Sure, there’s enough time for both of you, so I see folks here.

The numbers are going up and appreciate folks logging on welcome everyone once again to Cancer Center, grand rounds, and we’re really very privileged to have two of our exceptional physician scientists presenting.

You know, really and frankly, what’s exciting is it once again highlights the extraordinary work in immunology.

Immuno biology at Yale and at the impact on this ultimately.
In our cancer therapy and in our understanding of cancer biology, so let me turn to our first speaker to ensure we have time. Our first speaker is Doctor David Hafler, who is, you know, is the ugly professor and chair of the Department of the Rolla G and Professor of Immunology, Immunobiology, and David’s accomplishments are are really quite Legion. Renee actually prepared a synopsis, and I just said that I want to make sure David has time to present. I won’t.
Go through all of it,

but his accomplishments in

terms of understanding.

Advancing neuroscience and understanding

that human autoimmunity in an understanding

how to leverage our understanding

of immunology to impacting human

disease is really quite impressive.

And among his awards include the

distal Prize for Ms Research,

the University of Miami

Distinguished Alumni Award,

the American Urology Association,

Adams Lectureship.

And most recently,

and I think a year or so ago,
election to the National Academy of Medicine and David has really been an incredibly engaged member of our Cancer Center faculty. I think David’s leadership, I think, has advanced the cause of our brain tumor program, among other things, an David thank you for making the time to share your work with us today. Thank you Charlie. It’s really a pleasure to be here. And let me turn this on and. My cell phone, so I’d like to do today is to present some new unpublished
work which really epitomizes to me of
physician scientists of learning from
just in a nutshell,
what I'm going to show you is
very fundamental question,
which is what induces the checkpoint
inhibitors particular PD one Tim three lag,
3 digit on human T cells.
that's gonna be the nature
of the talk that the work has
been submitted for publication.
It was put online,
a bio RX being one's interest in
seeing the paper itself and upfront.
I want to really, now Stamos Amita,
who really really performed
00:02:54.220 --> 00:02:56.260 this work in our laboratory tone

NOTE Confidence: 0.9125635

00:02:56.317 --> 00:02:57.987 was now an assistant professor

NOTE Confidence: 0.9125635

00:02:57.987 --> 00:02:59.657 and then pursuing this work.

NOTE Confidence: 0.9125635

00:02:59.660 --> 00:03:00.680 It wanted knowledge.

NOTE Confidence: 0.9125635

00:03:00.680 --> 00:03:02.720 My long term collaborator, Vijay Kutru.

NOTE Confidence: 0.9125635

00:03:02.720 --> 00:03:04.420 Yes, you see a Yale,

NOTE Confidence: 0.9125635

00:03:04.420 --> 00:03:06.460 a sticker that he was here

NOTE Confidence: 0.9125635

00:03:06.460 --> 00:03:07.820 helping us recruit students.

NOTE Confidence: 0.9125635

00:03:07.820 --> 00:03:10.130 Don’t tell the people in Boston.

NOTE Confidence: 0.9125635

00:03:10.130 --> 00:03:12.270 Enjoy dulberg in the Softmod

NOTE Confidence: 0.9125635

00:03:12.270 --> 00:03:14.410 who did the computational work.

NOTE Confidence: 0.9125635

00:03:14.410 --> 00:03:15.902 So the question is,

NOTE Confidence: 0.9125635

00:03:15.902 --> 00:03:17.767 what are the regulatory mechanism

NOTE Confidence: 0.9125635

00:03:17.767 --> 00:03:20.035 for induction of a Co inhibitory

NOTE Confidence: 0.9125635

00:03:20.035 --> 00:03:21.865 receptors on human T cells?

NOTE Confidence: 0.9125635
But I'll show you is surprisingly type one, interferons induce Cohen Cohen

so that's the bottom line of what I'm going to show you over 30 minutes.

We worked through the in vitro transcriptional regulatory network for this interferon beta response and with type one interferon signature, which allowed us to perform an in vivo validation of the in vitro interferon transcriptional regulatory network Co inhibitory receptors. So that’s what my talk will be.
Now it’s been known for a number of years to work.

