

WEBVTT

NOTE duration:"01:00:36.5780000"

NOTE language:en-us

NOTE Confidence: 0.892465233802795

00:00:00.080 --> 00:00:09.110 So our first speaker is Anna Woot. She's coming to us from city of hope and she's the chair of the Department of molecular imaging in therapy.

NOTE Confidence: 0.830474436283112

00:00:09.740 --> 00:00:15.660 And I also want to mention that she's the L alarm, which is which is great, so we're really excited over here.

NOTE Confidence: 0.690622091293335

00:00:20.550 --> 00:00:24.650 Should I use this? I don't know.

NOTE Confidence: 0.419672310352325

00:00:27.830 --> 00:00:28.620 They truly.

NOTE Confidence: 0.694086074829102

00:00:32.260 --> 00:00:34.850 That sound gave everybody hear me.

NOTE Confidence: 0.882095456123352

00:00:37.630 --> 00:01:12.640 So thank you for the introduction. Thank you for the invitation to participate for me. It's always a thrill to come back to New Haven. I was a grad student in MB MB so I spent many hours in the Seawing basement during my graduate days and actually this place is very near and dear to me because I met my husband. He was a medical school. Yale Med school graduate and my daughter also came tails in undergrad so blue. Beulah always happy to come back to New Haven. My disclosure and this will come up later is that I'm the founder board member and consultant to a company called imagine app, which is developing translating in commercializing some.

NOTE Confidence: 0.826606869697571

00:01:12.640 --> 00:01:15.380 These antibody based imaging agents.

NOTE Confidence: 0.903297424316406

00:01:16.170 --> 00:01:46.420 And I feel like a stranger in a strange land because I'm not an immunologist. I was at MB and B so I'm a biochemist, but surrounded by immunology. So I'm not going to spend much time on this which are well familiar with which has been the on going what we've learned and how we started to implement what we've learned about the interactions between tumors and the host immune system and ways that we can. Hopefully modulate the host immune system to kick start that immunity and be able to use.

NOTE Confidence: 0.89582622051239

00:01:46.420 --> 00:02:16.430 More effective immunotherapy's against cancer and these are just some of the modalities that that many people are exploring vaccines cell based therapies checkpoint inhibitors by specifics for recruiting T cells to the tumor environment and I was inspired by good friend when I went to UCLA Toni Ribas, who many of you know is one of The Pioneers in Melanoma immunotherapy and as you know early on, just dramatic responses to checkpoint inhibitor therapy such as this patient shown here.

NOTE Confidence: 0.909126579761505

00:02:16.430 --> 00:02:47.900 Before and after FDG pet scans showing just the disappearance of this widely metastatic disease, setting but nonetheless. Tony also introduced me to some of the challenges in treating patients with immunotherapy and this is an example of pseudo progression where patients might look worse 1st in terms of tumor size tumors might be might get larger, they might be hot or hotter an FDG pet. But if you persist with treatment over 3 months, 6 months a year or sometimes even longer.

NOTE Confidence: 0.895618557929993

00:02:47.900 --> 00:03:05.510 They might get full resist response and he was a second example down here where you can see legions getting larger for quite some time before they finally uh resolve and you know the assumption was that this action was evidence of an active immune response Indian sales infiltrating into tumors.

NOTE Confidence: 0.914030909538269

00:03:06.250 --> 00:03:38.030 So what I'd like to do well first of all just to sort of back off a bit and just sort of frame. The challenges that I hear about and we're trying to address in terms of on the diagnostic and imaging side in terms of human oncology, distinguishing true responses from non response is. Delayed responses versus pseudo progression. I think one of the big challenges in oncology is tumor heterogeneity within lesions. And when disease spreads from lesion to Legion even within the same patient we have so much information.

NOTE Confidence: 0.898751795291901

00:03:38.030 --> 00:04:08.600 From rapid autopsy programs that shows us how shows how diverse disease. Camp can become an immune responses can also be heterogeneous. There are many examples where some lesions will respond other lesions won't respond to an immune therapy and we need to understand this we have a wonderful tool. Oncologix, which is FDG pet. But the challenge there. Of course, is that immune cells undergo similar analogous metabolic shifts as they become activated. They become very glycolytic. In fact, one of the main reasons you'll have.

NOTE Confidence: 0.904981672763824

00:04:08.600 --> 00:04:39.990 False positives in your pet scan is because of infection or inflammation will in reality. Those are true positives. Because you are measuring glycolysis, you not directly measuring malignancy, so that becomes a challenge and then we rely tremendously unviable sees and there's just been an explosion of sophisticated technologies and analysis, so that we can really access very, very detailed and complex information on patients tumor tissues, but our sampling imitation says.

NOTE Confidence: 0.892644584178925

00:04:39.990 --> 00:05:11.880 We're looking at a few slices of one lesion or we're looking at a core biopsy. We not accessing or assessing the entire spectrum of disease within a patient and biopsies are invasive. They're not typically done after initial diagnosis, except for in research settings or sometimes when they're clinically indicated but there are places that are difficult or you really don't want to be too biopsy blood sampling similarly very rich information. We can derive circulating tumor cells next sounds.

NOTE Confidence: 0.887070715427399

00:05:11.880 --> 00:05:43.190 Etc. But when it comes to immunology number that stood out to me was the fact that only 2% of your total T live. The sites are into circulation. The other 98% are elsewhere and you're not directly looking at those so those are some of the diagnostic challenges and then if we step back and we look at the field of Immuno Oncology. We have broader challenges in the clinic in terms of the fact that there are real, but modest response rates once we get beyond of Melanoma, some of their very.

NOTE Confidence: 0.885380566120148

00:05:43.190 --> 00:06:13.240 Responsive tumors and even in those cases there are patients. It don't respond. How do we select patients were more likely to respond? How do we know when treatments aren't working. You already touched on toxicities. So ritis colitis cardio toxicity is one of our good friends here. Larry young cardiologist had actually last time I visited a couple of years ago, mentioned the acute acutely ill patients that were showing up in to see see you in response to immunotherapy and so now there's this field.

NOTE Confidence: 0.920648753643036

00:06:13.240 --> 00:06:43.390 Cardio oncology and it's it's really, really important, and then I think we also realized that into therapies will probably work better in combination with other admitted therapies or in combination with other modalities. But there's so many possible combinations. How are we going to sort out how to sequence stage and dose combination therapies we have limited tools we need better tools. So what I'm hoping to show you today is that molecular imaging whole body image in look at very specific targets.

NOTE Confidence: 0.887595593929291

00:06:43.390 --> 00:07:14.840 Can offer a lot and it's becoming reality now so molecular imaging can offer whole body and non invasive imaging so we can assess the entire mouse or patient. An importantly, you know the immune system is a system. We can look at the full immune system not just what's happening in the tumor, but lymph nodes. Spleen bone marrow. We don't even know to what extent those changes might be observed in changes might be important spatial and temporal imaging and the ability to capture that heterogeneity.

NOTE Confidence: 0.891118884086609

00:07:14.840 --> 00:07:48.370 Specificity because we're developing imaging ages for very specific in Vivo. Biomarkers patties quantitative so it's not just yes. No, but we could actually measure up taken activity concentrations or do kine-tex and understand binding rates, etc. And So what I'd like to touch on in today's talk are to first talk about technology. What are the ways that people are imaging immune cells and biomarkers in vivo and then in parallel hope to introduce you to some of the biology biological questions that can be addressed.

