WEBVTT

NOTE duration:"00:52:19.1380000" language:en-us

NOTE Confidence: 0.934573233127594

00:00:00.120 --> 00:00:20.120 Well, good afternoon and thank you all for joining us today for Kansas Center. Grand rounds were really very fortunate to have a special speaker here today because many of you know today. We are recognizing the branch blanched.

NOTE Confidence: 0.87868469953537

00:00:20.920 --> 00:00:40.920 Sure, series for those of you are aware the blanched Almond series was endowed by doctor. Marvin Sears Professor Emeritus at Optomology in memory of doctor, Sears mother plans. Tolman and that a lectureship has brought to our center.

NOTE Confidence: 0.903895914554596

00:00:41.720 --> 00:01:01.720 Extraordinary talented individuals focused on research, particularly in the realm of Hematology and he even a logical agencies and we're very fortunate to continue that tradition today with our invited Speaker Doctor Michael Calijuri Doctor Calijuri is.

NOTE Confidence: 0.92762815952301

00:01:02.520 --> 00:01:22.520 Resident Inn physician in chief of the city of Hope National Cancer Center and who Mike's career as a physician scientist a leader executive mentor is really unparalleled. Mike was for 19 years at the James cancer ha.

NOTE Confidence: 0.931522071361542

00:01:23.320 --> 00:01:43.320 State and ultimately the CEO over the James where he took that institution to an altogether new level in terms of clinical care. Research philanthropy recruitment and really bring it to a great impact in cancer care regionally nationally and internationally.

NOTE Confidence: 0.871078610420227

00:02:25.720 --> 00:02:45.720 But for a mentor so I'm very pleased to welcome the 2019 blanched home and lecture doctor. Michael Calijuri probably. Thank you very much very kind introduction. I had the pleasure of having Charlie is the senior resident when I was.

NOTE Confidence: 0.848247230052948

00:02:46.520 --> 00:03:06.520 And he continues to do amazing things so it's a pleasure to be here. It's so good to see so many friends and so nice. Voluta comments such a snowy day, we were supposed to meet this morning but you got busy good. I realize some of you might have kids to pick up school closing early and then I.

NOTE Confidence: 0.867095172405243

00:03:07.320 --> 00:03:13.750 Was already closed yesterday for today, so that's impressive preventative Madison?

NOTE Confidence: 0.883585333824158

00:03:15.070 --> 00:03:35.070 I'm from Buffalo so this is like we used to roll in this kind of weather in the canal. You know, but it's a great pleasure and it's an honor to be here and especially because I'm going to talk about natural killer cells. I see that there's a great deal of interest and that's really neat for me because not a lot known when I started.

NOTE Confidence: 0.912439465522766

00:03:35.870 --> 00:03:55.870 And what I want to do is really take you through kind of my story of different discoveries and translation that we've made with natural killer cells through the years and finished with some clinical application. I'm not going to talk about its application in acute my Lloyd leukemia, which is perhaps the most exciting, but probably the best known.

NOTE Confidence: 0.895335793495178

00:03:56.670 --> 00:04:16.670 Going with natural killer cells as we learn more and more about the biology. My lab is really for focused since beginning is on understanding receptors because if you understand receptors that sit on the surface of a cell you can understand what that sells sees and if you understand what that liggen binding.

NOTE Confidence: 0.92108815908432

00:04:17.470 --> 00:04:24.560 Doing function really start to think about how do we manipulate these to help in cancer and infectious disease?

NOTE Confidence: 0.887633442878723

00:04:25.750 --> 00:04:45.750 So first just some disclosures here mine and I really won't be addressing any of this today, and so we'll talk about role of cytokines and natural killer cell development secondary lymphoid tissue, which is where we discovered it sells developed will talk about subsets.

NOTE Confidence: 0.869022369384766

00:04:46.550 --> 00:05:06.550 Applications of that receptor biology, so back along time ago. This is what was known about natural killer cells cover of Time magazine. You could take these CD 34 stem cells and pop them into Interleukin 2 and after.

NOTE Confidence: 0.868396997451782

00:05:07.350 --> 00:05:27.350 Based at 3 weeks 4 weeks, you get natural killer cells and really described by this CD 56 bright cell, which is an image here NK cell's becomes dim on the surface of the cell. It's it's mature in case I won't

hear bout. BCD 56 bright and dim but really the question was is this. The real growth factor for Isle to in feet.

NOTE Confidence: 0.887413382530212

00:05:28.150 --> 00:05:48.150 Because we knew that I'll 2 while Olympus cited Tropic hormone was very selectively released by T cells on activation. And yet you have billions and billions of NK cells in your body and turnovers fairly rapid so Mother Nature helped us with that with some genetic disruption experiments because the mouse when knocked out with oil 2.

NOTE Confidence: 0.824051737785339

00:05:48.950 --> 00:06:08.950 Find K cells in the mouse knocked out of the aisle 2. Alpha chain, which is one of 3 chains of the aisle. 2 Receptor had NK cells. Both the aisle to knockout and the aisle 2. Alpha chain knockout had NK cells. However, if you knockout. The beta chain of the aisle 2 receptor or the car.

NOTE Confidence: 0.86450457572937

00:06:09.750 --> 00:06:23.450 You know the aisle 2 receptor there were no NK cells, so that told us that something that isn't I'll too and doesn't require the Alpha chain, but does require the beta chain in the gamma chain is important for NK cell survival.

NOTE Confidence: 0.872559130191803

00:06:24.430 --> 00:06:44.430 And so that receptor schematically looks something like this, where you see the Beta Gamma and those of the signal transducing chains. That's where all the action is but for that Beta Gamma to sea. Isle 2 in your body it needs that third tiny Alpha chain that Alpha chain will not signal transducing confers very hief.

NOTE Confidence: 0.853180825710297

00:06:45.230 --> 00:07:03.390 Physiologic doses of I'll 2 and with that transduces a signal but by itself. The Beta Gamma chain can transducer powerful signal, but just requires enormously high doses of I'll 2 and that's why I'll 2 when given exogenously in high doses could make NK cells.

NOTE Confidence: 0.870167970657349

00:07:04.300 --> 00:07:24.300 And So what was subsequently discovered was another molecule that did precisely what I told you it had its own Alpha chain. I'll 15 Receptor Alpha chain and unlike the Alpha chain of I'll 2 the Alpha take about 15 by itself combined. I'll 15 with very high affinity and it sits on other cells a ninja presenting cells.