Button from Vijay Kutru and be ready given we’ve had a program Project Grant 2 program project grants looking Cohen inventory molecules valene sharp for well over 25 years that PD one Tim, three lag three and TIGIT ARCO, regulated and expressed as a module. So here we have. Hopefully you will see the pointer. I won’t advance the slide while I’m doing this, but you can see that there. Expression of PD one Tim,
three lag three and TIGIT on C4 and CD8 cells that their modulated together. And this is a new spot. I'll 27 here. We have the induction of Tim 3 not so much PD one but lag three and TIGIT by I'll 27 you knock down aisle 27 the mouse you lose. That's the upregulation and downregulation by the knock down. Now it's been known for a long time. That type one interferon signatures, or enriching chronic viral infection, and both mouse and humans, and that chronic viral infection
00:05:09.655 --> 00:05:11.923 induces T cell exhaustion.

00:05:11.930 --> 00:05:13.845 Really first identified by Rafi Ahmed in the HIV system and

00:05:13.845 --> 00:05:16.257 in El CMV infection and that’s associated with expression and Co

00:05:16.257 --> 00:05:18.807 inhibitory receptors such as PD,

00:05:18.807 --> 00:05:20.928 One Tim, three lag.

00:05:20.928 --> 00:05:22.868 Three antigen is interferon signature

00:05:22.870 --> 00:05:24.542 with the LC MP model suggesting that there may be an Association with type

00:05:24.542 --> 00:05:26.632 one interferons and these cone hitori

00:05:26.632 --> 00:05:29.024 molecules so wish to ask do they induce these receptors again here’s

00:05:29.024 --> 00:05:31.757 why I showed you in terms of mouse.

00:05:31.757 --> 00:05:34.547 An you know first experiments and when

00:05:34.547 --> 00:05:37.828 why I showed you in terms of mouse.
I googled a photograph of human,
I swear this is what showed up and I know
way mean to denigrate mouse immunologist.
By showing this picture,
but one can see is that in CD4 cells,
This market induction of Tim three lag
This market induction of Tim three lag
This market induction of Tim three lag
This market induction of Tim three lag
three and PD one. By interference.
So now we go into more depth to show this.
Here’s how the experiments were done.
We took CD4 CD 8 cells.
That was CD.
That were CD 45 negative positive.
That is a naive T cells and
stimulate them for non use.
Different different time points with CD3 plus minus. I'll 27 and interferon beta and one can see. The induction of here's a control. The market induction of lag three and Tim three with interfere on. Here's the control and he is looking at Tim three PD. One here is a summary of data with Tim three lag through in PD, one individually and the summary of Tim three lag 3P1 positive cells within this market. Induction by type one interferons interferon beta of these Co inhibitory molecules.
But surprisingly unlike in the mouse with digit is Co regulated part of the module? These other Co inhibitory molecules in human. We saw that TIGIT use digit expression markedly decreased from 25% down to four, 12% from 28% when look the RNA expression we saw there. In fact two modules, one with interferon with Lag, one increase with interferon beta and the other module with digit. The Jennifer subtest. Nine other modules, a CD 160 being decreased by
One interferon. So these data show that in humans there are two modules regulated by interferon that in fact go in opposite directions. Here’s a kinetex. Overtime the induction of Tim three lag, one with the decrease in digit. So just take a step back. Why do we have an interest in Tidjane? I mention this because under the leadership of Antonio Mora we’re about to embark upon a phase one clinical trial in patients with
glioblastoma with anti TIGIT or anti PD.

One or a combination of of the two, working with Jemal eternal and lead in my lab.

By Liliana Luca.

So why an interest in tinge of this work goes back to 2012 work done by S Duluth Lozano in the laboratory.

We’ve always been impressed with the biologic effects of blocking with anti TIGIT looking at Tibet.

The gamut of fear on Gata, 3RF-9 and and RRC expression.

