

WEBVTT

NOTE duration:"00:32:49.7630000"

NOTE language:en-us

NOTE Confidence: 0.895629823207855

00:00:00.580 --> 00:00:12.040 Alright so our next speaker is here from Yale. It's David rim. He's a professor of pathology in Madison and he'll be talking today about his work also in the area of visualizing tumor immunity.

NOTE Confidence: 0.175548627972603

00:00:20.180 --> 00:00:21.530 See.

NOTE Confidence: 0.885797739028931

00:00:33.960 --> 00:01:06.570 OK, great, thanks for inviting the honor to be part of this session as as a pathologist to be in a session on Immuno Oncology makes me a little nervous too. But thanks for inviting me and I look forward to sharing my approach to visualising tumor immunity in response, unlike doctor, Ru R resolution isn't in the millimeter ranges in the Micron range, but also unlike Doctor Who gave a beautiful talk. I can't get one piece of that issue. I don't get time courses and I don't get.

NOTE Confidence: 0.887018144130707

00:01:06.570 --> 00:01:37.880 Maybe when we start doing new advent or on treatment biopsies. I'll do more than that. But right now. We just get one piece of tissue so I want to start with my disclosures. These are the people that fun work in my lab and the people with whom I consult and this is kind of a long list. So I don't breeze through it in one second, but I want to share also that these are my partners because generally what I do in my lab can't become actually help patients and less there're commercialization avenues and these are some of the avenues for commercialization.

NOTE Confidence: 0.913672506809235

00:01:38.050 --> 00:02:10.830 Is what I want to talk to you about today? I want to talk to you first about the current assessment of visual how we visualize tumor immunity in the clinic today and that is P. DL1 HC I'm only talked about that briefly it's actually quite a nightmare, but I'll update you a little bit on where we are with that and then I want to talk about what are these things called immune cells which are an important part of the visualization and actually a criteria for response to therapy and breast cancer and then I'll talk to you about new tools that we have high Plex discovery tools.

NOTE Confidence: 0.911780834197998

00:02:10.830 --> 00:02:33.020 And our approach in my lab, which is to sort of not start with the immunology and go forward to drugs and diagnostics, but rather to start with the patients tumors have been treated with drugs and then

go backwards to try to find affects using high. Plex methods to look for potential diagnostics or mechanisms by which we can enrich the population, giving giving those drugs too.

NOTE Confidence: 0.878030717372894

00:02:34.200 --> 00:03:05.750 So this is where we stand today and there are multiple diagnostic tests. All of which are associated with different. Therapeutics made by different companies and the therapeutics are shown here it as Alism. Add value map. Vola map embolism lab and then their status with FDA approval or MA approval and what line of therapy and what criteria there are making the diagnosis. P DL1 positive and I don't have slides here. I'm going to show I'm sure my.

NOTE Confidence: 0.910193562507629

00:03:05.750 --> 00:03:36.600 Future slides will show that the P DL1 positive patients are generally the ones that respond, but how you determine whether patients are P. DL1 positives are quite a challenge so this is kind of how we read it. The pathologist actually look at it and I'll show you some pictures in a minute. But we either call things negative and all drugs like that category. But then what other category, you use? What percentage of cells are positive at any intensity depends on which truck and the pathologist don't know what drug the oncologist going to give so this is a little bit of a problem in diagnostics.

NOTE Confidence: 0.891063272953033

00:03:36.720 --> 00:03:53.880 And we also read into into regions. We read tumor cell standing and we read this thing called immune cell staining and I will talk a lot about what immune cells are but it's basically lymphocytes macrophages or any other cells that are not tumor cells that are in the stroma and not clearly fibroblasts.

NOTE Confidence: 0.732424080371857

00:03:54.470 --> 00:03:55.620 So here's.

NOTE Confidence: 0.909926533699036

00:03:56.220 --> 00:04:26.500 Another way that we read another thing that we have to do for some tests as we call combined proportion score. We look at the P. DL1 staining cells, including tumor cells lymphocytes and macrophages and then divided by the total more viable cells. So how many people think that's accurate. We can't do this. But it is actually FDA approved tests that were supposed to do as pathologists. So it's a problem that we can sort of estimate things and pathologist in general don't count every cell. We estimate how many we see and here's an example.