NOTE Confidence: 0.907882273197174

00:07:48.370 --> 00:08:19.840 And then by the end of the talk. I'm going to give you some examples of clinical translation, especially from our own work, and because I see this kind of is an overview. Talk to the program. I'd actually like to spend about 10 minutes sort of highlighting what other groups have been doing in the field because we could only do a few things and it actually is a rapidly expanding field. A lot of excitement and a lot of questions. I can now be addressed. So I'd like to go through as I said spend about 10 minutes for a snapshot of what's going on.

NOTE Confidence: 0.912572741508484

00:08:19.840 --> 00:08:27.980 Pre clinical and a little bit in the pre clinical world before turning to more specifically what we've been working on so? How do we image immune cells in vivo?

NOTE Confidence: 0.880242943763733

00:08:28.870 --> 00:09:01.410 I see at least 4 general approaches and one of them. We've already talked about with his image in altered Mataba Lism's immune cells undergo major shifts as they go from you know, naive or quiescent to activated proliferating and effector functions and so we have FDG which is useful. There's also plural thymidine, which many of you may be familiar with flooring 18 label 580, which is thought of as a good proliferation marker and then many groups. UCLA Stanford and then this is now moved into some companies are looking at.

NOTE Confidence: 0.884172141551971

00:09:01.410 --> 00:09:31.760 Additional nucleoside analogues because they've been able to probe the dinova synthesis versus the salvage pathways and have

found differences in how immune cells might upregulate these salvage pathways in particular, preferentially versus tumor cells. So we have these nucleoside analogs that have moved into the clinic and have potential also as metabolic probes for proliferation. Then there's a whole class of ex vivo labeling of cells?

NOTE Confidence: 0.897836565971375

00:09:31.760 --> 00:10:05.070 And this is something that clinicians have been doing for a long time with Indium. 11 Oxien If you're looking for the source of uh of infection that patient has a fever of unknown origin. Take the white cells out label India Maxine. Reinfused them, they will home in on sites of infection that has been around for decades. More recently, people have shifted over to moving forward with the pet version of that using zirconium 89, which is a positron emitting isotope 3.2 day half-life measured 20 marks see. I'm not sure if it's impatience yet, but it certainly made it through nonhuman primate taxes at these studies.

NOTE Confidence: 0.907553434371948

00:10:05.170 --> 00:10:37.800 Or switching to a different modality magnetic nanoparticles, including metal based or flooring 19 based nanoparticles to pre label sells reinfuse and see where they go. Then there's the engineered Reporter Gene approach where you can take cells out modify them ex. Vivo genetically so they incorporate some protein or something that will generate a recognizable signal whether it be optical or something you can recognize with radioactive tracer pro and I'll give you examples of these as we go through I just want to give you an overview.

NOTE Confidence: 0.905656278133392

00:10:37.810 --> 00:11:08.360 And finally there's the potential to directly image endogenous targets. For example, cell surface targets using antibodies. Nana bodies there's a whole slew of other additional protein scaffolds that people have used to do this So what I'd like to give you sort of examples of each of these and also the questions that people are asking so here's an example of metabolic imaging using FDG pet and it's a clinical study that was done by Steve Cho, who wanted to understand whether if you imaged.

NOTE Confidence: 0.887915253639221

00:11:08.360 --> 00:11:38.510 Early after initiating checkpoint inhibitor could you see a flair that was more representative of the immune response rather than later on, you're looking more at tumor response or combination of both and what they found in that study was that the FDG pet scan at 4 weeks was more predictive ultimate response in those tumors than waiting out to 4 months and some groups, including a horrible at Penn are looking even earlier as early as one week after starting checkpoint inhibitor.

NOTE Confidence: 0.872284948825836

00:11:38.510 --> 00:12:09.280 Therapy to see if there is an FDG pet flare so that's a metabolic imaging example. Here's an example of ex vivo labeling. I mentioned Cercone magazine, which is the pet equivalent of India mock seen this is a pre clinical study out of the National Cancer Institute from P Toy Keys lab, where they pre labeled took sells out pre labeled and put them back into mice. They looked at 2 different kinds of cells. They looked a dendritic cells. They looked at naive cytotoxic T cells and could show you that these different cells have different homing patterns.

NOTE Confidence: 0.864532589912415

00:12:09.280 --> 00:12:18.860 D CS go to the spleen and liver that's ET else. Go to the to the lymph nodes and spleen. So we can already see differences in how these cells are dragging.

NOTE Confidence: 0.87890750169754

00:12:19.750 --> 00:12:50.930 And here's an example of Reporter Gene Image Ng. This was, I think a massive collaboration between Stanford City of hope UCLA. Actually, a clinical example, where where I Christine Brown and colleagues have AOL 13. Zeta kind modified cytotoxic T cell for glioblastoma and they included the herpes simplex virus timing kind is Reporter Gene in those adaptively transferred cells and those can be picked up using flowing 18 labeled nucleoside.

NOTE Confidence: 0.8846555352211

00:12:50.930 --> 00:13:21.860 Iguana scene analog is shown here they had 7 patients they imaged six one was just a single injection only but 6 were image pre and post treatment and in one of these patients. You can definitely see an increase in signal in the brain when they image using this pet Reporter probe trying to pick up those genetically modified T cells. And here's an example that we actually displayed with a little bit at UCLA, where security Oliveira and colleagues at UCLA are not just genetically modifying.

NOTE Confidence: 0.876585006713867

00:13:21.860 --> 00:13:52.090 T cells, but also doing it in modifying stem cells somatic like stem cells. Instead, the idea being that in that case. You would actually give rise then to the different lineages, such as CD 4. Cedar NK cells that all have your car modification on them and since there construct included the truncated EGFR receptor protein. You could use radiolabeled anti EGFR antibody for detection and that also is a suicide gene you could use the cold antibody in math doses for ablation.

NOTE Confidence: 0.884335517883301

00:13:52.100 --> 00:13:59.320 So we demonstrated this in a mouse model where on the left, you can see the mouse that was in grafted with the human uhm.

NOTE Confidence: 0.847635447978973

00:13:59.950 --> 00:14:30.240 Hard transduced an EGFR tag stem cells and you can see the engraftment into the spleen and bone marrow of these mice and in parallel a set of mice wasn't grafted but oblated with cold syntax. Mab and in that case using the radio label stuck some abaza probe. You can see that the signal in the spleen in the bone marrow has disappeared, so these are the kinds of things that we could follow and finally I do want to focus on immuno pet because that's what my group and many others have been employing.

NOTE Confidence: 0.906520128250122

00:14:30.930 --> 00:15:01.370 I think that a lot of the issues with using antibodies and patients have been overcome and actually through protein engineering, which is really what my group specializes in so everything that goes into patients now is either humanized or fully human and what I will show you a little bit of how we have focus and optimizing the pharmacokinetics of antibodies. So we can use them for great radionuclide delivery and also how we can make those molecules biologically inert and enable side specific conjugation in labeling so we really can produce.

NOTE Confidence: 0.891636431217194

00:15:01.370 --> 00:15:10.820 Antibody based proteins that are truly optimized for delivering radionuclides for either imaging actually or for therapeutic purposes and then the.

NOTE Confidence: 0.914025187492371

00:15:11.430 --> 00:15:42.770 Radioactive side, the field is also been facilitated by the much broader availability of positron emitting radionuclides that have longer. Half lives than that of fluorine 18, but just just has a 2 hour half life like copper 64 with about 12.7 hour half life and then zirconium 89 and I want 24 with either 3.2 or 4.2 physical day, physical half lives. I have really extended our ability to look at biological products and biological processes in vivo without being limited to that very short 2 hour or hour window.