And one can see that with anti TIGIT antibody there’s a market loss of these cytokines in culture and if you
NOTE Confidence: 0.787109
00:09:00.345 --> 00:09:03.467 knock down ticket here within SHR Now
NOTE Confidence: 0.787109
00:09:03.467 --> 00:09:05.848 you have market increases engagement
NOTE Confidence: 0.787109
00:09:05.848 --> 00:09:08.824 affair on and decreases dial 10.
NOTE Confidence: 0.787109
00:09:08.830 --> 00:09:10.750 So comparing PD one antigen,
NOTE Confidence: 0.787109
00:09:10.750 --> 00:09:13.249 our hands in human systems been very
NOTE Confidence: 0.787109
00:09:13.249 --> 00:09:15.993 impressed with the effects of ticket and
NOTE Confidence: 0.787109
00:09:15.993 --> 00:09:18.405 also just comparing Ms two glioblastoma,
NOTE Confidence: 0.787109
00:09:18.410 --> 00:09:21.063 there really isn’t a big difference between
NOTE Confidence: 0.787109
00:09:21.063 --> 00:09:24.529 PDL one or PD1 between Ms and brain tumors,
NOTE Confidence: 0.787109
00:09:24.530 --> 00:09:26.828 but there is a virtual absolute
NOTE Confidence: 0.787109
00:09:26.828 --> 00:09:28.360 difference between TIGIT expression,
NOTE Confidence: 0.787109
00:09:28.360 --> 00:09:31.224 typically on the CD 8 cells in patients
NOTE Confidence: 0.787109
00:09:31.224 --> 00:09:33.727 with GBM virtually absent in Ms,
NOTE Confidence: 0.787109
00:09:33.730 --> 00:09:35.944 he was looking at teacher by
NOTE Confidence: 0.787109
00:09:35.944 --> 00:09:37.940 flow and tills versus blood,
suggesting the potential importance of digit.

NOTE Confidence: 0.787109

In the central nervous system for glioblastoma.

NOTE Confidence: 0.787109

So first one to work through.

NOTE Confidence: 0.787109

After that identification of the effect of type One interferons

NOTE Confidence: 0.787109

wanted to work through the in vitro transcriptional regulatory network.

NOTE Confidence: 0.787109

So we use the same model

NOTE Confidence: 0.787109

that would be regift.

NOTE Confidence: 0.787109

Near Youssef used in terms of setting up identifying the TH17A regulatory network,

NOTE Confidence: 0.787109

and this is work done by a soft in BJ’s lab,

NOTE Confidence: 0.787109

so we needed to have higher resolution transcriptomic data to construct the regulatory network.
00:10:14.750 --> 00:10:17.410 For those of you who aren’t engaging

00:10:17.410 --> 00:10:20.138 in terms of looking at RNA now,

00:10:20.140 --> 00:10:22.317 what we used to do is to

00:10:22.317 --> 00:10:24.760 take a T cell stimulate,

00:10:24.760 --> 00:10:27.298 measure the RNA 4 hours later

00:10:27.298 --> 00:10:30.129 and say this is what it is.

00:10:30.130 --> 00:10:32.800 We’ve learned that their complex regulatory

00:10:32.800 --> 00:10:35.870 networks and one needs to really do this.

00:10:35.870 --> 00:10:38.510 The kinetics overtime to construct

00:10:38.510 --> 00:10:40.622 a dynamic regulatory network.

00:10:40.630 --> 00:10:41.728 Such a performance.

00:10:41.728 --> 00:10:44.930 This network we took dive CD4 CD 8 cells,

00:10:44.930 --> 00:10:45.646 stimulate them,

00:10:45.646 --> 00:10:47.436 measure them in different time

00:10:47.436 --> 00:10:49.220 points with control versus type.
One interferon did bulk RNA sequencing.

We did 34 samples time three replicates with the same healthy donor and we decided that rather than looking at human variation, which is significant mediated by the genetics of the individuals, we do what mouse immunologists do, which is pick one strain of mice and study it in detail. And we measured are we did RNA seek RT PCR protein for flow so that this is a transcriptomic analysis of interferon beta high temporal resolution.

Differential expression of gene levels for eight different time
points with interferon stimulation.

Here’s a log 2 expression so we have differential expression patterns.

We have an early expression pattern here and here.

We have an intermediate expression pattern.

A late expression pattern over here and

finally a bimodal expression pattern goes up,

down and back up.