NOTE Confidence: 0.914752125740051

00:04:26.510 --> 00:04:59.060 So you think I could count every cell in this slide, not in a practical way. But what you can see is it pretty clearly strong tumor cell

staining in Mister Chemistry and also there's a lot of these cells in the stroma quote unquote immune cells. In fact, there's a great great correlation between immune cells and tumor cells and these are probably some kind of cell related to the immune response in the micro environment and not related to whatever alterations are occurring in the tumor. Here's another example of one. Taipower field and this is probably a 40X field and you can see.

NOTE Confidence: 0.91175776720047

00:04:59.060 --> 00:05:30.510 The head of the heterogeneity problems so we only get as pointed out by doctor. We only get a little tiny piece of tissue sometimes it's so small that it's only a few 100 or 1000 cells and even in this field of view, which probably has less than 100 cells. You can see some parts of it are really strong and some parts of it are really weak so we can talk about percent cells positive, but we don't even look at intensity. How light these cells are compared to how dark staining these cells are and so these are sort of the challenges of scoring P DL1 and what we would call them.

NOTE Confidence: 0.871909201145172

00:05:30.510 --> 00:05:37.380 And then we take and then we what we actually see are even things like this. So is this tumor cell staining or is immune cell staining?

NOTE Confidence: 0.922448992729187

00:05:38.170 --> 00:06:08.240 It's a little bit of a challenge. In fact, probably if you ask 10 pathologists they wouldn't all agree. But I would argue that what we see here is actually immune cells or macrophages surrounding the tumor cells and that this is actually immune cell staining not tumor cell staining in this particular case, there might, not be any real tumor cell staining or maybe a small percentage of tumor cells staining even though cases like this are fairly common, and easily could be confused so this is kind of where we are with the diagnosis along with the fact that.

NOTE Confidence: 0.925729393959045

00:06:08.240 --> 00:06:16.820 We have 4 different diagnostic tests one that's clearly inferior to the other three in terms of its sensitivity of detection so with this in mind.

NOTE Confidence: 0.917259693145752

00:06:17.350 --> 00:06:47.810 What we really need to do is come up with something new and that's what I'm going to talk to you about today. So to summarize where we are there's been only 2 statistically powered studies in lung cancer that looked at a number of probably 40 or 50 smaller studies that looked at these tests and what we find is that all the FDA these 3 FDA approved tests in this lab derive test which is what we actually do here at Yale are practically equivalent, whereas this test is lower sensitivity for both tumor cells and immune cells.

NOTE Confidence: 0.857539355754852

00:06:47.810 --> 00:07:20.080 With all this can actually read tumor cells. We have good concordance in reading the tumor cells, but are concordance in reading a immune cells is worse than a coin flip and CPS has not been tested at outside of vendor site tests but seems to and in fact failed for CPS 20, but it's unlikely to be better than immune cells scoring so with that in mind. What are immune cells and can we find out some more visual look at some visual observations to come up with ultimately a better way of predicting responders to therapy.

NOTE Confidence: 0.899644076824188

00:07:20.080 --> 00:07:51.580 And so here's here's the way we score and you can see that again. This is another summary from the FDA branch chief on companion diagnostics or complementary diagnostics and the drugs and then the scoring and you can see that for lung cancer where we started out there's two more proportion score tumor cells, scoring but then you can see all the rest of them have some component or many of the rest of them have some component. Either of immune scales or CPS scores, which are suggesting that the immune cells are probably really important so what's going on there well.

NOTE Confidence: 0.901900112628937

00:07:51.580 --> 00:08:22.490 If you look in the basic science literature. I'm not going to go in detail through these papers. Both of these papers showed that it was the P DL1 in the macrophages, not in the tumor cells that gave the gave sensitivity to PDL1 pathway inhibition. And here for example, they show that in patients with wild type macrophages and P. DL1 knockout mice. They did see a response that's by transferring in wild type macrophages, whereas if P DL1 negative macrophages that knocked out macrophages.

