4 weeks after treatment. This is actually days relative to surgery. The treatment starts 4 weeks before surgery in this trial so this is pre treatment. This is 2 weeks after initiation of treatment and this is right before. This is day of surgery and then they come down and we can postulate potentially that what's happening here is that cells are now beginning to.

NOTE Confidence: 0.91377717256546

00:49:00.260 --> 00:49:09.940 Exit the peripheral blood and circulate through the tissues and hopefully finding micro metastases so.

NOTE Confidence: 0.852830171585083

00:49:13.000 --> 00:49:43.030 Justina Kashi with help from Emily Zhao in Burg Vogelstein's group or actually turning Burt into a cancer immunologist. So my vision of world domination of cancer immunotherapy is in fact, coming true So what she did was to take a jerk at as a Reporter line.

NOTE Confidence: 0.893958330154419

00:49:43.030 --> 00:50:13.960 Crisper out the endogenous Alpha and Beta Jeans Jurkat still expresses the CD threes put in CD8 'cause. This is all looking at MHC class. One restricted responses and in fact, driven luciferase to make it

an easy read out in the old days. We used to look at, I'll 2 production by jerk at as a measure of TCR so this is essentially just read out for.

NOTE Confidence: 0.902783870697021

00:50:13.960 --> 00:50:46.610 Recognition of the peptide and this is just a test run with a pair of T cell receptors from and Edna for NP specific T cell clone and you can see when you use evona for NP peptide you see a nice dose response curve? When you look at luciferase expression and then this is Edna, too, as negative control. This is a very easy very clean essay.

NOTE Confidence: 0.901615679264069

00:50:46.640 --> 00:51:12.580 And actually after taking all the trouble to make these lines and everything. This actually can be done in very high throughput actually with electroporation. She can basically query 96. TCR pairs at a time so this is really getting to be a reasonably high throughput approach.

NOTE Confidence: 0.927756905555725

00:51:13.080 --> 00:51:44.530 So for this particular clone. This is the dose response curve so this is the final formal 100% proof that this clone really is specific for this neo antigenic peptide 'cause. This is taking the Alpha invaders out and reconstituting the jerk at one interesting question is what is the functional avidity? Which is not the best term in the world, but it's essentially the dose response curve against Neo Antigen.

NOTE Confidence: 0.889268159866333

00:51:44.600 --> 00:52:16.250 Versus sort of the ultimate memory affect your response, which would be to a viral antigen such as EBV an interesting. Lee, the functional avidity again measured by the dose response is. Almost identical, which is really not what I would have predicted but what it says is that there are these tumors civic T cells.

NOTE Confidence: 0.893471837043762

00:52:16.250 --> 00:52:30.710 Have pretty reasonable T cell receptors. It's not like these really wimpy low affinity T cell receptors that you can often see against auto antigens.

NOTE Confidence: 0.92203015089035

00:52:31.490 --> 00:53:02.750 So what happens now when you overlay. These clones on the you map plot for this particular responder patient and what you see. And so the red triangles are one of the clones and Blues. There were fewer of these I can tell you to see these clones took 80,000 cells on a single cell and if anybody here uses 10X you're probably gasping.

NOTE Confidence: 0.915233671665192

00:53:02.750 --> 00:53:33.790 I guess when I saw the bill, but in any case about 50% of these clones. You can see our clustering in this one cluster in this patient

cluster 16 and the clustering map So what is this cluster? What are these clones and these turn out to be this cluster is the tissue resident memory T cell cluster so about half.

NOTE Confidence: 0.908894300460815

00:53:33.790 --> 00:54:04.150 Of these clones are classic TRM's CD103 CD 69 CX CR6. They Express' Hobbit, which is the transcription factor that maintains TR ends in their sort of poised state. This got me very excited because I'm a Tolkien fan and I always wanted to work on The Hobbit so particularly exciting for me, but the question is what about.

NOTE Confidence: 0.912510216236115

00:54:04.150 --> 00:54:36.030 These other clones in different places and actually these have signatures of acutely activated T cells. And when you actually use these programs like fate map, which is version of so-called pseudo time, which gives you can activities. You can actually see connections that go from this sort of resting TRM cluster.

NOTE Confidence: 0.903360486030579

00:54:36.030 --> 00:55:07.520 2 into activated cells as a continuum So what about clones and Nonresponders. So I'll show you one example of that? what I can tell you is that when we do. The manifest analysis. We see as many look in the till we see as many Neo Antigen specific cells in the nonresponders.