So in performing it just

transcriptomic analysis,

we looked divided into transcription factors.

Here CD four cells with different kinetics and these are different transcription factors.
Again, we can see early transcription factors immediately, and we identified different Co inhibitory receptors and different T cell related genes for both the CD four and for the CDA population. Again, in looking at the effect of interferon, and what it does in terms of the transcriptional networks is critical to look over time ’cause there’s a dynamic change in these transcription factors and Co inhibitory receptors overtime. So we identified the most differentially
expressed transcription factors and about 20 of them here and these are transcription factors that were differentially regulated and decreased in both CD4 and CD8T cells, and we as a reality check we asked of these word known. Interferon responsive gene. So here’s the IFN responsive gene score overtime and then the green represents regulators for Co inhibitory receptors until the yellow HIV signatures in progressive patients. And then I’ll 27 regulators. So we we want to examine these
transcriptional for these transcriptional factors in detail.

So in order to do this and presented dilemma, we had to develop new technology because I called the Heisenberg uncertainty principle of immunology.

The process of examining the cell with activation perturb the system. Some of looking for an electron after hitting it with HV. So we had to develop a gene knockdown the early time points and primary T cell without activating T cells and again this is all work developed by Tomo by Thomas Anita.

We used an efficient lentiviral vectors
NOTE Confidence: 0.810376
00:13:46.656 --> 00:13:48.768 that developed by wearing a green.
NOTE Confidence: 0.810376
00:13:48.770 --> 00:13:50.795 And basically one takes a
NOTE Confidence: 0.810376
00:13:50.795 --> 00:13:52.820 viral like particles V LP’s
NOTE Confidence: 0.810376
00:13:52.906 --> 00:13:55.276 which is incorporated with TPX,
NOTE Confidence: 0.810376
00:13:55.280 --> 00:13:57.878 which degrades Sam Sam HD one,
NOTE Confidence: 0.810376
00:13:57.880 --> 00:13:58.824 removes restrictions,
NOTE Confidence: 0.810376
00:13:58.824 --> 00:14:01.184 you can transfect primary human
NOTE Confidence: 0.810376
00:14:01.184 --> 00:14:03.530 T cells with this Sam S1,
NOTE Confidence: 0.810376
00:14:03.530 --> 00:14:06.554 which now allows transfection with SH RNA,
NOTE Confidence: 0.810376
00:14:06.560 --> 00:14:08.730 HIV, HIV, lentivirus and all.
NOTE Confidence: 0.810376
00:14:08.730 --> 00:14:12.636 This can be done in an activated T cells.
NOTE Confidence: 0.810376
00:14:12.640 --> 00:14:14.896 Could knock down the gene and
NOTE Confidence: 0.810376
00:14:14.896 --> 00:14:17.410 then do the the incubation.
NOTE Confidence: 0.810376
00:14:17.410 --> 00:14:20.098 So here we have night CD.
NOTE Confidence: 0.810376
00:14:20.100 --> 00:14:21.664 Or cells incubated without
NOTE Confidence: 0.810376

24
CD3 CD 28 with this procedure, knocking down the different genes
and then there is stimulated with and without interferon beta and then measured five days later and then we perform fax GFP of we sort of the GFP positive cells were knocked down and did bulk RNA sequencing and you can see very efficient knockdown in the GFP positive cells. With these different transcription factors. This is a monumental amount to work. Performed by tomo. So we perform principal component analysis to changes in the total RNA expression after the interferon
00:14:58.343 --> 00:15:00.618 signature associated with each knockdown.

00:15:00.620 --> 00:15:03.406 So let me just say that again,

00:15:03.410 --> 00:15:05.400 so these are PCA plots.

00:15:05.400 --> 00:15:07.405 We knock down each transcription

00:15:07.405 --> 00:15:09.803 factor and then looked at all

00:15:09.803 --> 00:15:11.747 the RNA expression and then put

00:15:11.747 --> 00:15:14.179 that into a principle component.

00:15:14.180 --> 00:15:15.724 One in principle component,

00:15:15.724 --> 00:15:19.065 to what that revealed is that the interferon

00:15:19.065 --> 00:15:21.440 one stimulated genes are positive.