NOTE Confidence: 0.879043281078339

00:07:25.100 --> 00:07:45.100 Since the molecule I'll 15 to the beta gamma chain, and that sends a signal and then we can see this here. When we take I'll 15 pop

it into culture. Those CD 34 positive cells and now we're looking at a histogram here and you could see the CD 56 bright cell, which is the image here NK cell.

NOTE Confidence: 0.870112836360931

00:07:45.990 --> 00:08:05.990 What we noticed was that even though there were pure cells from this uncommitted metaphorical Jenna Tercel there were very few in number without 15 alone and so we asked ourselves. What other receptors might there be in the CD 34 cell that augments the growth of the.

NOTE Confidence: 0.906829953193665

00:08:06.790 --> 00:08:13.900 To do that, we actually looked at this cells and what sells what receptors are on this cell that might tell us a little bit more about that.

NOTE Confidence: 0.878338158130646

00:08:14.950 --> 00:08:34.950 By the way I'll 15 knockout mice at all 15 receptor Alpha chain. Akamai slack NK cells, confirming what we found in humans. All the work. I'm going to talk about is in humans and then I refer back to the mouse for studies. We go to the mouse for but we work primarily in human natural killer cell development. So to look at what else could be involved in the growth of NK.

NOTE Confidence: 0.888212203979492

00:08:35.820 --> 00:08:55.820 We went back and we found that this receptor receptor tyrosine kinase seek it was also on the service of just the bright NK cells. Not on the surface of the more abundant dim NK cells that are floating around in your blood. But when collected on the bright eyed kids if you could see fading as the cells become dimmer.

NOTE Confidence: 0.839532315731049

00:08:56.620 --> 00:09:16.620 You look to see if Kit Lige end stem cell factor also known or mass cell growth factor and its sister flip 3 lie again could be important in NK cell development and so here you see kit lag. Endorf Litli again. Here's total cell number from those CD 34 cells initially and here's the eh.

NOTE Confidence: 0.860722899436951

00:09:17.420 --> 00:09:37.420 Soak it log into flet login along with CD 34 cells gives you growth here. We're looking at FL alone. But as you can see, there is no natural killer cells, though no natural killer cells here. I'll 15 I showed you very high numbers of very low growth that so together.

NOTE Confidence: 0.828433990478516

00:09:38.220 --> 00:09:58.220 NK cells, but combine kale or FL when combined with their look at 15 in both of these stromal cell factors shows that not only do

you get the purity of NK cells? But you get synergy and growth. Total numbers so you get high numbers of cells here, so the.

NOTE Confidence: 0.894943594932556

00:09:58.270 --> 00:10:17.480 Date that FL in Cal work on the NK cell without affecting it by itself is Twofold. One is through the proliferation pathway, which I'm not showing you but the other is it up regulates the beta and gamma chain on more CD 34 positive cells, allowing a greater number of those cells to be responsive to Interleukin 15.

NOTE Confidence: 0.816220164299011

00:10:18.520 --> 00:10:32.400 So the picture, then began to unfold is such where an earlier sell a projector has on its surface kit in or flip 3 and sees flip 3 or kit lie again and.

NOTE Confidence: 0.895013868808746

00:10:33.540 --> 00:10:45.520 Pops up, I'll 15 Receptor Beta and gamma chain, and then in the presence of Isle, 15 would produce the natural killer cells and exogenous. I'll 2 and high doses could do this same thing.

NOTE Confidence: 0.919511139392853

00:10:46.450 --> 00:11:02.130 So this is interesting, so we wanted to go after this cell to see if we could identify the true natural killer cell precursor in humans because if we could we could start to figure out the developmental pathway beyond this we could figure out a couple of things where to human NK cells develop.

NOTE Confidence: 0.890242755413055

00:11:03.240 --> 00:11:23.240 And then what are the molecules involved in their development so in looking for this cell we so let's just look for this? I'll 15 Receptor Beta and gamma chain on the surface of the CD 34 cell. We couldn't see that by flow cytometry at all but we knew it had to be in the 34 population because of this pathway.

NOTE Confidence: 0.893519341945648

00:11:24.040 --> 00:11:44.040 As we took the 34 positive cells and using a variety of markers. I'm not showing you. On 3 different populations from these 2 markers 3:45 RA in 3412 and 3 and we popped each of those into culture with Kyle 15 or high dose. I'll too and you see only the third population over here.

NOTE Confidence: 0.889221131801605

00:11:44.840 --> 00:12:04.840 Number 3 labeled here CD 34 low are a positive was the one that became natural killer cells and indeed. We further took that population and said what other receptors are on the surface of this population to tell us where this cell might live what we found uniquely was that.

NOTE Confidence: 0.879060208797455

00:12:05.640 --> 00:12:25.640 Population of CD 34 positive cells expressed high levels of L selection in beta 7, Integrant and these 2 molecules were known at this time to be important for homing to secondary lymphoid tissue such as tonsil and spleen and lymph. Notes so they've never been CD 34 cells found in any of these tissue.

NOTE Confidence: 0.889945566654205

00:12:26.440 --> 00:12:46.440 Time, but we decided to take a look and so we first speech our system work. We had a CD 34 column. We put blood through CD 34 column and you see all 3 populations when enhanced through this. Selective column found in your circulating blood when we did the same thing with the collection of human lymph nodes or tonsil you.

NOTE Confidence: 0.886772572994232

00:12:47.240 --> 00:13:03.600 We see this population so the receptor biology brought us to the right population and in fact when you take that population out of the fresh lymph nodes or console and you put it in. I'll 15 you. See you get your CD 56 bright natural killer cells right here.

NOTE Confidence: 0.890671253204346

00:13:05.340 --> 00:13:25.340 So that was really interesting and then we said well. Why are they going to lymph nodes and we looked at lymph nodes and we found that there were CD 56 bright immature NK cells in the lymph nodes as well, and they lived around the parafollicular T cell. Rich region of the lymph nodes and so we asked ourselves well if you have the early NK cell here and.