NOTE Confidence: 0.899653851985931

00:08:22.600 --> 00:08:54.460 In P DL1 positive RP on negative mites, no response, so that the macrophages are what was generating response, not the tumor in this study is similar. I won't go into details but basically pudding in PL-1 positive tumors and P. DL1 knocked out or negative. Macrophages results in a lack of response so with that in mind. Maybe we should actually try to figure out what those immune cells are this would suggest maybe their macrophages. and I would argue that even though I'm reasonable pathologist. I can't tell you what those 2 styles with the stars are.

NOTE Confidence: 0.878333628177643

00:08:54.730 --> 00:09:24.030 Are they macrophages or are they tumor cells? Where they kind of look like tumor cells and they're clearly expressing P DL1. Maybe that one's a macrophage? How do I know while Fortunately in the science world not in the diagnostic world. But in the science world we have ways of telling those apart by simply staining with CD 68. So here's a slide

very similar to the one Marcus Bosenberg showed in grand rounds in pathology yesterday where we see the H&E of a Melanoma and then the P. DL1 staining of the Melanoma. So is this macrophage staining or is it tumor cell staining.

NOTE Confidence: 0.902988433837891

00:09:24.550 --> 00:09:55.690 I would argue there's no way we can tell and in fact, but if we'd stain with CD 68. We can see huge number of macrophages. The macrophages are yellow in this side and white. In this lung cancer spent section seeing that. A lot of what we're seeing is probably macrophages, not tumor cell staining and in fact, it may be that macrophage staining hiding under the tumor cells. It's what really the diagnostic tool. That's guiding us on which patients will respond to therapy so let's go to confocal microscopy. We can take a little closer look at what's going on?

NOTE Confidence: 0.891831278800964

00:09:55.690 --> 00:10:27.140 And here in this in this image of this lung cancer case you can see the multi Plex fluorescence. And here's the Diaby so that's the cell. We're going to look at right there, OK and so, if you take that sell and then you look at it and confocal you can dissect it apart and you can see that the cells positive for PD L1 along with a bunch of other cells, but it's also positive or CD 68 and negative recital character so that means it's a macrophage that staining now that's not to say they run a lot of tumor cells standing with P DL12, but I think this is an important example. Here's another example.

NOTE Confidence: 0.882766723632813

00:10:27.160 --> 00:10:43.000 Using confocal microscopy at high power you can see this little region here and here's this sheet of tumor cells. But here's a sort of an infield trading macrophage and that's with staining with P DL1. In fact, the rest of this case isn't P DL1 positive, but the macrophages, P DL1 positive.

NOTE Confidence: 0.908541977405548

00:10:44.380 --> 00:11:15.020 So let's count that's what we do in my lab, we always count stuff up and measure stuff and so we have actually a tool that what we're going to do is look at the target positive cells divided by the total sales and we're going to do that in the regions of tumor in the regions of stroma and then a double positive cell will be positive for Saito Karen NP. DL1 and that's going to be a tumor cells positive and if it's CD 68 positive in P. DL1 positive will call that a positive macrophage. I know there's other things that are positive or C 68, but I would argue that that just for the sake of.

NOTE Confidence: 0.877767145633698

00:11:15.020 --> 00:11:24.080 These will just call that macrophages, even though it's really CD 68 positive then we'll look at CD 8 double positives with P. DL1 and C56 and as a surrogate for NK cells.

NOTE Confidence: 0.897119641304016

00:11:24.580 --> 00:11:55.850 So how do they come out so here's an example of some different cases and you can see what the case look like here and then quantifying the number of cells in this field of view that are positive for cytokeratin MP DL1. These are all positive for PD. L1 so they all have some P. DL1 present in them and then the bar shows where it is so here you can see it's a lot of CK but a little bit in the macrophage and in the stroma. It's essentially on the macrophage. Here's a case where everything is in the macrophages. Here's a case where in the tumor. It's in the site of keratin positive and instruments in the macrophages.