00:15:21.440 --> 00:15:25.075 Regulated by we call interferon

00:15:25.075 --> 00:15:28.716 regulated module one, this modulator

00:15:28.716 --> 00:15:31.628 increased the downstream interferon.

00:15:31.630 --> 00:15:36.340 Stimulated genes with module 2 represented

00:15:36.340 --> 00:15:39.480 transcription factors that negatively
00:15:39.579 --> 00:15:43.699 regulated the interferon interferon genes.
NOTE Confidence: 0.79101753
00:15:43.700 --> 00:15:46.388 So to go into more detail,
NOTE Confidence: 0.79101753
00:15:46.390 --> 00:15:48.625 we first have the interferon
NOTE Confidence: 0.79101753
00:15:48.625 --> 00:15:49.966 regulated module one,
NOTE Confidence: 0.79101753
00:15:49.970 --> 00:15:52.586 so a something that a transcription
NOTE Confidence: 0.79101753
00:15:52.586 --> 00:15:55.317 factor that knocks down the gene
NOTE Confidence: 0.79101753
00:15:55.317 --> 00:15:57.587 will lead to decreased expression,
NOTE Confidence: 0.79101753
00:15:57.590 --> 00:15:59.830 which means as positive regulating.
NOTE Confidence: 0.79101753
00:15:59.830 --> 00:16:02.812 So the interferon regular module one
NOTE Confidence: 0.79101753
00:16:02.812 --> 00:16:04.800 regulates the conical interferon
NOTE Confidence: 0.79101753
00:16:04.874 --> 00:16:06.818 stimulated genes over here.
NOTE Confidence: 0.79101753
00:16:06.820 --> 00:16:10.012 Where is interferon regulated module two over
NOTE Confidence: 0.79101753
00:16:10.012 --> 00:16:12.839 here regulates these non Canonical jeans?
NOTE Confidence: 0.79101753
00:16:12.840 --> 00:16:15.080 Interferon stimulated genes perhaps
NOTE Confidence: 0.79101753
00:16:15.080 --> 00:16:18.975 a greater interest was looking at the
NOTE Confidence: 0.79101753
00:16:18.975 --> 00:16:21.687 Co inhibitory receptors so we have.
Interferon regulated module one over here which is bath map. ETS2 SP 140 which differentially regulate lag 3. PD1 PD L1 slam F6 and other transcription factors. And then we have stat one and stat three which positively regulate Tim three but not lag 3. So we see that these different transcription factors differentially regulate different Co inhibitory receptors. And here's a summary. The data just showed you, which is the effect of these
transcription factors.

Interferon stimulated stimulation,

so again there are two modules of transcription factors based on the global effects on interferon stimulated genes,

thereby directly regulated by different modules,

Co inhibitory receptors are also regulated by interferon associate

regulated by interferon associate

transcription factors and which up and down regulate these receptors.

So we have for example, a MoD in module one, the which is a bath.

ETS2 math one which positively
00:17:33.695 --> 00:17:37.868 regulate lag 3 Tim three and PD one

00:17:37.868 --> 00:17:40.378 but negatively regulate a TIGIT.

00:17:40.380 --> 00:17:43.355 BTL BTL A and CD 160 again.

00:17:43.360 --> 00:17:46.335 Going along with the flow cytometry data.

00:17:46.340 --> 00:17:49.238 And again this I showed you step one and three here.

00:17:49.238 --> 00:17:51.030 Positively regulate Tim three but negatively regulate PD one.

00:17:51.030 --> 00:17:53.202 So then we performed a hierarchical backbone network analysis transcription factors.

00:17:53.202 --> 00:17:55.917 I'll just go over this very briefly, but basically looked at gene expression, overtime, differential expression, protein, DNA bonding,
a transcription factor database

Those data looked at a rank list

Those data looked at a rank list

Those data looked at a rank list

Those data looked at a rank list

Those data looked at a rank list

Those data looked at a rank list

Those data looked at a rank list

Those data looked at a rank list

Those data looked at a rank list
The first 16 hours over here which then the dominance of down regulated transcription factors over here.