NOTE Confidence: 0.927941203117371

00:55:07.520 --> 00:55:38.370 As in the responders which to me is actually very exciting because it says that in non responding patients. It's not like they don't have T cells. So we can figure out what's really going on in the nonresponders. The substrate of tumor. Reactive T cells is in those patients so to me again. That's actually very exciting at least in lung cancer and but I think we're going to be able to obviously query a lot of cancers.

NOTE Confidence: 0.0366096496582031

00:55:38.550 --> 00:55:39.220 Um.

NOTE Confidence: 0.910069167613983

00:55:39.780 --> 00:56:04.870 So this was a non responder interesting. Lee this particular T cell Clone, which again you can see clustering here fact. This particular cluster. You see here and sort of winds up here. This is this orange cluster here, which was called just cluster 0.

NOTE Confidence: 0.927103817462921

00:56:05.940 --> 00:56:36.670 This particular clone also validated through the jerk. It transfer actually sees a hotspot P 53 Mutation again. This is a non responder. So just because the T Cell Clone? Is there doesn't mean the patient is going to have a response so the question is what is the transcriptomic profile

of this patient and these are some of the Top jeans here and what's interesting is that?

NOTE Confidence: 0.898229956626892

00:56:36.670 --> 00:56:42.580 It doesn't parse out into a simple.

NOTE Confidence: 0.919635713100433

00:56:43.100 --> 00:56:57.860 Definition as per the literature, it's somewhat of a combination are a mix of tissue resident memory. It does have Hobbit it does have CD 103.

NOTE Confidence: 0.919874012470245

00:56:58.990 --> 00:57:29.380 Together with activation ready or activated you see the Grand Zymes that are up and this is relative to all CD8 cells. You see that HLA Class 2 is up. Perforant is up. But you also see something that you didn't see at all in the manage specific T cell clones that I showed you from the responder patient, which is now a number in red shown here.

NOTE Confidence: 0.911494016647339

00:57:29.380 --> 00:58:01.690 Of exhaustion molecules, including lag 3 CD 39 and also it's interesting because PD one comes up on this which says that the levels. It's not these aren't the only cells that are PD. One positive but it says that the levels of PD one on this on these cells are higher than when you take the sum total comparat are of all CD8 cells, which again fits with the notion that exhausted.

NOTE Confidence: 0.872519433498383

00:58:01.690 --> 00:58:16.540 Sells sort of lock on very high levels of PD one and you also see a one of the heat shock proteins coming out HSP A1B.

NOTE Confidence: 0.382065951824188

00:58:17.060 --> 00:58:18.410 So.

NOTE Confidence: 0.940169751644135

00:58:18.960 --> 00:58:49.850 So I think what the picture that's emerging here is that and again. This is just looking at the T cells. So one could argue it's like you know trying to look at the elephant and we're focusing just on the trunk or just on the left ear, but it's certainly an important piece of the anti tumor immune response. We're seeing are some patterns that at least give us a potential working model.

NOTE Confidence: 0.9186772108078

00:58:49.850 --> 00:59:20.690 For differences going on in T cell transcriptional programs for responders versus Nonresponders and I'm going to propose that a

lot of this has to do with whether or how much stress exists in the tumor microenvironment again. There's hypoxic stress oxidative stress, ER stress metabolic stress.

NOTE Confidence: 0.92695939540863

00:59:21.510 --> 00:59:42.500 This is something we're just beginning to parse out, but certainly we see this as a very strong signature selectively in Nonresponders and I showed you some examples that leads to energic or exhausted or dysfunctional T cells.

NOTE Confidence: 0.887597441673279

00:59:43.090 --> 01:00:14.420 There are some hints that glycolysis is lower just looking at some of the levels of jeans associated that encode Enzymes link to glycolysis checkpoints are up exhaustion transcription factors like talks are also up. You also saw nab one which will remember from his PhD thesis.

NOTE Confidence: 0.893028914928436

01:00:14.420 --> 01:00:33.330 And you see an increase in T Reg number as well as jeans. Encoding suppressive factors that allowed tier egg cells to suppress eficient. Lee we're obviously going to have to go back in fact.

NOTE Confidence: 0.885957300662994

01:00:34.410 --> 01:00:53.210 Where is E during your talk? I actually texted zsa Zsa to look at the lactate transporters that's a great thing about having these great. Bioinformatics colleagues as you can text them and usually within 2 hours, you get an answer back and you've got a new discovery.