NOTE Confidence: 0.901887953281403

00:11:55.910 --> 00:12:28.560 And here's a case where there's no CD 8, Arnaud P. DL1 is in the macrophage compartment. But it's all in a tumor compartment except for this little tiny bit here and the other thing you notice is in all. The cases there's very little staining with CD56 or CD8 so most of the immune cell staining seems to be with macrophages. And if you look at 126 different lung cancer cases. You can summarize him like this. This is distribution in the tumor and you can see that the P. DL1 in the tumor is 41% in macrophages and that's what Marcus was showing yesterday as well.

NOTE Confidence: 0.905581593513489

00:12:28.560 --> 00:12:59.190 Even though it looks like it's in the tumor cells. It's really there's a bunch of macrophages that we can't morphologically distinguish from tumor cells that are probably bearing a lot of the P. DL1 expression and although there is a lot in tumor cells as well. and I don't want to diminish that 'cause that combination may well be important whereas in the stroma. It's essentially all in the macrophages. There's about 10 or 12% that's in tumor cells or epithelial seek positive cells in the stroma, which might be individual tumor cells and then there's little tiny bits in CDA Tour CD.

NOTE Confidence: 0.903026223182678

00:12:59.190 --> 00:13:00.370 56 compartments.

NOTE Confidence: 0.870284497737885

00:13:00.890 --> 00:13:31.420 So, In summary the immune cell expression is more productive than in tumor cells in some cancers. In fact, gastric cervical and bladder had a neck and breast. We don't even look for tumor cell staining were just looking for immune cell staining to predict response to therapy except for the CPS which kind of combines it in there and some non reproducible way in mouse models anti PD. L1 medium that therapies only work when the host can express P DL1 in the macrophages, independent of tumor cell expression.

NOTE Confidence: 0.883388638496399

00:13:31.510 --> 00:14:02.200 And finally are dated that I just showed you said the Pijuan is expression is high in macrophages, so that leads to this hypothesis that we're going to try to test in patients. Pedia one expression in macrophages

carries the predictive power of the biomarker So what I really should get is a keynote try Laura Checkmate trial. but I can't get those. I'm an academic investigator So what we have to do is retrospectively collect a bunch of patients that have been treated here by Roy and Scott and Sarah with a Tessa Lysmata, Nevala, mab or federalism app in the.

NOTE Confidence: 0.91010046005249

00:14:02.200 --> 00:14:33.970 Meta static setting here at Yale, where we have the tissue biopsy from their pretreatment specimen and you can see. Unfortunately, there's only 67 patients that had mono therapy, including only 62 with a pretreatment biopsy. So it's a little bit. Maybe in order of magnitude smaller than the keynote or Checkmate Studies. But at least it can give us a little bit of pilot information about and we can measure every cell in these in these patients and maybe this pilot information can point us in the direction of the importance of PD L1.

NOTE Confidence: 0.905290186405182

00:14:33.970 --> 00:14:53.650 In macrophages versus tumor cells and here it is here is P. DL1 in the tumor cells measured the same way. I showed you before, and in fact, Pedia wanted the tumor cells in this cohort is not associated with outcome, whereas P. DL1 on the macrophages is statistically significantly associated with outcome, suggesting that it might actually be the P DL1 in the macrophages.

NOTE Confidence: 0.871650755405426

00:14:54.310 --> 00:15:24.770 So how's the model work? Here's a model. This is a little bit pie. In the Sky and excuse. The pathologist approached immunology here. But what you can see is that the general Canonical. Mont mechanism if you will is that when P D1 interrupts the PD L1 or when the antibody either anti. PD one or an anti. PD L1 interrupts this interaction, then that inactivates the checkpoint and then the T cell can kill the tumor cell and so that's clearly one approach and that's what?

NOTE Confidence: 0.907969415187836

00:15:24.770 --> 00:15:55.650 What I learned and I think we all learned is the potential mechanism of action of these checkpoint inhibitors well? I'd like to propose an alternative approach based on the data that I just showed you which I show you here that may be what happens is that macrophages are the controllers of inflammation in the tumor and then in fact, the macrophages are moderating that level of iteration of inflammation by their interaction with PD one on the T cells. And when the macrophages, quote unquote see the T cell present by binding from.