NOTE Confidence: 0.870813846588135

00:15:43.160 --> 00:15:50.520 And I will also talk more about the many bodies and I bodies antibody fragments, but also alternative scaffolds.

NOTE Confidence: 0.893302977085114

00:15:51.260 --> 00:16:21.370 And I think Cercone in 89, has really blown up in the field because it means that you can take intact antibodies and label them with your conjugate and the radioactivity persist long enough that you can wait out the long physical long biological half-life of those intact antibodies, So what are the kinds of things that people are looking at I won't go through this in great detail but I do think that you know for us was attractive was all of the CD antigens that are representative or informative for Lineage Differentiation and activation of different immune cells subsets.

NOTE Confidence: 0.90115350484848

00:16:21.370 --> 00:16:51.500 And then in parallel there are functional March is of course, including the checkpoint inhibitors and other related molecules and they become very important, especially when there are drugs targeting those particular cell surface markers and so there. Actually, the whole cottage industry now around imaging. PD one and PDL one of these are just samples of papers that have come out in the literature pre clinically looking at that and I just want to give you a couple of specific examples. These I think are actually both out of Stanford on the left just taking the drug pen, bro.

NOTE Confidence: 0.846467912197113

00:16:51.500 --> 00:17:05.730 Labeling with copper 64 in a mouse model. These are MSG mice that either. They all have a 375 Melanoma tumors on them. These to mice on the right wearing grafted with human immune systems and here is the tumor.

NOTE Confidence: 0.348653465509415

00:17:06.560 --> 00:17:07.260 Hum.

NOTE Confidence: 0.87677538394928

00:17:08.020 --> 00:17:27.440 Anti PD one antibody showing that signal in the tumor of the Humanized Mouse and a blocking control showing confirming the specificity of that binding so that's just straight labeling. the FDA approved drug and on the right is an example of a little more creative use where the the PD one itself.

NOTE Confidence: 0.874413788318634

00:17:28.160 --> 00:18:01.110 Was used as the tracer a modified domain. It was regular copper 64 and similarly you can image you? Can detect the PD. L1 positive tumors. You don't see the negative tumors and you can block confirming the specificity so they're pre. Clinical examples and I think it even more interesting preclinical example actually comes out of company David Donley in Bristol Myers Squibb, where they used in Anti. Human P DL1. Add nekton so this is a small protein scaffold and what they did I was not to label with copper 64, but rather use click chemistry.

NOTE Confidence: 0.890231430530548

00:18:01.110 --> 00:18:31.620 And very rapidly label, it with flooring 18 for pet imaging and they also confirmed in mouse models that they could distinguish in this dynamic scan between tumors that are low. Expresser's or negative for the marker or higher. Expresser's and then in the middle is the blocking control, so keep that one in mind because I'll return to it, and then moving on to them. What kind of questions. Can you ask if you have these specific markets not just is the target there for your drugs? Can we for example?

NOTE Confidence: 0.855523943901062



00:18:31.620 --> 00:18:48.670 Image early, but more specifically for the infiltrating T cells and will that predict ultimate response and want to ask that first in a mouse model so these are this is work from Bedlam are at Omar Mahmoud's lab in mass general or out of pity clues group also in Boston, where they?

NOTE Confidence: 0.859178245067596

00:18:49.240 --> 00:19:19.350 Either use anti CD 3 or anti CD 8. Ant antibodies in mice and in this example below from Ben Larimer. What they found was that image. Early and distinguish between high tumor uptake and low tumor uptake so high tumor uptake being the presence of the CD. 3T cells in the tumor and then the ones with the higher tumor uptake where the ones that ultimately went on to respond to the immunotherapy so they could image earlier an predict later.

NOTE Confidence: 0.846202194690704

00:19:19.350 --> 00:19:20.020 Response.

NOTE Confidence: 0.849067509174347

00:19:21.040 --> 00:19:53.430 And finally to be more directly engaged in a are the T cells. There are they doing anything. The Larimer, an enema mood group turned to one of the more direct readouts of T cell activation. T cell cytotoxic T cells when they actually attack and kill their targets released Granzyme B and perforin since Granzyme B is an enzyme. They developed a substrate analog that they radio labeled so they can look for binding of that peptide to the grand sign.

NOTE Confidence: 0.85742175579071

00:19:53.430 --> 00:19:59.250 And could show in a responding tumor. You do see signal that the Grand Slam is active versus the non responder or vehicle.

NOTE Confidence: 0.898503184318542

00:19:59.980 --> 00:20:30.310 OK, so that's kind of what's going on in the field now. I want to 0 in on what we're doing with antibodies for immuno pet and this is where we've spent, many years in my group versus city of Hope then off to UCLA and now back at city of hope working on these engineered antibody fragments. Really, the reason was to get around the challenge of intact antibodies that have a really long circulating half-life typically if you want to image with an intact antibody you have to inject the patient and wait a week for the circulating activity to clear before you can come back and.

NOTE Confidence: 0.858413577079773

00:20:30.330 --> 00:21:02.340 Image so we developed engineered antibody fragments of actually the dia body format was developed by Greg Winters Group at the MRC and also Pete Hudson and colleagues down in Australia. We also developed a slightly larger fragment called mini body, which also includes single chain. Fe fragments, but the CSS3 domain so the key factors are we focus on

both we use, both of these the key characteristics are that these are bivalent molecules and they retained specificity. Infinity of the parent antibody. Well, they have accelerated clearance.

NOTE Confidence: 0.884451270103455

00:21:02.340 --> 00:21:32.370 For the dia body primarily because it's below the threshold for first pass renal clearance. So it disappears within a few hours, as do most small proteins or small molecules are peptides then anybody at 80,000 molecular weight is just above the threshold for pinot clearance. So it clears via the liver, but it clears faster than intact antibodies because it's lack from the full FC region. So this actually does 2 things we do not have binding to the FCRN Receptor, which is the recycling receptor that maintains antibodies persistence in the Serum.

NOTE Confidence: 0.876466274261475

00:21:32.370 --> 00:21:55.460 It also means that these many bodies don't interact with FC Gamma receptors on immune effector cells. So it is biologically inert, which is really important feature if you're trying to do just an image Ng agent. Everything humanized her fully human we can steer the clearance to the kidneys or liver, depending on exactly what we want to look at in a tumor setting for immune cells.

NOTE Confidence: 0.897101223468781

00:21:56.080 --> 00:22:26.160 As well, but the important point is we can achieve high contrast at early times after injection much earlier than we can with intact antibodies so this is just one example from the tumor targeting world where we're making antibodies and fragments against prostate stem cell antigen, which is upregulated in prostate pancreatic and bladder cancer here in the mouse models. You can see excellent targeting of intact mini body or diabatic formats label with I-124 for pet. But importantly, what you'll notice is that in order to get this.

NOTE Confidence: 0.859309196472168

00:22:26.160 --> 00:22:33.350 Contrast using intact antibody, we waited a week with them anybody. It was the next day with Oduya body. We could do it the same day.

NOTE Confidence: 0.756662547588348

00:22:33.870 --> 00:22:35.530 All right.