NOTE Confidence: 0.895708739757538

00:13:26.140 --> 00:13:43.850 Earliest precursor here in secondary lymphoid tissue. Maybe this is where human NK cells actually develop and mouse. They develop in the bone marrow and it didn't seem to be the case in humans, so a lot of work and by the way I'm showing different pictures of people that responsible for the work.

NOTE Confidence: 0.855249583721161

00:13:45.020 --> 00:14:05.020 In the lab we narrow this down to 3 different markers acquisition of CD 34, no seek it. No see 94 CD 34 with Scikit. No seating 94 Los of CD 34 CC kitten CD 94 negative and then just CD 94.

NOTE Confidence: 0.868023812770844

00:14:05.990 --> 00:14:25.990 When you took these populations. You said let's look at CD 56 and this is from lymph nodes. You see that we see absent some a lot and then CD 56 bright cells. So we name. These stages 123 and 4 of NK cells develop we could find these all fresh and lymph nodes and tonsil.

NOTE Confidence: 0.906342446804047

00:14:26.790 --> 00:14:46.790 Interesting journey characterizing these various stages of NK cell development for number reasons. One reason is when we characterize this population here, we found out that actually before it transforms to an end kasell. It's actually what's called an innate lymphoid cell type 3 ILC threes?

NOTE Confidence: 0.905610740184784

00:14:47.590 --> 00:15:07.590 Important in gastrointestinal physiology, and pathophysiology secreting abundant. Interleukin, 22, which drives the epithelial cells of the gut to secrete defensins for protection against bacterial invasion when this cell actually loses a transcription factor called HR.

NOTE Confidence: 0.889446020126343

00:15:08.390 --> 00:15:15.920 Drives all the way to do natural killer cell so this. I'll see 3 and NK cells actually live in balance, depending on which tissue there in.

NOTE Confidence: 0.852671504020691

00:15:17.420 --> 00:15:35.180 But the picture began to sum up something like this, where T cells. T cells developing in the Thymus B cells in the bone marrow and NK cells, having a projector that left the bone marrow's went to secondary lymphoid tissue and then really preceded in this pathway here.

NOTE Confidence: 0.912949800491333

00:15:36.580 --> 00:15:56.580 And over the years, just to summarize work from our lab, predominantly in some work from other labs. We've gone on to further dissect how not only NK cells develop but how human innate lymphoid cells of which there are at least 3 types developed within secondary lymphoid tissue with a variety of different types of discovery.

NOTE Confidence: 0.889629662036896

00:15:57.380 --> 00:16:17.380 Focus on one of those I'm here you see we've learned that we go from a projector here to about 3 or 4 different stages. Here we get a projector for both I'll see 3 and NK and when you get to this stage 4, eh, it's quite a unique population. I'll show you how this works so.

NOTE Confidence: 0.870886087417603

00:16:18.180 --> 00:16:38.180 Acquire CD 117 that's shown here, then you require and acquire NKPAT and then you acquire CD 16 and once you get to CD 16. You gotta mature NK cells that floating around in your blood remember CD 16 is the receptor for human globulin? What's important for a toxin and or so.

NOTE Confidence: 0.8690345287323

00:16:38.980 --> 00:16:58.980 Mediate antibody dependent, killing and this all occurs through these inside shoe. These pathology flow. Cytometric pictures of

normal lymph nodes and tonsil as well as well as all your lymph nodes and of course, you have kilograms in kilograms of your lymph nodes. So lots and lots of these cells are develop.

NOTE Confidence: 0.900504350662231

00:17:00.070 --> 00:17:20.070 What's interesting is that this stage for a As you seen here we actually have found something very interesting to show you where this work goes into the clinic and into the lab and so, if you look it. Turns out here's your C 4, A is only in tonsil or lymph nodes. You don't see it.

NOTE Confidence: 0.886951088905334

00:17:20.870 --> 00:17:40.870 Or bone marrow or other places as well, what we found is that there's a malignant car apart to stage for A and that's this EDDTNK cell lymphoma. This God awful tumor that you see here and it turns out that if you look at 4 different patients with this and you look at their tumors whether it's Blood Bowl.

NOTE Confidence: 0.8783820271492

00:17:41.670 --> 00:18:01.670 Blood here or central nervous system with CNS involvement. All of them have their malignant counterpart in this stage for a originally found it's part of the NK Cell Development Paradigm. So we're actually doing lots of work on the Genomic, an epigenomic differences between these normal and malignant popul.

NOTE Confidence: 0.868423223495483

00:18:02.470 --> 00:18:06.560 Very interesting findings and moving this out publication soon.

NOTE Confidence: 0.87370091676712

00:18:07.960 --> 00:18:19.620 So now you're experts on NK cell development. There's no quiz. But I'm sure you all get. It now will talk a little bit about survival and what goes wrong with NK survival so.

NOTE Confidence: 0.881406664848328

00:18:21.140 --> 00:18:41.140 Once we understood it, I'll 15 was critical for the development. We asked you know what? How to NK cells sustain just like your hemoglobin. They remain very, very constant your body despite tremendous dynamics. And we ask the file 15 could be the factor responsible for that. So just taking NK cells and placing them and I'll 15.

NOTE Confidence: 0.874306380748749

00:18:41.940 --> 00:19:01.940 Media You can see the tiny amount of pile 15.01 nanograms per mill will sustain their survival for over a week, so that gave us a hint but it's only of course in in vitro experiment to do this properly. You'd want to take normal NK cells. Popham into a mouse that has no I'll 15 and see if.

NOTE Confidence: 0.872123956680298

00:19:02.740 --> 00:19:22.740 Switch can't develop in that mouse still surviving that mouse and so we did that experiment. Here's your normal NK cells from a wild type mouse coat them with and I put them into a knockout mouse and say do they survive in the picture tells the story because here's normal mice going into normal mice, so you took him out. You label them with die.

NOTE Confidence: 0.892068684101105

00:19:23.540 --> 00:19:43.540 And 36 hours later, you see their .12 is what's in there and a week, 5 days later. It's .1 want essentially the same. You do this into an aisle 15 knockout mouse wild type cells into another 15 and within 36 hours. You're drastically down and within 5 days. They're completely gone, so this told us that I'll 5th.

NOTE Confidence: 0.884484648704529

00:19:44.340 --> 00:19:51.120 Very very important for not only in case of development, but in case cell homeostasis survival in people as well.