NOTE Confidence: 0.885423243045807

00:15:55.650 --> 00:16:26.140 P1 to P DL1 then they don't have to produce anymore. T cells or bring any more T cells to the site of information, but when

the map when you inhibit that interaction with anybody, then the macrophage thinks there's not enough. T cells around even though there are and so it upregulates perhaps EX EL9 in CX CL-10. I don't know that we're doing that right now, but that would be a way to make the tumor hot and in fact that's what doctor would just showed that after you treat with P. DL1 you increase the inflammation increase the number of T cells that go to the site.

NOTE Confidence: 0.895509600639343

00:16:26.140 --> 00:16:45.570 And so this, I would posit is an alternative mechanism of action for PD L1 and maybe how we're seeing some of the mechanism of PD L1 and Pract. This might be a better mechanism to predict response to therapy then just looking at IHC for for PD L1 using tumor cell standing.

NOTE Confidence: 0.898013770580292

00:16:46.650 --> 00:17:18.440 So conclusions we have other support this actually this paper just got accepted in clinical Cancer Research. You can read the details. We had other supporting mechanisms as well, looking at other methods or other asset conditions and then a third method called Nanostring Digital Spatial profiling, which all support. This data, and I'll show you a little bit of that next because what I want to talk about next is high. Plex discovery tools that is OK. If we found this kind of came out. This one from the clinical side and then we found the importance of macrophages.

NOTE Confidence: 0.913793325424194

00:17:18.480 --> 00:17:48.590 But now I'd like to talk about tools that we can use especially new tools to visualize tumor immunity because that's that's sort of the context is uh? What are the other ways of visualizing our immunity so 1st I'm going to talk to you about digital spatial profiling and then I'm going to talk about image in mass cytometry and I'm going to assume that some of you in the audience. Maybe many of you don't know what these tools are so explain him a little bit and then show you a little bit about discoveries. We've made so far, which are completely pilot level discuss studies.

NOTE Confidence: 0.904642403125763

00:17:48.600 --> 00:17:58.440 And need to be validated, but open our eyes as to new potential biomarkers that may be better than P. DL1 HC in determining which patients are likely to respond to noon therapy.

NOTE Confidence: 0.880092620849609

00:17:59.060 --> 00:18:29.110 So this is the Nanostring Digital Stick Facial profiling mechanism and this is the nanostring DNA tag that binds to a bar code so that we can count exactly how many of DNA tags. We have in a solution and we can also hook him up to an antibody through a photo cleavable linker, so here's the tag and then here's the antibody with a photo cleavable. Linker and so that we can take that antibody with the photo cleavable. Linker on it,

and put it right on a slide just like immunohistochemistry, but we can do 44 or 800 at once.

NOTE Confidence: 0.915507733821869

00:18:29.110 --> 00:19:00.260 Our experiments, we've only done 44, but the theoretical limit is actually thousands because instead of detecting them. You can sequence them, but either way, you have to put them within compartments were out making the imagistic chemistry. I showed you is it about .5. Micron resolution this technology's at 10. Micron resolution so we're not going to be able to make an image. We can make a heat map. But we're not going to be able to make an image. But we can do is define the image and define regions of the end of interest in the image with immunohistochemistry or immuno florescence rather.

NOTE Confidence: 0.890043914318085

00:19:00.260 --> 00:19:31.250 And then point that laser at those immuno fluorescent positive regions and cleave off that tag and then sip it up with a little sipper and put it in a in a 96 well plate and then count it where this machine where we hybridize accounting device and then count so we're counting every single one of these of these tags. So it's a way of quantitatively looking at multiple antibodies. At one time on one section of tumor. And so to do this we're going to use a cohort of Melanoma patients, all treated with immunotherapy.

NOTE Confidence: 0.887776792049408

00:19:31.320 --> 00:20:02.830 Collected by Harriet cougar and Pack Faye Wong as a grad student in my lab and again not a clinical trial. But a pretty respectable collection of retrospectively treated patients with Pam brow leave or ipini. Vo as many as 117 where we're going to use a TMA so in order to make a TMA they had to be bigger tumors. These are all pretreatment biopsies. And so they had to be bigger tumors, but we have 60 of those that were large enough to biopsy and make TMS of which we could make for master blocks for which we will get at least 100 different assays from each.