NOTE Confidence: 0.899284541606903

00:22:36.230 --> 00:23:06.350 I look at the time here and it's a platform and this is really why I wanted to work with the antibodies because there's so many specificity is available because they're larger proteins. You can modify them. You can add labels without really perturbing their ability to bind their targets. So we and others have really been able to take starting antibodies with a variety

of different specificity's reformat them attach a positron emitting radionuclide and have yourself an immuno pet probe so we've used.

NOTE Confidence: 0.866072475910187

00:23:06.350 --> 00:23:36.850 Flooring 18 copper 64 is Jacona meeting 9 on 124th. We really have can pick and choose. What isotope reusing and this allows us to him is as I mentioned earlier next day with many bodies or even the same day with the diabetes. So I like to think of this as a kind of in vivo immunities to chemistry staining for specific phenotype. But it has to be self. Surface phenotype in this setting since we're looking at living cells in living organisms now beyond oncology.

NOTE Confidence: 0.814933717250824

00:23:36.850 --> 00:23:41.900 Antibodies for imaging immunology, we have the challenge that FDG pet is not specific.

NOTE Confidence: 0.643816113471985

00:23:42.770 --> 00:23:44.360 For tumors.

NOTE Confidence: 0.885454177856445

00:23:44.890 --> 00:24:16.020 We have the CD antigens, we can put those together. The antibodies anpadh isotopes and really open the door to applications in immune response is. An inflammation and in particular in checkpoint in cancer immunotherapy. So my group initially focused on 3 targets. CD8 CD 4 and actually human CD 20, so I'd like to walk you briefly through our pre clinical studies. These are all done in collaboration with Owen. Where do you Tony Ribas and Jonathan Brown?

NOTE Confidence: 0.898506164550781

00:24:16.020 --> 00:24:24.820 At UCLA because I'm the technologist. They're the ones with the biology. The Physiology of the disease model so we really.

NOTE Confidence: 0.849485337734222

00:24:25.480 --> 00:24:37.080 Profited from being able to work together to get these to move. These studies forward and we were initially inspired to focus on CD 8 because it's Toni Ribas said.

NOTE Confidence: 0.903546094894409

00:24:37.660 --> 00:25:07.760 You may have a variety of immunotherapy strategies, but in many cases they converge on the cytotoxic T cell as the active agent as Tony said the site as he says the cytotoxic T cell is the drug so let's have a look. We were inspired by the people doing early on treatment biopsies in patients undergoing checkpoint inhibitor therapy. So on the Top is a paper from Tony Tony's group where they looked in Melanoma patients at the pre treatment and.

NOTE Confidence: 0.893451035022736

00:25:07.760 --> 00:25:33.660 Early on treatment biopsies that are usually done, 3, four, five weeks after initiating therapy an in responding patients. One one of the better. Biomarkers was the appearance of that massive CD 80 cell infiltration into the tumor and they showed that this change. This increase in CDA T cell density. Correlated very well with decrease in tumor size in those patients and here's an analogous study out of MD Anderson.

NOTE Confidence: 0.880615472793579

00:25:34.220 --> 00:26:09.230 Well, they also looked at pretreatment biopsies early on treatment biopsies in nonresponders across the Top looking at CD. 84 P DL1 and in responding patients across the bottom and you can see this very significant signal of the CD 80 cell infiltration in these early biopsies and patients at ultimately went on to respond so he asked. The question or Tony's a question, we directly image CD8T cells in vivo So what we did was and this is the work of Richard cover a very talented post doc in the lab. We ended up we did different formats and different labels. We ended up with this being our workhorse, which is that smaller fragments of.

NOTE Confidence: 0.881239414215088

00:26:09.230 --> 00:26:43.420 This diabatic and it has cysteine residues a sea turtle cysteine residues that we can use for site specific conjugation in radio labeling with zirconium 89 and So what you see here is an image of a normal mouse first of all you will see hot kidneys because that's where the protein clears and the radioactive mataba lights are retained their charged but we will also see a beautiful map of the normal mouse immune system here is the spleen. And here are the lymph nodes. We can even see the popliteal lymph nodes in many of these scans and those away like a half a milligram to a milligram they're really, really tiny.

NOTE Confidence: 0.880168616771698

00:26:43.420 --> 00:27:14.110 But we can pick them up and in the example. I'll show on the right among the models. We did was a metal poetic stem cell transplant model in the mice. Here are the recipient mice that we imaged at 2 four or 8 weeks after reconstituting their immune systems you of course, see the kidney all the way through because that's the organ of clearance. But if you look at the errors. You can see at 2 four and 8 weeks or the lymph. Nodes are filling in as the mouse gets re populated with OC DAT cells. The spleen also fills in but it's kind of sitting.

NOTE Confidence: 0.904425084590912

00:27:14.110 --> 00:27:27.850 In front of her on Top of the kidney so sometimes it's hard to directly visualize but anyway. We're able to detect T celery population and now the real question can, we image infiltrating T cells in models of cancer immunotherapy.

NOTE Confidence: 0.867338478565216

00:27:28.470 --> 00:27:59.000 So this is now the CT26 syngeneic colon cancer model in valve. See mice treated with anti CD 137 antibody or 41BB, which is a general Indian stipulatory antibody and these tumors are very immune response if so, if you feed with anti CD 137. The tumors will regress and eventually disappear by day 20. We look at Day 15. If you take the tumors out of date 15 an stain for those anti CD 80 cells here. They are in massive amounts in the treated.

NOTE Confidence: 0.876323938369751

00:27:59.030 --> 00:28:29.120 Tumor and the question was can, we see that in the mice. So on the left is a control mouse. You'll see kidneys all the way across as I mentioned but here is very large tumor and this is the MIP so it's looking at the full mouse and you can see kind of diffuse and sort of heterogeneous looking up take if we go slice by slice in cross section what you see down here in the transaction. View is a large tumor with very cold center and here's the treated mouse a much different picture tumor is smaller.

NOTE Confidence: 0.900156676769257

00:28:29.150 --> 00:29:01.840 It's hotter it's hot through and through as you can see with the transactional sections and interesting you can see the entire immune system of this mouse has been activated the spleen is very large and just full of immune cells. CDA T cells. But throughout the body. The lymph nodes are much more active and in particular concede to draining lymph node here. Also in the transactional. View much larger much higher activity of CDA T cells. So maybe we're looking in the wrong place. If we're only looking in the tumor. Maybe we should be looking at the draining lymph node. That's where the re education of the T cells are taking place.

NOTE Confidence: 0.844794809818268

00:29:01.980 --> 00:29:09.640 This came up at Asmara couple weeks ago. Maybe we shouldn't be taking out the Sentinel lymph nodes who knows anyway.

NOTE Confidence: 0.8665971159935

00:29:10.170 --> 00:29:41.260 And 2 more examples, so anti PD L1 and again with Toni Ribas, those of you who work in the field know that this can be challenging because you can groups of 20 minus you by the mall or breed them all the same place same batch of tumor cells. Same batch of antibodies. Anti PDL anti. PD L1 antibody and only 25 to 33% of the mice will respond so these are mice. Both of which were treated. But one responded better and the other one kind of films in the non responding category.

NOTE Confidence: 0.825693488121033

00:29:41.260 --> 00:29:44.010 But you could still see a similar.

NOTE Confidence: 0.856443822383881

00:29:44.560 --> 00:30:15.170 Story in the tumors in that in the nonresponders. We do have these large tumors with cold centers and in the responding tumors. We do have smaller tumors that are hot through and through when we're looking for the CD45 cells and finally in an example of adoptive transfer of T cells. This is the OT 10 valve. Even specific model. We have E L4 tumors that are parental over negative or transduced to express ovalbumin implant. The tumor cells, we introduce the OT 10 T cells boost them with I'll too.