NOTE Confidence: 0.908018410205841

01:00:54.840 --> 01:01:25.230 In contrast, if you have a low stress environment when you block PD one if you have enough T cells. You get productively activated T cells again. We think they start in the lymph node and then ultimately make their way back to the tumor. They traffic to the tumor where a lot of them, take up a tissue resident memory transcriptional program.

NOTE Confidence: 0.875555336475372

01:01:25.230 --> 01:01:55.500 But then can also with increase activation like Alesis and cytotoxic machinery can turn into an active killer cell and ultimately kill the tumor. Obviously much more to be done, but working model that some of this profiling is allowing us to do so.

NOTE Confidence: 0.911880671977997

01:01:56.290 --> 01:02:26.340 I want to thank certainly the patients who have been great are collaborating clinical trial centers in particular, Sloan Kettering for the trial behind the data that I showed you support from number of groups certainly tremendous thanks to Sidney Kimmel and the Bloomberg Foundation actually this was taken a few years ago.

NOTE Confidence: 0.897544682025909

01:02:26.410 --> 01:02:37.540 We need a wide angle lens now because everybody in the Kimmel Cancer Center in Hopkins is an immune a therapist, thank you.

NOTE Confidence: 0.811911523342133

01:02:46.250 --> 01:02:53.490 I have a quick question about the oncogenic Benz.

NOTE Confidence: 0.858117818832397

01:02:54.090 --> 01:03:08.820 And whether you saw specific patterns and the types of oncogenic mutations that you were detecting for which you are detecting responses in your study so.

NOTE Confidence: 0.890503227710724

01:03:09.880 --> 01:03:11.260 We don't.

NOTE Confidence: 0.922566056251526

01:03:11.920 --> 01:03:41.230 Yet see an obvious pattern other than the fact that we can find T cells. More frequently than we had expected now again in some cases, they're relatively infrequent and it's just because we have a very sensitive assay to pick them up. But as of now, and again this may just be numbers. There's no obvious pattern that jumped out.

NOTE Confidence: 0.914072096347809

01:03:45.040 --> 01:03:59.680 That was a beautiful talk, I wanted to ask you how much of the nice gene signatures that you see in responder versus nonresponders within the tumors environment? Can you detect in the peripheral blood of these patients?

NOTE Confidence: 0.790359914302826

01:04:01.030 --> 01:04:02.610 Yeah, so.

NOTE Confidence: 0.943021059036255

01:04:03.440 --> 01:04:17.860 The problem with peripheral blood and you actually see this when you follow the frequencies of Maná specific T cells is that they are extremely deluded.

NOTE Confidence: 0.900140106678009

01:04:18.420 --> 01:04:48.550 An so I have to say we haven't looked with single cell analysis because of the frequency. You can find these as you saw you can find these manage specific T cells by the bar coding. But I don't know if you noticed the Y axes for frequency among the till versus the PBL, but there are 100 to 1000 full lower that said.

NOTE Confidence: 0.900649964809418

01:04:48.550 --> 01:04:51.230 My neighbor Jonathan Powell.

NOTE Confidence: 0.890298306941986

01:04:51.950 --> 01:05:20.540 Is working on? What looks like it could be a more of a global metabolic signature so actually we haven't looked but my prediction is that these are so environments specific that I don't know that we would would necessarily see them and I just think the purple blood is the relevant T cells are just so diluted out.

NOTE Confidence: 0.744986951351166

01:05:23.350 --> 01:05:25.220 So great stuff drew?

NOTE Confidence: 0.874347567558289

01:05:25.730 --> 01:05:36.660 In the one of the strongest coral. It's with PD one in the responders was RBPJ Kappa. So what's going on with notch in the responders.

NOTE Confidence: 0.767443656921387

01:05:37.980 --> 01:05:40.040 Yeah.

NOTE Confidence: 0.919548392295837

01:05:40.680 --> 01:06:11.910 I don't know, but anybody who has some good ideas for us to follow up. We certainly need to obviously look at downstream notch regulated jeans an that particular one. I think it's just a subset. It's not all of the not related sort of an offshoot. It's something that I have to read more about. But if you have an interesting ideas were really Needless to say notch is.

NOTE Confidence: 0.889028489589691

01:06:11.910 --> 01:06:43.700 Not uninteresting this also actually came up in the pee pee in the responder when you actually looked at the Maná specific cells. I don't know if I had it on the list, there, but it's on sort of the list of the Top 30 or 35 jeans. There so it's not just globally but actually when you look at the Maná specific clone within the in the responder so please any.

NOTE Confidence: 0.927900373935699

01:06:43.700 --> 01:06:46.730 Any interesting thoughts in terms of following that up.