NOTE Confidence: 0.905005872249603

00:20:02.870 --> 00:20:35.060 So that we can spare for this crazy new DSP technique and here's what we found is that we take. Here's an example of the nanostring DSP masks and so you can see the regions of interest that we're going to pick our Melanoma. The green CD 68, the macrophages are the purple and CD. 45 is red so this is the only image you're going to see. Now after that, we're just going to see counts. It's interesting how well the first technology that I showed you when I was doing fluorescence was the Aqua technology and they're very parallel technologies.

NOTE Confidence: 0.896333694458008

00:20:35.610 --> 00:20:47.240 And what we found in fact, is that it was PDL one in the CD 68 compartment. Not P DL1 in the Malana site compartment. Now,

this is not lung cancer, which is what I just showed you this is now Melanoma.

NOTE Confidence: 0.886767327785492

00:20:47.950 --> 00:21:18.860 But the same result that the patients do better if they have high PDL one of the CD 68 region of interest compared to low P. DL1 and they CD68 region of interest. Whereas P DL1 in the Malana site region of interest really doesn't show the same result so that's a first look now. Let's look back at that lung cancer covered so in the lung cancer. Cohort we looked at 40 targets and 40 independent in 4. Independent compartments and you can see they don't all work so the ones in red or targets that failed.

NOTE Confidence: 0.90450781583786

00:21:18.880 --> 00:21:48.910 So we're still in the very early stages of this technology, but I think it's a really inner technology and we can look in the side of keratin compartment that will be the cancer cells CD 45 compartment all immune cells and the CD 68 compartment for macrophages. And probably MDS ease and maybe some other cell types. Dendritic cells and then we can look in the rest of the stroma, but we can look with this whole list of tags and using this same approach so here's the example of a picture of the compartmentalization.

NOTE Confidence: 0.942173182964325

00:21:48.910 --> 00:21:58.580 And you can see how the compartmentalization works and it works pretty well. But it's giving you all the cells together so this is not a single technology, but as a group of cells technology.

NOTE Confidence: 0.833973050117493

00:21:59.160 --> 00:22:04.500 And here's the cohort that we use. I already showed this cohort. This is the lung cancer cohorts. I'll move.

NOTE Confidence: 0.892992496490479

00:22:05.380 --> 00:22:15.100 Through Next One and look at some of the markers that we found that were interesting so in the CD 45 compartment. That's the compartment that is all lymphocytes.

NOTE Confidence: 0.885852336883545

00:22:15.670 --> 00:22:46.430 We found that C6056 CD 4 and large one all cut at various cut points where associated with benefit from immune therapy in these both univariate analysis. Anna multivariate analysis and so it's kind of the beginning and clearly this is not the end of the story. We can try to validate it a little bit. You can see CD 4 patients in the same cohort now this is looking at.

NOTE Confidence: 0.874939918518066

00:22:46.430 --> 00:23:17.320 The survival curves showing better outcome in the CD 56 high patients and in fact, we can then take that data and do regular quantitative. Immuno florescence now only 4 Plex with CD 56 and you can see

some C 56 positive cells here and then you measure them and the patients with high CD 56 in CK negative cells. So Melanoma in some lung cancer expressed CD 56. But if there CK negative. That means they have to be lymphocyte Oregon.

NOTE Confidence: 0.905658960342407

00:23:17.320 --> 00:23:47.370 Some sort of leukocyte and you can see that the high CD 56 cells do better and then the patients with low CD56BFS Nos so early work but provocative and more importantly. I think illustrative of the kinds of things that you can do with digital spatial profiling, which is a sort of a new high. Plex technique what we showed here was just a 44 Plex and even that was probably stretching the truth. That was probably closer to a 35 Plex since some of the molecules didn't work, but

NOTE Confidence: 0.914506793022156

00:23:47.370 --> 00:24:03.630 It leads to provocative suggestions that CD 56 and CD. 4. Maybe something worth pursuing in more detail as a quantitative approach to look for activation. Now, less thing to talk about in the last 5 minutes or so is image in mass cytometry.