NOTE Confidence: 0.88930481672287

00:30:15.200 --> 00:30:39.420 And then we come back an image so the cells going cold. We haven't modified the cells in any way. We just put them in. We wait 5 days. Boost then we asked. Where did they go and what did they do and what we can see is that they've preferentially localized in an are proliferating in or accumulating in the over positive tumor, presumably in an antigen driven responsive way.

NOTE Confidence: 0.889705896377563

00:30:39.450 --> 00:31:12.520 Therapy, which is for example, our collaborators showed us told us that you would get a robust CD. Four response so this is the DSS colitis model 5 days of dextran sulfate sodium in the drinking water versus an influx of neutrophils. But by day, 11 or 12 is a massive influx of CD 45 cells. This is all known from Histology in human history. Chemistry can, we image it and the answer is yes. Here's the control mouse, beautiful map of normal immune system in the mouse plus kidney here is the college now and now, what you see is very prominent signal coming from the mesentery.

NOTE Confidence: 0.873358190059662

00:31:12.520 --> 00:31:38.060 Notes as well as from the colon very visible here in the sagittal view because we put CD contrast in the colon and you can see in the lytic mouse mesenteric lymph nodes and activity definitely in the cone in the live mouse. We did take those tissues out an image them. XP vote to confirm with that signal is coming. From here is the control here are the clinic mice with the cecum that colon the mesenteric nodes are very hot and again confirmed by immunohistochemistry that those are the CD 45 cells.

NOTE Confidence: 0.894575476646423

00:31:38.760 --> 00:32:09.750 And So what are the potential applications of being able to image CD 45 in cancer immunotherapy well if you have a therapy? Which is for example, our collaborators showed us told us that you would get a robust CD. Four response so this is the DSS colitis model 5 days of dextran sulfate sodium into drinking water versus an influx of neutrophils, but by day, 11 or 12 is a massive influx of CD 45 cells. This is all known from Histology and his chemistry. Can we image it and the answer is yes. Here's the control mouse.

NOTE Confidence: 0.875667631626129

00:32:09.750 --> 00:32:39.800 Beautiful map of normal immune system in the mouse plus kidney here is the colid mouse and now, what you see is very prominent signal coming from the mesenteric lymph nodes as well as from the colon very visible here in the sagittal view because we put CD contrast in the Colon and you can see in the click mouse mesenteric lymph nodes and activity definitely in the cone in the live mouse. We did take those tissues out an image them ex vivo to confirm where that signal is coming. From here is the control here, the clinic. My swear the cecum that colon the mesenteric nodes are very hot.

NOTE Confidence: 0.883711814880371

00:32:39.820 --> 00:32:43.590 And again confirmed by immunohistochemistry that those are the CD 4T cells.

NOTE Confidence: 0.891654908657074

00:32:44.290 --> 00:33:15.340 And So what are the potential applications of being able to image CD 4 and cancer chemotherapy well if you have a therapy? Which is for example, altering CD 4 and CD tray shows. There are some CD 4 based therapeutics and some of the car tip preparations are mixtures of CD4 CD 8 summer more just CD 8 focus so it kind of depends on what your therapy is and do we have the possibility of looking at CD4 driven toxicities such as colitis? Do we have the possibility of looking at CD8 driven toxicities. We don't know yet?

NOTE Confidence: 0.908385634422302

00:33:15.340 --> 00:33:46.010 But of course, there is a huge challenge that the CD 4 cell surface marker includes many subsets with different activities, including the helper subsets and of course, the T rags right now there's no obvious cell surface marker that will distinguish specifically T regs so again. We have to be driven by what's known from the biology and the clinical setting where this might be useful, but nonetheless. It's a tool that is available, OK, so now finally I want to get to the clinical.

NOTE Confidence: 0.894937694072723

00:33:46.010 --> 00:34:16.130 Translation of these types of markers in Immuno Oncology and what I'm going to do first of course, is backed up, and acknowledge the tremendous work. That's going on. Elsewhere, not just our work and in particular. In particular in Europe and the Netherlands. So I hope many of you have seen this these 2 papers that came out a year ago out of the Netherlands. This first one, is from Elizabeth to freeze this group in Groningen, where they imaging using it as alism map labels are conium.

NOTE Confidence: 0.889623045921326

00:34:16.130 --> 00:34:47.020 89 shown here, so one millicurie and one milligram of protein and they were imaging. Uh basically to look for the target of that antibody in these patients so a mixture of malignancy's and what they did was

well first I will point out. They did image serially from day one out. Today, 7 to find out when would be the best time point to image. These patients and they settled on Day 7 because of that challenge that I mentioned to you. Earlier, the long persistence of activity in the blood pool.

NOTE Confidence: 0.872819244861603

00:34:47.050 --> 00:35:08.320 Normal tissues for several days so they did their image. Ng at Day 7 and these are pre treatment. Then they would have do a biopsy eat shortly after that day 8. Today, 10 prior to the patients going on to therapy and they found a mix they found high uptake. The heterogeneous uptake. I think this one is so you can see some examples of uptake that they saw in these patients.

NOTE Confidence: 0.87349933385849

00:35:08.940 --> 00:35:17.890 This I think is interesting here is the core biopsy and you can see that there was heterogeneous radioactivity in the biopsy and if they looked at hot and cold.

NOTE Confidence: 0.867371320724487

00:35:18.560 --> 00:35:50.150 Sections of the biopsy by H&E and by staying you can see the hot areas are indeed the ones that had the P. DL1 expression and the CD 80 cells, whereas the cold sections were were negative by staining but it really again sort of reiterates the heterogeneity that you see in your biopsy and is that really reflective of what might be going on in the entire patient and after a lot of work. They actually did correlate the imaging compared to his chemistry versus RNA. Seq and they were starting to find was at the correlation between the.

NOTE Confidence: 0.874420046806335

00:35:50.150 --> 00:36:20.360 Image Ng an ultimate response was stronger than using those other markers and then the other study out of Amsterdam goes back to that agent I showed you before which was that out of BMS. This ad nectin protein that was labeled with Florian 18 by click chemistry. I showed you the mouse studies without what they did was they translated this into the clinic. Not only imaged the PD L1, but also the PD one so they have a flooring 18.

NOTE Confidence: 0.833553075790405

00:36:20.360 --> 00:36:28.680 Probe for PD L1, they use nuvola mab labeled with zirconium 89 to look at PD one and in their patients they looked at FDG pet.

NOTE Confidence: 0.886253774166107

00:36:29.400 --> 00:37:00.470 P DL1 and one all in the same case and it really was a tour divorce in terms of image. Ng and again heterogeneous uptake of both tracers. They correlate with him and his chemistry. They had positive signals and positive. I'd see versus negative. And so that all was very, very promising



but also I think what was promised thing was they did see accumulation in some brain metastases in some of these patients. Normally antibodies do not cross the blood brain barrier, but of course in the setting of.

NOTE Confidence: 0.88887494802475

00:37:00.470 --> 00:37:32.040 Either maybe a primary or metastatic lesion in the brain. There's alteration of the blood brain barrier. There's differential access of maybe you know this is something that people are starting to look at in greater detail and so now I'd like to tell you about our efforts to image CDN and this is where I have to reiterate my financial interest in imagine EB all of these clinical agents were developed at the company so this is the image. Ng agent for detecting human CD8T cells. It is a mini body that recognizes human CD8 with.