NOTE Confidence: 0.888649702072144

00:19:54.920 --> 00:20:14.920 So where is the Isle 15 coming from obviously not the marrow. These aren't developing the marrow something in the left out and we figured this was an antigen presenting cell most likely dendritic cell so we ended up doing experiment again going to the mouse to prove that when you knockout dendritic cells, which I show here this is the dendritic cell.

NOTE Confidence: 0.885340094566345

00:20:15.720 --> 00:20:35.720 There knocked out through this mechanism actually NK cells are drastically reduced if not almost absent so the picture is for 3 log end is important not only for that CD 34 cell, but of course, it sustains the survival and growth of dendritic cells. It induces I'll 15 or sustains out.

NOTE Confidence: 0.884568750858307

00:20:36.520 --> 00:20:52.880 The brain surface through that I'll 15 receptor Alpha that high affinity chain and then that with precursors leads to NK cell development and then as NK cells develop and trickle through your secondary lymphoid tissue constantly. They get a hit from Isle 15 and continue with their survival.

NOTE Confidence: 0.887161910533905

00:20:53.900 --> 00:21:13.900 What's interesting about I'll 15 is that the M RNA is in virtually every tissue in your body is abundant in brain heart, liver kidney, but very, very little protein anywhere. And so it's modification. It's impossible detect most methods.

NOTE Confidence: 0.888150095939636

00:21:14.700 --> 00:21:27.050 For example, so one of the things it must be is that it's being translated post transfer posttranslational regulation post transcriptional regulation and so we looked at that.

NOTE Confidence: 0.881478071212769

00:21:28.060 --> 00:21:48.060 And you can see here that what we did is we did a little jockeying with this gene and put basically some constructs into the gene to make it so that the transcript. The abundant transcript was transcribed and not only transcribe but abundant. Lisa created and developed this transgenic mouse simply to ask the question when you alter this site a kind.

NOTE Confidence: 0.859591901302338

00:21:48.860 --> 00:22:08.860 K cells as you can imagine NK cells are abundantly increased as shown here here, you're looking at a mouse and you could see a wild type mouse has this much NK same mouse transgenic for the Isle 15, where it's getting abundantly transcribed translated. It's created his huge numbers of NK cells so.

NOTE Confidence: 0.892624914646149

00:22:09.660 --> 00:22:29.660 People driving development, sustaining survival you're going to get lots of NK cells. What was perhaps more interesting, however, is this is, after about 3 to 6 months. These mice developed TNKSLLGL leukemia. I think was the first time a single sided kind manipulation was shown to be.

NOTE Confidence: 0.87018620967865

00:22:30.460 --> 00:22:50.460 In a mouse and these bice developed massive Lucas Cytosis, sometimes 5600 thousand and I from overwhelming accumulation of these blasts in the body and as I say it was NK cells is shown here, but also NKT which is another innate immune effector cell.

NOTE Confidence: 0.858625590801239

00:22:51.260 --> 00:23:11.260 When we looked at the NKT cells. We could determine who is clonal by looking at the T cell gene rearrangement and we could show here that it was in fact model here is an example of the lymphoblast you can see prominent nuclei prominent nucleoli and very blast like picture different from the large gradient lymphocyte in normal.

NOTE Confidence: 0.832088768482208

00:23:12.160 --> 00:23:18.240 Further very much very easy to adopt complete transfer and lethal in the syngeneic mice.

NOTE Confidence: 0.877191603183746

00:23:19.420 --> 00:23:39.420 That was interesting and specially because we also study AML, which I'm not talking about today. But the leukemia. Ologist Here

know that about 30% of AML in some LL have constituent of activation of flip 3, the receptor tyrosine that I talked about.

NOTE Confidence: 0.86890572309494

00:23:41.220 --> 00:24:01.220 And when it's the ideas present that is can step receptors constituent. Lee active as if there's abundant. Liggen, always activating the Receptor and that's at least a precursor to malignant transformation into AML and sometimes they LL but fully 30% of AML has this particular mutation usually.

NOTE Confidence: 0.857456803321838

00:24:02.020 --> 00:24:22.020 Operative Mutation was interesting to us because we didn't have a constituency active receptor like the TD. But we had constituent Lee active lie again in this transgenic mouse. So it was like the aisle. 15 Beta Gamma Chain was always on and sure enough, we saw this with just this.

NOTE Confidence: 0.881630718708038

00:24:22.820 --> 00:24:42.820 Phone we saw this transformation, so this became interesting and relatively easy for us to study and I'll make a Long story short about that kind of working backwards. We notice that the blasts in both human LG L&R. Mice with this, LG L leukemia had a Barent and abundant centrosomes.

NOTE Confidence: 0.867004990577698

00:24:43.620 --> 00:25:03.620 Look at the pathway that's important for centrosome development and maintenance and it involved mic and so to summarize what we determined was that I'll 15 was driving NF. Kappa beta increase mic and then together, we're driving up Aurora kinases aid be and that led to.

NOTE Confidence: 0.8458052277565

00:25:04.420 --> 00:25:24.420 Ability through this centrosome apparency and part of the story for Leukemia Genesis. What we also found which was interesting was that NF cap would be in Mick. We're teaming up with H Dec, one and this trimer repressor complex pop down and drove near 20.

NOTE Confidence: 0.848439574241638

00:25:25.220 --> 00:25:45.220 To the depth which mere 29, B regulates DNMT 3 B in the leukemia, Docs now. This is often mutated in AML and BI. Lo mere 29 be an high DNMT 3. P we got tremendous amounts of meth. Elation at the 5 Prime regulatory region of jeans, which led to further chromosone instability gene silencing.

NOTE Confidence: 0.84805816411972

00:25:46.020 --> 00:26:06.020 Yeah, they put this pathway together for the Genesis of LG L leukemia through overexpression of Isle 15 an to focus on

this therapeutically. We had determined that bart is a map actually broke up this repress are complex and could lead to dry.

NOTE Confidence: 0.855647385120392

00:26:06.820 --> 00:26:26.820 Time be up and EMT 3 beat down and so we focused on that and here you see that simplistic schema where you have partism abt hitting on their oppressor complex. This disappearing near 29 be going way up driving DNMT 3 be way down and so we treat the LG L leukemia.