NOTE Confidence: 0.905244052410126

00:24:04.280 --> 00:24:35.260 So imagine mass cytometry is a way of taking the antibodies and labeling with a heavy metal and heavy metals. Unlike fluorecents, which overlap a lot. The heavy metals don't overlap in spectrum at all. When you look at mass spec. So we can take these heavy metals and actually blow him off the input connector heavy metals to the antibodies put them in the machine blow him off with a laser in this machine here running down the time of flight to which is in this machine here and then plot them out and then map them back to where they were so that you can have.

NOTE Confidence: 0.90058434009552

00:24:35.260 --> 00:25:05.270 Essentially, a multi multi Plex assessment using these different heavy metals and I add this slide because the Cancer Center through the closer to free bike ride is how we got this machine in the 1st place. So thanks to the Cancer Center for providing that here's what the images look like and you can see it doesn't look quite as nice as an immediate chemistry image because first of all we're mapping, it back and then Secondly, we're mapping it back to each one. Micron pixel so remember them. It has to chemistry sort of at the limit of diffraction has about a .2 Micron resolution.

NOTE Confidence: 0.892278492450714

00:25:05.270 --> 00:25:33.800 This technology has one Micron resolution and the DSP technology that I showed you has 10 Micron resolution. So they're not competitive you get to ask different questions with different tools. And so I think this is another tool that you can use and you can see how sort of

pixelated disappears for various biomarkers that were in the mix and in fact, I didn't include that slide it must've fallen out of the carousel the number of.

NOTE Confidence: 0.874838650226593

00:25:34.330 --> 00:26:06.670 Anybody's that we use in this was a 26 Plex so we had 26 different antibodies labeled with 26 different heavy metals and what we did is then look do. That same thing that is look at which of those antibodies are associated with response to therapy in the Melanoma cohort in the lung cancer cohort in this case is actually the Melanoma. Cohort talk about the lung cancer. Cohort next or Kurt shelter will tell you about that when you listen to him speak, but what you can see is that HLA ABC and made it to my crabby and have the?

NOTE Confidence: 0.87500673532486

00:26:06.670 --> 00:26:40.190 Most other highest hazard ratios or if you invert these one over these is the protective level. CD8 of course. We know is significant. That's the most significant one and we've actually validated that in previously published that. But even some other things in the tumor compartment. CSF one R like 3 HLA are also interesting biomarkers to probe in the future, and then in the CD 8 compartments. CSF one R so perhaps regulating. Perhaps that someone will ask me or someone will say, Well isn't this just a surrogate for Gamma Interferon, signaling.

NOTE Confidence: 0.878780663013458

00:26:40.190 --> 00:27:10.710 Maybe it is, but maybe that's what we need because P DL1, which is a surrogate for Gamma Interferon, signaling as well isn't a very good biomarker for predicting responsive therapy at least not by HC so here's how these biomarkers actually look. It's not quite as pretty. It's a binary thing color. These are it's a binary metal heavy metal signature and what we can find then is looking at survival. Curves of various techniques that so here's These Top 4 all in tumor cells HLA DRB 2M with.

NOTE Confidence: 0.904015183448792

00:27:10.710 --> 00:27:41.080 Progression free and over all survival progression free and overall survival looked very promising in the struggle compartment. Beta 2M looks fairly promising and in the CD 68 compartment CD 163 looks like a fairly promising biomarker again, discovering from the patient side back to the molecular side that I hope to partner with some immunologists and actually come up with potential mechanisms for some of these findings because we have both toys. We can compare them. Here's image in mass cytometry and digital.

NOTE Confidence: 0.884320080280304

00:27:41.080 --> 00:28:11.430 Spatial profiling and you can see for some biomarkers like CDA to CD4 and CD3. There is pretty good. Correlations whereas some markers like Ki 67, which is both in the tumor cell and in the immune cell is confusing. It's confusing. The Technologies 'cause. They have different resolution.

And so you see not such a great correlation and similarly for grants. I'm be so with that. These are kind of overall conclusions that 3 potential predictors for IO response in Melanoma are beta 2M.