NOTE Confidence: 0.849886834621429

00:37:32.040 --> 00:38:05.150 Affinity of .4 nanomolar and it's fully humanized, so it should not be emitted genic impatience. Its conjugate in radio label with your conium 1889 and a lot of work went into the IMD package that was sent to the FDA including just in vitro and in vivo assays to look for any alterations in immune cell populations or activation markers or cytokine release, etc and also studies in Humanized mice, so this is one of those studies in Humanized mice.

NOTE Confidence: 0.896294713020325

00:38:05.150 --> 00:38:36.260 Where I started with MSG mice and reconstituted them with human PBM CS and over about a week you get engraftment of a human immune system in these adult mice, but then. After about 4 or 5 weeks that that human immune system. Of course, recognizes the mouse as foreign and will develop graft versus host disease, So what does that look like using this human specific T cell probe here is a one week image and what you see? Is there is the spleen. Those are the human T cells that have taken up residence in the spleen.

NOTE Confidence: 0.881197214126587

00:38:36.280 --> 00:39:08.150 But also you see signal in the liver, which is actually a combination of probably 2 different things. One is that it's a mini body. So it's a larger protein and it clears to deliver but there also is a matter of voices in the liver anyway. There's the liver and spleen and if you wait for weeks. Look at this additional haze of signal that is appeared in the lung the lungs is one of the organs that is attacked in GVHD in this mouse model and so we see it in the scan and we also see it when we take those tissues out and stain them for human CDA T cells. They are on the spleen, where they belong.

NOTE Confidence: 0.924866259098053

00:39:08.150 --> 00:39:10.350 But there they are in the longer they don't belong.

NOTE Confidence: 0.8819580078125

00:39:11.080 --> 00:39:42.610 So I'm delighted be able to now show you uh just an update of what has been seen in the clinic. So the Phase 1 study in 15. Patients was completed last year. It was reported. The first six patients at S&M and then the full 15 were presented in abstract formats. Etsy and actually a week ago, the 1st paper has now been published on line on the first six patients. This work primarily was done at Memorial Sloan Kettering led by needed pendant taskbar.

NOTE Confidence: 0.774094223976135

00:39:42.610 --> 00:39:46.590 On the nuclear medicine side and Jen Walchak on the myeloma side.

NOTE Confidence: 0.894342303276062

00:39:47.200 --> 00:40:17.630 So to show you what that Phase 1 study looked like of course. The objective was the safety and tolerability and whole body distribution of this human CD 8 specific imaging agent and this was also designed to help determine what the optimal imaging parameters would be this is a brand new agent in patients so that patients were image. Serially, 1 to 2 hours, 6 to 820, four 48 and then 6 days later.

NOTE Confidence: 0.895590484142303

00:40:17.660 --> 00:40:51.750 So that we could try to understand that the optimal imaging time, but also to understand the optimal protein dose. They all received 3 Villa Curries of zirconium 89, but the initial study was a rapid dose escalation in 6 patients where he went from .2 milligrams to 10 milligrams per patient and then we zeroed in on a dose expansion at, I'll give you the take home message. Now the optimal settled on as the optimal image in protein does is 1.5 milligrams. So the expansion looked at one to one point 5 milligrams.

NOTE Confidence: 0.858663976192474

00:40:51.770 --> 00:41:23.280 15 subjects half of them were Melanoma, many of the rest were not smocza lung cancer. One had a cellular they had a mix of treatment histories to the patients were treated naive 3 had prior checkpoint inhibitor therapy, but had gone off therapy and the rest were on checked when inhibitor therapy either for different extensive time. One patient I had just gone to check one hitter 2 weeks. Prior to the CD8T cell image. Other patients, 2 years or longer than they were.

NOTE Confidence: 0.878684341907501

00:41:23.280 --> 00:41:45.820 Inhibitor therapy and overall the safety profile profile. We were very happy with no adverse events know cytokine release. No blood test abnormalities. One patient had a transient anti-drug antibody. That then disappeared so not not a major immunogenic signal and there shouldn't be because it's fully humanized protein small doses of protein.

NOTE Confidence: 0.91617876291275

00:41:46.430 --> 00:42:17.180 And as I mentioned earlier biologically inert, so here are the first six patients from point 2.5. One one point, five five and 10 milligrams of protein and what you see right off the bat are there's the spleen. There are the lymph nodes. There's the bone marrow so we are seeing the expected tissues lighting up another thing you'll notice is that actually very good image and we were just shocked that with only 200 micro grams of protein.

NOTE Confidence: 0.900602996349335

00:42:17.180 --> 00:42:47.520 In the patient that we could see this, I mean, we put 100 micrograms into mice. So to see this was such a small protein does. We're really looking at trace doses of the protein. We also saw that by 5 or 10 milligram total protein doses were starting to push activity out into the blood pool. We're seeing longer persistence in the circulation and higher backgrounds. So we decided not to go that high and that's why in the expansions days. The rest of the patients were either put on one Meg or 1.5 milligram of protein.

NOTE Confidence: 0.812108278274536

00:42:47.520 --> 00:42:49.870 But again all with the 3 Miller Curries of.

NOTE Confidence: 0.868620634078979

00:42:50.490 --> 00:43:21.220 Of zirconium 89 an all 6 dose levels were safe and not to go through some of the individual patients of this is one of the patients, a gentleman with had a cellular carcinoma. He was the one that started treatment 2 weeks. Prior to the CDA. Timmy Nope, it and actually what you see here is the day one image and the day 6 image so this shows you can image early and you can also have a stable image for quite some time. But you can clearly see these 2 lesions standing out in the liver.

NOTE Confidence: 0.89416891336441

00:43:21.220 --> 00:43:56.230 And then if we look more closely on the contrast, see T there was this one known lesion in the liver. But when we looked with the CDA Timmy notepad. We definitely saw that lesion. But In addition, we saw an additional second sight of activity in the liver. There were not biopsy's in this study, so there's no confirmation as to act exactly what that signal was do too. But I think the things that we found our first of all we find that we actually can image very early in many of these patients after one or 2 hours. You already have a good sense of what's going on so you can image from one to 2 hours all the way out to one week later, basically everything is done in the 1st.

NOTE Confidence: 0.910192787647247

00:43:56.230 --> 00:43:59.590 2 hours, the protein disappears very rapidly from the blood.

NOTE Confidence: 0.896935880184174

00:44:00.120 --> 00:44:30.570 And we could also see these very strong signals against liver background in the liver is the organ of clearance. So all that was encourage ING so that was one patient here is actually this was our first patient the with the .2 milligrams of protein women with metastatic Melanoma. She had some small lesions in the muscle and 1st of all if you look at the again. The whole body scan actually forgot to point this out, but she was our first patient and we are very pleased to see very little activity in the brain lungs.

NOTE Confidence: 0.883896172046661

00:44:30.570 --> 00:44:59.300 Liver kidney so the normal tissue activities very low immune tissues are very high when I first saw this. I thought, What are those spots the liver but those are actually the tips of the ribs so that's the kind of detection that you're able to achieve with this agent and importantly. Here's the FDG pet. The lesion in the deltoid muscle in the shoulder and here are some of the serial CDA images showing clear Co localization of that signal with the FDG pet.

NOTE Confidence: 0.881916046142578

00:45:00.010 --> 00:45:13.050 And subsequently that lesion actually was biopsied. It was not part of the study. But for other reasons. They did biopsy the lesion. So here's the IHC showing tumor and here are those CD8T cells that were in the tumor.