NOTE Confidence: 0.821109175682068

00:26:27.620 --> 00:26:47.620 UCL the DNMT 3 B disappears up here in this gel, so seem to be working. We took this then to the clinic and had huge success, kidding, but didn't give up actually turned out the PK was off.

NOTE Confidence: 0.858004927635193

00:26:48.420 --> 00:27:08.050 Developing a nano part is a map and ended up showing that in our animal model. We could get 100% cure of this, LG L leukemia with this nanoparticle map in this gentleman still at OSU is further developing this compound for the clinic. The other thing I just thought I'd show here is.

NOTE Confidence: 0.891907632350922

00:27:09.090 --> 00:27:29.090 Very interesting actually came in my lab instead so that happens in 30% of the transgenic mice. What happens, the other 70%. Of course I had never been to the mouse room. So I said, I don't know so she went to the mouse room in a Long story short, she found that these mice were getting this progressive alopecia and ultimately this.

NOTE Confidence: 0.830910205841064

00:27:29.890 --> 00:27:49.890 Cutaneous problem denuding of the fur etc. An again telling a long story in a single slide. This turned out to be a great model for CTCL cutaneous T cell phone with something studied so, so incredibly well here.

NOTE Confidence: 0.86176460981369

00:27:50.690 --> 00:28:10.690 And as you can see here, the pathology is classic foresee TCL and I could replace these with normal and see. TCL patient and quite indistinguishable. The infiltrators a CD 3, Knoll or a CD 3 CD 4 T cell and again lot of work being done comparing.

NOTE Confidence: 0.885007321834564

00:28:11.490 --> 00:28:22.110 In this mouse model and got great strategies now on their way to the clinic to both slow down or prevent the disease very exciting epigenetic modifyers.

NOTE Confidence: 0.864114105701447

00:28:23.050 --> 00:28:43.050 So that's a bit about NK cells survival deregulation by the way I'll 15 is abundantly overexpressed in CTCL and you may now know there's a peptide being test that blocks I'll 15 binding and they've had some maybe it's open the trials opened here, but there's been some dramatic responses and multiply relapse.

NOTE Confidence: 0.909443438053131

00:28:43.850 --> 00:28:51.310 Suggesting that what we're finding in this transgenic mouse is quite quite important in the human pathogenesis of the disease.

NOTE Confidence: 0.869299113750458

00:28:52.510 --> 00:29:12.510 So moving on to a little more defense of what are these NK cells normally doing in the body here you see this is a typical flow of your blood your T cells and B cells are down here. Your NK cells. A few of them in mature ones up here that bright cells as they become dim they acquire CD.

NOTE Confidence: 0.867849230766296

00:29:13.310 --> 00:29:33.310 That molecule it hooks up with antibody to do the ADC, killing and so one of the things we were wondering is does it bright cells floating in your blood versus largely in the lymph nodes and the dim cells? Which you don't find in the lymph nodes, but are in your blood abundantly do they have different immune regulatory properties and so?

NOTE Confidence: 0.860984325408936

00:29:33.390 --> 00:29:53.390 One of the things we knew is that NK cells can make gamma interferon in a heartbeat like within a minute if their tweak the right way, with Mana Kinz. I'll 12 by 18 IA one. I'll 15 they could make lots of Game Interferon and we knew that monocytes need game interference to contain obligate intracellular.

NOTE Confidence: 0.878222942352295

00:29:54.190 --> 00:30:14.190 CB and pneumocystis and listeria etc. And so we said well if they don't make gamma they must get it. Early on from NK cells and could there be a difference between the bright selling them cells to what cells providing that monocyte macrophage with that requisite gamma, interferon to contain these.

NOTE Confidence: 0.85563987493515

00:30:15.020 --> 00:30:35.020 And we know these infections are contained because people that lack either the Gamma Receptor Organ Interferon die of overwhelming obligate intracellular pathogens such as tuberculosis so we asked are they the same or different. We set up an assay where we put monocytes in a well needed the bright cells are that.

NOTE Confidence: 0.84758335351944

00:30:35.820 --> 00:30:55.820 And then we added the pathogen and to see Monica is delivered is the bright so the dim cell that cell that's delivering the game interferon and it turns out you can see from this slide, even when you just give the monic kinds or if you do that ass. I showed you it's really the bright cells that are making all of the gamut.

NOTE Confidence: 0.850536048412323

00:30:56.620 --> 00:31:16.620 Cells that are more abundant in the blood art that made more sense to us because of course. The dim cells live in the blood and Bryce largely live in that parafollicular T cell. Rich region of the lymph nodes so one model is within your lymph node pathogens enter and case.

NOTE Confidence: 0.861733436584473

00:31:16.670 --> 00:31:36.670 Those are tweaked with Mana kinds, they make lots of Gamma Interferon, there more Mana cards produce and it's a very nice cycle. We also learned I'm not developing the story here at all today is that In addition to that. Those NK cells that sit in the parafollicular T cell. Rich regional lymph node actually get a signal from T cells as well and.

NOTE Confidence: 0.859449446201324

00:31:37.470 --> 00:31:57.470 Tiny amount of Interleukin, 2 that the T cells secrete when it's activated because just the bright cell not the dim cell has the high affinity. I'll 2 receptor on its surface. In addition to the beta gamma itself, so it can compete for I'll too. With the T cell so if it gets the energycap presenting cell for example, gives 12.

NOTE Confidence: 0.88014680147171

00:31:58.270 --> 00:32:18.270 Who is Shawn this in our culture constructs you actually get abundant game interferon that can drive this to a TH one important adaptive immune response to certain pathogens so the NK cells involved in both of those the bright cell. I should say is involved in both of those in its niche in the secondary lymphoid.

NOTE Confidence: 0.315466046333313

00:32:19.070 --> 00:32:19.500 Sets.

NOTE Confidence: 0.870350539684296

00:32:20.480 --> 00:32:40.480 So that's here bright cell doesn't have many granules again receptors tell the story produces lots of side of different cytokines to stimulate the innate and adaptive immune system certain receptors that suggest homing etc and very little CD 16.

NOTE Confidence: 0.838379383087158

00:32:41.280 --> 00:33:01.280 Very little granules, so not a killer in contrast, the dim cell was floating around in your blood abundant granules. Lots of different receptors. Here's The Cure Cure Globulin receptors, which are inhibitory signal

largely tells NK cells don't kill and I'll refer to that a little later very few cited kinds.