NOTE Confidence: 0.905167102813721

00:28:11.740 --> 00:28:42.930 HLA ABC and 163 in the CD 168 and the CD 68 compartment and there's there's good correlation with some but not all between IMCQIF quantitative fluorescence in the DSP and that I think these are powerful tools for biomarker. Discovery that I look forward to being able to come up with better biomarkers. Then P DL1HC, which is where we started with respect to visualization so before I end. I want to thank the people that did all the work in the lab and former lab members aniel collaborators.

NOTE Confidence: 0.863432168960571

00:28:42.930 --> 00:28:50.450 Shown here, an also then just show a picture of my lab group at our party. Thank you very much.

NOTE Confidence: 0.86174613237381

00:29:00.600 --> 00:29:09.570 That's great David I think you showed that or were suggesting that orange. One also expression correlated with response in the CD 68 compartment.

NOTE Confidence: 0.885672867298126

00:29:10.120 --> 00:29:41.310 Yes, I mean, it's interesting, too because the identification of myeloid subtypes in that era micro environments been a mess for quite awhile between M1M2 and MDSCI think your data actually is probably the best data. I've heard suggesting a suppressive role because the P. DL1 expression being important in the macro Fage compartment, but from our mouse studies. We also find that large one correlate very closely with P. DL1 expression an OS 2 Orion OS is another marker that you might.

NOTE Confidence: 0.878913581371307

00:29:41.310 --> 00:29:59.320 Think about it, we can talk about that. Another time, but I think it's pretty exciting as you were saying to as a proxy. Perhaps for local kind storm or interferon. Gamma expression in the tumor microenvironment. So I think the approach looks really great you know, I think this is a great.

NOTE Confidence: 0.880301773548126

00:30:00.000 --> 00:30:13.680 Many of you are some of you might have been Marcus is grand rounds yesterday and Sosa very similar results in a mouse model. So I'm anxious to try to compare and contrast what we're seeing in our patient cohorts versus the mouse model.

NOTE Confidence: 0.845727920532227

00:30:16.300 --> 00:30:29.190 I knew that was amazing actually I can't wait to see these things start to replace single IHC's in the path lab.

NOTE Confidence: 0.867169439792633

00:30:29.980 --> 00:30:42.450 So I couldn't help but notice that one of your slides PD L2 had high predicted value.

NOTE Confidence: 0.890713632106781

00:30:43.700 --> 00:31:14.370 And so my question is when you say stroma do you have more granularity is 2 cell types and how does it be in macrophages, but I didn't I kind of glossed over that and sharpen picked it up. We actually have some PD. L2 data from M RNA, but we're worried about the PD. L2 data here because I'm not sure how specifically antibodies. RPD L2 has escaped the attention of a lot of investigators because.

NOTE Confidence: 0.895900666713715

00:31:14.370 --> 00:31:33.930 So many of the antibodies don't validate and it's not clear that this anybody is not cross reacting which is the problem with many other PDL too. Many bodies they actually cross react with other species, including P. DL1 and so this worries me a little bit that this isn't truly PD, L2 and so I kind of skimmed by that.

NOTE Confidence: 0.811558425426483

00:31:34.510 --> 00:31:38.540 'Cause of P values pretty good, yeah, it is very good.

NOTE Confidence: 0.884835600852966

00:31:39.360 --> 00:31:51.440 That was great, I'm wondering about spatial localization and taking this, who's next to who have you guys get thought about how to possibly integrate that into these kinds of analysis?

NOTE Confidence: 0.876316249370575

00:31:52.080 --> 00:32:22.160 Distances between so we haven't actually for had met who may be in the audience of post. Doc in my lab. I spent a lot of time determining the space. The distances between macrophages. Ain T cells and T cells and tumor cells and what we found so far is nothing it turns out that the T cells are important. But they don't fractionate into different spaces. And when they get far enough away, then they're not important.

NOTE Confidence: 0.899282872676849

00:32:22.160 --> 00:32:43.190 But it's a broad range of distances and finding them close to tumor cells. For example, or close to macrophages is no no better. A prognostic marker or predicted marker than finding them far to a certain distance, which is sort of the distance. We can measure out to about 50 microns or so 40 or 50 microns thanks.

NOTE Confidence: 0.903152823448181

00:32:46.560 --> 00:32:48.200 OK, thank you very much.