NOTE Confidence: 0.889689445495605

00:45:14.430 --> 00:45:45.340 And finally this is one that finally second to last patients. I want to show you which is actually I think is important negative control, so this was a newly diagnosed lung cancer patient treatment, naive and so first of all you'll see here is the FDG pet scan were used to looking at confirming she notice. This lump in her neck. And then FDG pet. Another studies confirm primary lung cancer. Local spread here is the lesion in the neck very hot here is also.

NOTE Confidence: 0.891753435134888

00:45:45.340 --> 00:45:56.960 Another lesion in the liver it that's very hot on FPG. But when we did. The CD8T cell imaging prior to treatment. You can clearly see the lesions on the CT but they are not hot.

NOTE Confidence: 0.881484806537628

00:45:57.860 --> 00:46:27.990 So to me, this shows that we could actually measure a range of of activities from low or negative 2 quite hot. Of course, all of this needs to be confirmed by biopsy, but it does reassurance that the update that we saw in the tumors was not due to non specific retention of the protein in tumors. We actually have a range from actually quite a Reigns over which we hope to be able to detect and quantitative and finally this patient quite interesting.

NOTE Confidence: 0.832553267478943

00:46:27.990 --> 00:46:29.820 And I think it was here, I'm not sure.

NOTE Confidence: 0.902971982955933

00:46:30.440 --> 00:47:02.810 Part of the expansion of at one to one point 5 milligrams patient with Melanoma with numerous lesions, but was not known to have any brain metastases. In fact here is the see T scan prior to the patient entering the CDM you know pet. But what you can see is here by even the 6 hours. Can we see a little bit of activity showing up here in the brain, which really is much more clear as we wait for background to clear but very clear lesion in the brain so they went back and did it MRI. And Unfortunately there is a lesion in the brain of this tumor, but we're also encouraged by the fact that we can.

NOTE Confidence: 0.86939263343811

00:47:02.810 --> 00:47:08.120 Demonstrate that there are T cells there as well, so summary of the Phase 1 study.

NOTE Confidence: 0.891740679740906

00:47:09.190 --> 00:47:40.360 Rapid clearance actually much more rapid than we expected when we had Anti Tour. MIDI bodies into patients. We have approximate usually around a 24 hours circulating half-life, which is what we expected you know from the mouse models, but instead with these with CD8 imaging agent. It's gone from the blood of these patients within 1 or 2 hours and that most likely is because the antigens present in the circulation on those T cells so it immediately hits AT cell.

NOTE Confidence: 0.895739734172821

00:47:40.360 --> 00:48:12.370 And actually the T cells must go somewhere because it disappears in the blood or it may also be that were circulating through very well vascularized tissues, such as the bone marrow and the spleen. And so the radio tracers, taking up very quickly so we do see up taking T cell rich tissues very low uptake in the very low background in normal organs and tissues. We do see a range of tumor up takes in this initial series of patients, as I mentioned earlier. We zeroed in on the most favorable by a distribution around the .5 to 1.5 milligram.

NOTE Confidence: 0.89960378408432

00:48:12.370 --> 00:48:39.950 Total protein tells and this is good, too because it's a very, very low. It's officially a microdose by FDA standards? Is it really is a radio tracer amount and it also means that you know, we always had concerns that what happens? What is the impact of the radio tracer perhaps on your therapeutic T cells. The last thing you want to do is to perturb this cells that are actually.

NOTE Confidence: 0.895071923732758

00:48:41.060 --> 00:49:14.350 Active but when we go back and use these low protein doses. The tracers really only hitting a small percentage of the T cells in the body who really just taking sort of a snapshot so that kind of lays any concerns about interfering with the therapeutic effects, and settled on 24 hours as the most favorable imaging time, even though we can see a lot by one to 2 hours and better at 6 to 8 hours and that's really more of a matter of patient convenience and just executing the clinical trials. It's easier to infuse the patient and have him come back 20, the next day.

NOTE Confidence: 0.868989527225494

00:49:14.350 --> 00:49:25.980 That is confused them and have a weight around 6 to 8 hours to get that same day image and the quality was a little bit better, so here all 15 patients and we are now in Phase 2.

NOTE Confidence: 0.911878883838654

00:49:26.480 --> 00:49:35.130 This is the study. We wanted to do in the 1st place, which is to compare the pretreatment baseline. CDA Timmy no pet scan with biopsy.

NOTE Confidence: 0.882491171360016

00:49:35.800 --> 00:49:52.760 Have the patients initial initiate community theater piece of in this case is standard of care checkpoint inhibitor therapy and then 4 or 5 weeks later, do that early on treatment CD 8 pet scan with the corresponding biopsy and really to look at the safety of dosing.

NOTE Confidence: 0.866169393062592

00:49:53.700 --> 00:50:24.790 Twice instead of once primary objective, then it's to correlate the CDA pet signal with even history chemistry to what's the threshold over? What range of SUV's? How does that correlate with? What range of T cells per millimeter squared and that ultimately, we do want to look at correlation without come by Standard recist criteria will also ask those questions is there a Delta is the Delta informative is the baseline scan informative.

NOTE Confidence: 0.908557415008545

00:50:24.790 --> 00:50:46.630 Is the on treatments can by itself informative? We don't know it's very early in the process but we already have 8 or 9 sites open for the Multicenter Phase 214 patients have been enrolled in probably 11 or 12 completed both scans. We are working with Rich Carson and Bernadette. Marcus here to do an investigator initiated study. I don't think either that Bernadette are you here today.

NOTE Confidence: 0.895321190357208

00:50:47.930 --> 00:51:11.920 Shout out to Bernadette so will really love to bring this agent to Yale and open it up to 2 additional studies as well so that's when I could bring back to Yale for all that. Yale has given to me, I would

be delighted. So I'm not going to read. You everything on this slide except to focus on potential role in cancer chemotherapy, perhaps in patient selection.

NOTE Confidence: 0.904833853244781

00:51:12.480 --> 00:51:43.650 Early on treatment response because the way I look at it. the CD 80 cell is the relevant PD marker for all of these for many of these immunet therapies and this. I think is going to be very helpful in terms of optimizing combination therapies again. If you need to have T cells there before your T cell activator or whatever can work to be able to understand that process is important. I'm hoping it'll be interesting in the management of toxicity certainly as we image more and more places. We're going to be looking very closely at the normal organs.

NOTE Confidence: 0.908280491828918

00:51:43.650 --> 00:52:14.420 To see if there's anything unusual to understand what normal and is there anything on user going on and then of course, potential role in other immune mediated conditions and diseases. I already showed you inflammatory bowel disease graft versus host disease wouldn't transplant rejection, but about arthritis. Multiple sclerosis and there are many, many ways that you could envision once you have these tools in hand are very specifically quantitate those immune cells and or those activation markers or functional markers. I really think.

NOTE Confidence: 0.917791903018951

00:52:14.420 --> 00:52:44.800 It opens up to what we're going to be able to study and understand you know the immune system and people are whole systems and we need to be looking at the whole system as a complement to what we can do in the laboratory with our biopsies with our blood analysis, etc. So I will stop there. Thank you very much for your attention. My lab past and present by collaborators at UCLA. The clinical trial was done at a memorial Sloan Kettering pen and honor health and of course, with tremendous efforts.