NOTE Confidence: 0.86200350522995

00:33:02.080 --> 00:33:22.080 To do an abundance CD 16 to bind all that antibiotic and so there's been a beautiful story developed when the cure is Michmash Mismatched in allogeneic transplant. Many of you hematologist know that certain not going to talk about today is fascinating there's increased survival in patients that uh.

NOTE Confidence: 0.89169979095459

00:33:22.880 --> 00:33:42.880 Call T cell depleted mismatch transplant that have cure mismatch as well to talk about the CD 16 receptor largely today. But I'm going to refer back to these inhibitory receptors for a moment and talk about some discoveries. We've made and how we're taking them to the clinic about CD 16, which as I say we know is important.

NOTE Confidence: 0.845894932746887

00:33:43.680 --> 00:34:03.680 We could try to get inside the mind of the killer. This is an NK cell is the tumor cells puncturing holes from its perforin release and then cytoskeleton of the tumor and the NK cell recovers. After that can regenerate and kill again? So very interesting tumor license. So I'm going to talk about glioblastoma bad tumor.

NOTE Confidence: 0.865578770637512

00:34:04.480 --> 00:34:24.480 Now very fail, we've all known friends and family have had this devastating disease. Horrible survival with this been working on this in our lab now for awhile because I teamed up with a neural neurosurgeon.

NOTE Confidence: 0.840439796447754

00:34:25.280 --> 00:34:45.280 Call Kyoka is that Ohio State and he kept noticing that there were Gamma Interferon, producing cells when he would try to treat GBM with uncle lytic virus uncle herpes virus. I'm going to tell you how that stories involve for us, so just the Digress. We do have a car T program very act.

NOTE Confidence: 0.845201849937439

00:34:46.080 --> 00:35:06.080 Will soon have car MK 4 GBM at city of hope and this has been very, very interesting study that continues so here's what happens with uncle lytic virus is couple videos in here and just to show you So what we do is you create a virus that only becomes lytic.

NOTE Confidence: 0.875462412834167

00:35:06.880 --> 00:35:26.880 Context of a tumor specific promoter which is found in the GBM and not in your normal brain tissue. So herpes virus is, you know is a neurotropic virus right kids die of herpes encephalitis adult style

represent satellites. It's attracted to the nervous system so it's a perfect virus to consider therapeutics because it goes to neural tissue and with.

NOTE Confidence: 0.846186339855194

00:35:27.680 --> 00:35:47.680 It'll only become lytic in in a GPM versus normal tissue. So what happens is the virus comes along it will infect one cell and then what's critical is that those viruses able to spread along it spreads along as its president. It then becomes lytic so it need.

NOTE Confidence: 0.865482866764069

00:35:48.480 --> 00:36:05.600 To do this, it needs time to infect and it needs time to spread before it will become lytic problem with this and why my friend in Tokyo called me is that it turns out NK cells protect you against dying of herpes.

NOTE Confidence: 0.873141765594482

00:36:06.680 --> 00:36:26.680 Um anybody gets a cold sore it's a limited infection. It's not ebola right. Whatever you have an infection. But you don't have any be seller. T cell immunity to it. Your first exposure, but it's very limited and it turns out that NK cells are critically important to limit that infection as as evidenced again by Mother Nature.

NOTE Confidence: 0.886654794216156

00:36:27.480 --> 00:36:35.860 And the problems we have when NK cells aren't around. This has been shown in several different examples, but how and why they work was really unknown.

NOTE Confidence: 0.877955436706543

00:36:37.090 --> 00:36:57.090 So what we found Neo Cap collimated gamma producing cells is that the NK cells were flocking into the brain tumor before the herpes virus got a chance to work on the brain tumor. Massive numbers of NK cells going in there and killing the virus and I'll just illustrate that so here's your.

NOTE Confidence: 0.877549052238464

00:36:57.890 --> 00:37:12.730 In the context of normal neural tissue, you get your herpes virus comes in. It's going to again go to infect your GBM and start its processes spreading before it even gets a chance to spread your NK cell is there to kill it.

NOTE Confidence: 0.881107449531555

00:37:13.790 --> 00:37:33.790 Such that it's ineffective and we showed in rat models that you deplete NK cells or mouse models. You can get great response of the brain tumor. So we knew we had something but understanding what to do really had to understand what's going on here to really start to and no clue despite that other paper. I think being published in the early 90s.

NOTE Confidence: 0.832256495952606

00:37:34.730 --> 00:37:54.730 So gentlemen in our lab function when it had any cloned every single gene of the herpes genome into a GBM cell line and then he took and that is about 70 or 80 jeans and then he took NK cells and he did this, killing assay he said. Which of these jeans are the NK cells getting activated?

NOTE Confidence: 0.857472598552704

00:37:55.530 --> 00:38:15.530 And, which ones are they being inhibited by the GPN it turned out there about 3 of the ladder but about 5 of the former the front about 5 jeans that you put that single gene in the GBM. All this messy stuff and you tilt. The scale and that GBM goes to history with the NK cell so he found the jeans and.

NOTE Confidence: 0.834135413169861

00:38:16.330 --> 00:38:34.320 And I'm going to refer to 2 called glycoprotein E and glycoprotein, I and these 2 were the most potent if you have GE GI GI by itself did nothing but GE with Georgi alone. You get the kill it and the killing would release the grain size, you get the cell death.

NOTE Confidence: 0.529873907566071

00:38:35.360 --> 00:38:36.070 So.

NOTE Confidence: 0.844231903553009

00:38:37.100 --> 00:38:57.100 Just going to explain how this work and how we're exploiting it so I said normally what happens is the topic of your NK cell and there's it's CD 16 stick it off of the surface. It binds FC and of course, your tumor cell has tumor specific immune globulin protoxin per se.

NOTE Confidence: 0.811416029930115

00:38:57.900 --> 00:39:17.900 CS either CD 20 Hertz, dude on the Antigen specific way. The Fab Fragment of fines to that specific antigen and the end. K cell then binds to a different region. It binds to the FC region here as you could see FC.