NOTE Confidence: 0.931162655353546

01:06:47.330 --> 01:07:06.630 In your model you very nicely showed the movement to the lymph node and I wondered whether you were also thinking about the possibility of tertiary lymphoid tissues in the tumor themselves, which and some cases have predicted better outcomes better prognosis.

NOTE Confidence: 0.909182906150818

01:07:07.950 --> 01:07:38.820 Yeah, that's a really good question. You do get you do see tertiary lymphoid structures in lung cancer. We are actually working

with a CD and actually there was one recent paper that can actually do RNA scope specific for the Cdr threes an when you actually combine it. The Alpha CR3 in the beta Cdr 3, you can actually get some.

NOTE Confidence: 0.920284330844879

01:07:38.820 --> 01:08:06.780 Some real specificity, we've actually piloted it with them. It actually works impressively well. Sometimes it takes some tweaking so that's so I'm hoping to actually have that answer for you soon. It's actually it's at the Top of our list of interest in terms of where we're interested to look to be able to be able to find these clones in sections. Thank you.

NOTE Confidence: 0.89127790927887

01:08:10.140 --> 01:08:40.370 Really nice talk through the you talked about climate type frequency and it looked like you had paired pre therapy and at 4 weeks. Time point samples that you have single cell RNA seq with TCR evaluation is that right now, so the untreated are a separate group. Now we have gotten better at actually doing single cell on pretreatment bronchoscopic biopsies, so we have about 3 cases now.

NOTE Confidence: 0.898799896240234

01:08:40.370 --> 01:09:08.900 In the expansion so we will be able to have pre and post. But those are just lung cancer resections from patients that didn't get any new agentry well. I guess related to that was wondering was if you look at the clona types that are expanded at the four week time point. If you have any of those relative to the pre therapy. Do they have a higher frequency of tumor reactivity or anything like that with the therapy? Do you actually enrich for 2 more reactive T cells?

NOTE Confidence: 0.94104140996933

01:09:09.760 --> 01:09:40.710 Yeah, so very good question and there are some interesting patterns that we see when we take sort of the most highly represented clones. One of which is that the expansion of those clones that we find in the periphery between week. Two and week. Four is actually very highly correlated with response when we've actually.

NOTE Confidence: 0.909078180789948

01:09:41.800 --> 01:10:14.430 Looked with the manifest validated clones in the ass a we don't see them in those those very common clones. We do see some clones because we always do in eabf flu positive control in the manifest as we do see some of those now that doesn't mean that the man, a specific cells aren't in there, because we the statistics that we do for the essay.

NOTE Confidence: 0.9254270195961

01:10:14.440 --> 01:10:41.760 Is set up to have as low as possible of false positive rate but I'm sure we have a lot of false negative rates. With this jerk. It transfer actually what we are going to do is simply take the Top clones and just do the

transfer and then just test the peptides that might actually turn out to be easier to see but right now, we haven't seen overlap.

NOTE Confidence: 0.920092165470123

01:10:43.310 --> 01:11:13.660 One of the therapeutic modalities that has worked in cancer is to amplify the tumor infiltrating lymphocytes from the tumor and in your data, it looked like there were just as many tumor reactive T cells in the adjacent normal lung than in the tumor itself and I'm wondering if that's the case and you also talked about that they might be in the adjacent limp node and then home to the tumor when they are activated in the checkpoint blockade situations, so should we be getting draining lymph nodes instead of tumor.

NOTE Confidence: 0.846909582614899

01:11:13.660 --> 01:11:16.090 When were isolating tumor reactive T cells.

NOTE Confidence: 0.901766002178192

01:11:17.400 --> 01:11:48.120 Absolutely absolutely and you do indeed find so the example. I showed you of the responder. I didn't talk about it. But if you notice that you could find those clones in the normal one just as he said. Sharp eyes and they also clustered you know the majority of them similarly clustered in that TRM cluster an one of the things that's come up.

NOTE Confidence: 0.926085650920868

01:11:48.120 --> 01:12:18.250 Actually, we're going to ask the patient for a skin biopsy because 1 question is some of this somewhat tissue. Specific there are now there is data and experimental models that there are tissues. Specific components of the TRM program so that's something we're actually very interested in looking at that.

NOTE Confidence: 0.795754432678223

01:12:18.270 --> 01:12:19.410 Question.

NOTE Confidence: 0.790915071964264

01:12:20.650 --> 01:12:25.760 Well let's think doctor part all for a really great talk.