NOTE Confidence: 0.902521133422852

00:52:44.800 --> 00:53:03.970 On the part of the team that imagine tab and of course to thank our patients because this is an image Ng study. They volunteered for it's an obnoxious imaging study with all these serial time points is no benefit to them, but still you know they're willing to participate to bring advance the knowledge. We hope so. Thank you very much for your attention.

NOTE Confidence: 0.48948809504509

00:53:10.750 --> 00:53:11.940 Questions.

NOTE Confidence: 0.912312924861908

00:53:12.880 --> 00:53:31.000 Ana thanks very much that was a really great talk. I really enjoyed that I'm particularly excited about the Phase 2 study that you

described which it sounds like you're doing pretty therapy imaging and then at 4 to 5 weeks some studies and neoadjuvant settings have shown that.

NOTE Confidence: 0.91940975189209

00:53:31.520 --> 00:54:01.460 Earlier time points on therapy may be more informative like about a week or 10 days out and that 4 or 5 weeks is what people are looking at now for complete pathological response to predict overall survival is it possible to reimage after that first dose at like 6 days in this trial, it probably wouldn't be. But since you already loaded them with that. You can just go back and image him again. Yeah, it actually is in this study, there's an optional scan after the first administration.

NOTE Confidence: 0.892942130565643

00:54:02.630 --> 00:54:37.640 The idea is to inject the tracer scan and then start treatment and because the half life of the rated nuclide is so long. We have an exploratory image to see if we can detect redistribution of the T cells that were labeled prior to treatment and I can't tell you what the results of those are yet, but these are all excellent questions. We're just beginning to learn how to use the other thing is that we're very interested. Of course in doing a new edge event study because then you get tissue. So we don't know what's the optimal time to image. We went with this because the biopsies indicated that this would be a relevant interesting time and logistically to it worked.

NOTE Confidence: 0.861787378787994

00:54:37.640 --> 00:55:09.630 For the clinical trial, but yeah, all good questions and we want to move towards addressing them. And so that's why it's an exciting time to be to have access to this so thanks. Yes, interesting question is to think about or how do you rule out a possible effect of the CD 8 antibody mini body or whichever with Alpha beta CD 8IN vivo that could actually compromise app stream binding to the MHC or downstream to LCK and I think the major point is that in healthy patient or unstimulating.

NOTE Confidence: 0.878749430179596

00:55:09.630 --> 00:55:44.640 In addition, they still geometry of the complex may be very different so you may actually modify the response somehow impatience an invade also be HLA specific so it could be patient specific? How do you do you have any in vitro data or something to show that the CD 8 binding doesn't compromise or effect in any negative or positively. The response so we do know that the place at the anybody buys the CD does not interfere with CD. MHC complex formation and we do know, especially then at these low protein doses were only going to hit a fraction of the CD 80 cells. We do know that.

NOTE Confidence: 0.867067098617554

00:55:44.640 --> 00:56:15.290 There's neither in vitro nor in vivo in Humanized mice. You don't see alterations up. You don't see upregulation CD 69 or cytokine release or anything like that, and we're looking in the patients what



we do see is we do see transient internalization. 'cause its bivalent of the CDA, but it reappears after 2 or 3 days in the mice so clearly. If not completely completely inert it doesn't induce internalization but.

NOTE Confidence: 0.909482181072235

00:56:15.290 --> 00:56:31.690 At least to the extent that we've looked no functional outcomes from that, and again as I mentioned I think we're everybody is relieved that we're able to do the imaging with such small approaching doses. So we don't think we're going to be perturbing the immune system but those are all good questions.

NOTE Confidence: 0.899172127246857

00:56:33.170 --> 00:57:05.760 I think that was great, so I'm wondering about the sensitivity. How many cells do you think you can get down to really good? Question and it helps if they're all in the same spot in the mice. You can see 50,000 or 20,000 cells if they're all in one spot like those popliteal lymph nodes that are really, really tiny in the patients were thinking more in terms of correlating to the sort of standards cells per millimeters squared quantitation, where it looks like and imagine that they've done.

NOTE Confidence: 0.892461597919464

00:57:05.760 --> 00:57:36.190 They did some reconstitution studies with Maitre gel plugs to try to detect threshold. I think from like 20 views the study. It looks like baseline might be around 100 for millimeters square but they go as high as 10 or 12,000 T cells per millimeter squared and that information will come out through the current Phase 2 study. I really can't tell now, what it's going to look like in patients what we also will say, though, is that even though the clinical scanners have resolution of 4 to 7.

NOTE Confidence: 0.834880650043488

00:57:36.220 --> 00:58:06.750 Mm if your probe is good enough in your signals hot enough you can pick up smaller lesions. You just can't quantitate them as well. His partial volume affect so you don't recover all the counts. But you saw the his chemistry. I showed you think of how small that lesion was like a small lesion in the shoulder and small percentage were T cells. And yet it did show up in the scan so we're looking into all of that. We do see signals even saying prostate cancer targeting agent.

NOTE Confidence: 0.876618325710297

00:58:06.750 --> 00:58:37.830 You would have normal size lymph nodes that would be 6 to 8 millimeters that would be negative considered normal by CTR MRI. But we did the a PS may mini body scan and we could detect very small lesions of tumors within those nodes. So I think their sensitivity is good and we just have to be more systematic about it. It's just going to be more patient before we understand truly what we can. The other problem is, I mean, this dinner or maybe another conversation. I had earlier this week.

NOTE Confidence: 0.895096480846405

00:58:37.830 --> 00:58:39.090 Yeah, how do you know your?

NOTE Confidence: 0.877095341682434

00:58:39.600 --> 00:58:58.370 False negative rate the problem is, we're not going to biopsy those negative lymph nodes. So there's not going to be a gold standard because the negative understand. We're not to take them out and say, Oh, was there tumor there. We just not going to do that, so that's going to be a shortcoming that were not to be able to address but we're going to work on the positive.

NOTE Confidence: 0.898456275463104

00:58:59.000 --> 00:59:17.550 Predictive value of the scans that it's your question I hope yeah. Yes, I can even describe this are you. Doing anything in the field of Nano sized titanium dioxide as a way to destroy organic molecules tumor cells.

NOTE Confidence: 0.891575396060944

00:59:18.080 --> 00:59:49.550 We are not, but there certainly are many people working in nanomaterials in the nanoparticle field and even targeted nanoparticles to either directly kill tumor cells or use some kind of induction heat induction or something to try to destroy tumor cells so absolutely. It's a very active field. It's not a field. I can report on except to the extent that we did collaborate with one guy at UCLA is doing a nanoparticle. It was amisa porous silica particle that had.

NOTE Confidence: 0.290423959493637

00:59:49.550 --> 00:59:50.030 Hum.

NOTE Confidence: 0.892337918281555

00:59:50.750 --> 01:00:22.060 Oxaliplatin to induce immunogenic cell death and then in Docs Emma to boost immune systems and we image. the CD eight response to that, so in that sense that was published its work with Andrew nail from UCLA and that was published like a couple of years ago. If you want to look it up, but actually we think beauty of this imaging approaches that you actually can apply it across different types of therapies if your therapy also induces an immune response and I think we're understanding more and more the importance of those immune responses that we induce.

NOTE Confidence: 0.909934103488922

01:00:22.060 --> 01:00:27.930 Even with just chemo or radiation potentially so all of that is.

NOTE Confidence: 0.852530241012573

01:00:28.440 --> 01:00:33.030 It's complicated, but, hopefully we're getting better tools. Thank you.