NOTE Confidence: 0.856120765209198

00:39:17.930 --> 00:39:36.150 Non antigen reason, Anet Locks and loads when you get dimerization shoots a signal to the NK cell. It kills the tumor cell and that's at least in part, how things like Herceptin and Rituxan are working in an anti tumor. It's called antibody dependent cellular site successfully been known for 40 years.

NOTE Confidence: 0.865779519081116

00:39:37.390 --> 00:39:57.390 What we found was something completely different and irrelevant to herpes and other pathogens that I'm not going to talk

about. But so here's your CD 16. It's binding to that. FC portion of the antibody here. It turns out the GENGI of the herpes genome wooden infects the GBM or whatever it sells infect.

NOTE Confidence: 0.847438752651215

00:39:58.190 --> 00:40:05.470 Add GENGI go on the surface that forms a protein that also binds FC.

NOTE Confidence: 0.843174576759338

00:40:06.690 --> 00:40:16.330 It's called an FC binding protein so just like CD 16. It's binding FC. But it's binding it in a different region.

NOTE Confidence: 0.832225561141968

00:40:17.330 --> 00:40:37.330 And it allows the CD 16 to still bind wenig lab in his Brown to the FC binding protein or the dimer up here of CD 16 with any immune globulin on services limited in your body turns out, I'll show you NK cells are covered in a globulin without any antigen specificity.

NOTE Confidence: 0.876406967639923

00:40:38.130 --> 00:40:58.130 The system working before you have any edge of specificity. It binds here it finds this receptor on the surface of the HSV infected GBM and then they lock and load and again. The key with this is this without any engine specificity so before you have immunity to herpes with B cells and T.

NOTE Confidence: 0.859893560409546

00:40:58.930 --> 00:41:18.930 Your NK cells, which are floating around with limited globulin stuck to their CD 16 use that dimer to CFC binding proteins. And I'm not going to talk about it today, but FC binding proteins are also on staff. A and they're on strep. So it's a broad mechanism of innate immune recognition of pathogens any pathogen that encode.

NOTE Confidence: 0.915949761867523

00:41:19.730 --> 00:41:23.280 See binding protein is recognized by this mechanism.

NOTE Confidence: 0.84367311000824

00:41:24.730 --> 00:41:44.730 And then you get your license and we did. This crystal graphic structure of this region was known CD 16 binding to human FC and of this FC binding proteins bind inhuman FC and as it shown here in the crystal in the in silico representations. Here's Gigi from her.

NOTE Confidence: 0.86059433221817

00:41:45.530 --> 00:42:05.530 Here CD 16 from the NK cell no overlap whatsoever, so this can and does occur structurally and have lots of proof of this. I'm just going to show you a couple things. Here's how this would work in our

cartoons. So now you got your GBM here in the midst of your normal tissue and again, you're HSV is coming in and because of that tumors.

NOTE Confidence: 0.812385320663452

00:42:06.330 --> 00:42:25.380 Here it combine only infects will become lytic and only those cells and so the first cell goes to lice, but it can't because the NK cell comes in quickly. Anne Lise is that HSV infected GBM before it can really spread throughout the whole tumor.

NOTE Confidence: 0.825606107711792

00:42:26.350 --> 00:42:46.350 So that's why is it goes like the next one notice there's no spreading of the virus. Here's an up close? What's going. This is GB a membrane HSV infected so there's GE. HSV infected there's GI they come together and form a high affinity FC binding protein for emitted globular then the NK cell, which is.

NOTE Confidence: 0.863325297832489

00:42:48.830 --> 00:43:04.080 Up there with it, you'll see popping up all over the surface of the GBM here and K cell has CD 16 on its surface. It's gotta admit globulin stuck to it, and it has as I said, this binding area called the bridge area.

NOTE Confidence: 0.845138728618622

00:43:05.180 --> 00:43:16.650 Binds the CD 16 and this called CH 2. CH 3 region that binds the high affinity. FC binding protein and then you get your dimerization your activation in your kill it.

NOTE Confidence: 0.52803760766983

00:43:18.830 --> 00:43:19.450 So.

NOTE Confidence: 0.848981857299805

00:43:22.400 --> 00:43:42.400 Is this real so here we're looking now were staining NK cells to say? Do they have a big lobby on the surface? When they're floating around in your blood and the answer is they do here is imminent globulin staining. Here's your NK cell. Here's your T cell. You can see the drastic difference and that's what it would look like. But it turns out, we're all different we.

NOTE Confidence: 0.888840734958649

00:43:43.200 --> 00:44:03.200 Infinity so here's 3 different donors In addition to this donor you see look at the numbers here. This is just indicating the amount of immigrants binding relatively high low low so depending on who you are is how much immunoglobulin is on the surface of your NK cells and if that's the case then.

NOTE Confidence: 0.888868570327759

00:44:04.000 --> 00:44:24.000 Killing mechanism I showed you is the case. Then there should be variation between all of us. When we actually see a herpes infected cell whether it's GBM or otherwise. There should be a difference because we have so much only so much in many globulin on the surface or abundant in Munich globulin on the surface of the NK through the CD 16.

NOTE Confidence: 0.857327818870544

00:44:24.800 --> 00:44:44.800 It took 25 people randomly and just measured their intensity. How much in England, they have on their surface and the disaster cells do. They all killed GBM the same or is it different? Strikingly different highly correlated so the more I'm going to have a new surface more activation or killing you have at the GB.

NOTE Confidence: 0.883820116519928

00:44:45.640 --> 00:45:05.640 Perfect correlation you take that same 25 people and you have them kill an NK sensitive target. K 562 that doesn't have Gigi on its surface. No correlation whatsoever. Same people so that told us that in fact. This is a functional mechanism. That's relevant to people and you can understand.

NOTE Confidence: 0.874162793159485

00:45:06.440 --> 00:45:26.440 I don't understand why at least in this case why different people respond to different pathogens differently in this case this by the way is called we've we've got a name for this. It's called FC bridging ADC so because the FC Fragmente is bridging between the end case.

NOTE Confidence: 0.834577143192291

00:45:27.240 --> 00:45:47.240 Herpes infected cell so how about in vivo does FC bridging Cellular State, Texas. He protected because we know mice have CD 16. They have NK cells. They bind their own immunoglobulin. This should be able to see weed affect the mouse with herpes NK cells should see it and prevent it well, it turns out that this.

NOTE Confidence: 0.861390292644501

00:45:48.040 --> 00:46:00.550 When pathogen and it this FC binding protein doesn't see the mouse. CHCH 3 region, so in fact, you don't get recognition and you get 100% fatality.

NOTE Confidence: 0.846922159194946

00:46:01.530 --> 00:46:21.530 But we should be able to take that same mouse and inject it with human human globulin. And if there's enough. It will compete for the CD 16, it does bind human globin mouse CD 60, Bynes went above and we know that CH 2 CH 3. This whole complex recognize the SD binding protein so now if I give one.

NOTE Confidence: 0.823308289051056

00:46:22.330 --> 00:46:31.120 This antibody and it could be irrelevant antibody. It should provide protection when I give a fatal dose of the HSV one.

NOTE Confidence: 0.869800806045532

00:46:32.270 --> 00:46:52.270 And in fact, I should be able to cleave off the Fab Fragment and just give FC because this is a complete innate immune function and indeed that's in fact? What is the case as you can see here whether I give Retaks and whether I give Derek to mmap weather, I just give the FC.

NOTE Confidence: 0.874751389026642

00:46:53.070 --> 00:47:13.070 Portion of any imminent globulin, you get nearly complete or complete protection against the HSV infection. Fatal infection in the mice further if you knockout. The NK cells as shown in the bottom line with something called a sale. GM one before you do the exact same experiment you completely lose the protection because now.

NOTE Confidence: 0.849477112293243

00:47:13.870 --> 00:47:27.610 FC fragment before you give the herpes. You've taken out the NK cell that CD 16 is not there and so even though the FC fragments binding to the herpes FC binding protein. There's no killer cell to come in and clear it out.

NOTE Confidence: 0.850825369358063

00:47:30.420 --> 00:47:50.420 So therapeutically what could we do to delay those NK cells from killing that GBM and it turns out that there's a natural bacterial pathogen that struck progenies that makes an endopeptidase that cleaves globulin and this actually has been given in vote patients.

NOTE Confidence: 0.881557464599609

00:47:51.220 --> 00:48:11.220 This endopeptidase to stop hyper acute rejection of kidney transplants and in vivo in the human body. It cleaves this so you're just left. With this piece, which binds to CD 16 or this piece, which binds that FC binding protein and so, if you purify this you should be able now to take your cartoon and what you should do is.

NOTE Confidence: 0.851715981960297

00:48:12.020 --> 00:48:32.020 This FC piece and it should bind only to the herpes infected FC binding protein and block the NK cell from seeing that free site. Here's the FC Fragmente coming in blocking GE GI so now when the NK cell comes by that sites occupied.

NOTE Confidence: 0.897212445735931

00:48:32.820 --> 00:48:52.820 Get that sell the virus has time to spread replicate and lice and you could prolong your survival and so we've been working on this to do this in vivo and ultimately in patients for this another pathogenic diseases

that I'm not going to talk about today that are relevant to this mechanism used surveillance.

NOTE Confidence: 0.847275078296661

00:48:53.620 --> 00:48:54.340 They discovered

NOTE Confidence: 0.855499744415283

00:48:55.540 --> 00:49:15.540 So one thing is our lab is developing EGFR car for GBM going to talk about it, but it's relevant to what I'm going to tell you about and it turns out you can get a nice little bump in survival and Xenogeneic. Human GBM when you take NK cells and Expresa car on the surface that can find both mute.

NOTE Confidence: 0.763500869274139

00:49:15.580 --> 00:49:18.660 In wild type EGFR in this car that we have can.

NOTE Confidence: 0.864199757575989

00:49:19.700 --> 00:49:39.700 It turns out that if you give HSV and NK car sequentially you can get this actually start to get a survival curve with these mice. But what we found is that we took our original herpes virus, which we now have in patients to do start clinical trial.

NOTE Confidence: 0.926917731761932

00:49:40.500 --> 00:49:43.000 We actually added to that herpes virus.

NOTE Confidence: 0.769755423069

00:49:44.190 --> 00:50:04.190 An inhibitory lie again that finds the NK cell cure like molecules. I was telling about is called Cal. GR one KLRG. One so we added this eat cat here in to the purpose virus when it infects the GBM it secrete salivated expresses a log in.

NOTE Confidence: 0.859023690223694

00:50:04.990 --> 00:50:24.990 In case cell from killing it so it actually without needing to do that difficult cleaving experiment talk about if you change your herpetic virus to express an inhibitory lagom NK cell it holds them off for a bit and we've now shown that actually just by itself. This virus can be cured of in these models.

NOTE Confidence: 0.863380551338196

00:50:25.790 --> 00:50:45.790 Wow, scaling up to move this virus to the clinic in the mean time. This is this the so in the mean time. We started our first clinical trial for AP 01. We have with our original herpes virus that is specific for GBM and the idea is that we will next do GBM with EGFR car.

NOTE Confidence: 0.806166529655457

00:50:46.590 --> 00:51:06.590 Ultimately, we will combine the HSV one for GBM followed by a NK car therapy or if we have it in time. We will move to are treating recurrent GBM with our uncle itic herpes virus that expresses eat coherent and then.

NOTE Confidence: 0.820190608501434

00:51:07.390 --> 00:51:27.390 Car NK cells so I'm going to stop there. I want to talk about today or cited kinds in NK development specifically the role of I'll 15 how we use receptive biology to find where the precursor is where it traffics to wear NK cells develop we talk to live.

NOTE Confidence: 0.880323350429535

00:51:28.190 --> 00:51:48.190 Malignant counterparts to those we talked about human NK cell subsets and their role in the immune regulation the immune system prior to Antigen specific immune responses and then clinical application. Receptor biology understanding how in case else herpes virus trying to prevent that.

NOTE Confidence: 0.854232430458069

00:51:48.990 --> 00:52:06.230 Like herpes virus from working in GBM and then ultimately combining that with an NK cell car in this particularly terrible disease. So I'll stop there. Thank all the people in my lab 15 of whom move with us to city of hope. This is the real mastermind here Jim why you?

NOTE Confidence: 0.702141523361206

00:52:09.150 --> 00:52:09.520 There.

NOTE Confidence: 0.886914372444153

00:52:10.740 --> 00:52:17.630 We are responsible for a lot of the work in our laboratory so. Thank you very much for your time in such an honor to be here appreciate.