And just a summary. So there were dominant transcription factors that bridge each wave to the next. So the green circles represent transcription factors that are differentially expressed in one transcriptional wave. Where is the purple circles represent transcription factors that differential expressed in all transcriptional waves. So Cal offense tattoo are early intermediate transcription factors.
00:19:15.880 --> 00:19:17.428 Math blimp one?
NOTE Confidence: 0.80999196
00:19:17.428 --> 00:19:20.008 An MIP are intermediate transcription
NOTE Confidence: 0.80999196
00:19:20.008 --> 00:19:24.304 factors and stat one hit 1A and T bet or
NOTE Confidence: 0.80999196
00:19:24.304 --> 00:19:26.351 bimodal transcription factors apart show
NOTE Confidence: 0.80999196
00:19:26.351 --> 00:19:29.444 this it just to get the bigger picture
NOTE Confidence: 0.80999196
00:19:29.444 --> 00:19:32.720 of the what nature does in terms of the
NOTE Confidence: 0.80999196
00:19:32.807 --> 00:19:35.677 biologic complexity of these systems.
NOTE Confidence: 0.80999196
00:19:35.680 --> 00:19:38.320 So a dear friend of mine,
NOTE Confidence: 0.80999196
00:19:38.320 --> 00:19:42.168 somebody may know of one of the great.
NOTE Confidence: 0.80999196
00:19:42.170 --> 00:19:44.198 Textbook authors of immunology.
NOTE Confidence: 0.80999196
00:19:44.198 --> 00:19:47.240 Abul Abbas would say to me,
NOTE Confidence: 0.80999196
00:19:47.240 --> 00:19:51.344 in Vivo Baratas and then in vitro maybe.
NOTE Confidence: 0.80999196
00:19:51.350 --> 00:19:54.102 So the challenge for us was to find
NOTE Confidence: 0.80999196
00:19:54.102 --> 00:19:56.739 an envy both system which replicate
NOTE Confidence: 0.80999196
00:19:56.739 --> 00:19:59.529 all this lovely in vitro data.
NOTE Confidence: 0.80999196
00:19:59.530 --> 00:19:59.895 So.
Like to show you it in Beeville.

Model that we did not develop a nature developed for us with the viral load.

Strongly correlate with interferon T cell signature which is COVID-19.

So this is work that is presently under revision.

That nature communication, led by a team of individual or for two at the end where we perform single cell.

Now sis of patients with healthy controls and various COVID-19.

samples of individuals with mild, severe or moderate severe disease and basically for the purpose of this talk.
But we found this out as a very strong correlation between the interferon score and the viral load, as measured by PCR. Nasal swabs, in fact, if you look at the correlation time difference between here and the respective change interferon score, we had a remarkable $R^2 = 0.9$.

So nature had accidentally given us a in vivo model of type one interferons in their effect on T cells. So if you look at the interferon signature, it’s higher in progressive Covid patients, his controlled,
stable progressive CD4 CD 8 cells.

One can see that the type one interferon score went up with more progressive disease,

Looking at these, the interferon stimulated T cells in ex vivo with a similar to what we saw in vitro with our interferon transcriptional signature and the answer is yes.

So here is CD4 cells CD 8 cells this this column. Here are the controls, stable and progressive patients. So we see this module too.
Upregulated these are highly upregulated.

Precisely what we saw in vitro in CD4 and CD8 cells, whereas module 1.

Which led to downregulation again of TIGIT BTL ACD 160 and such.

So we had a extremely. Could the recapitulation what we saw on in vitro.

Here’s expression of Co inhibitory receptors for the controls and COVID-19 patients.

Just to summarize, here’s like 3 going up to three going up,

whereas TIGIT Slam 6 and layer one all went down.

Similar to what we saw in vitro.
So we looked at the T cells induced in vitro, which led to with an interferon score and asked that really mirrored the transcriptional wave aren’t dividing covid CD4 and CD8T cells and basically one can see then dividing CD four and eight cells that the in vitro interference core very much recapitulate if we saw in vitro. And finally we looked at the relation between regulators that we saw in vivo and in vitro in this intermediate wave network. The positive regulated transcription factors in red, negative and blue, and we saw that SP.
00:23:11.200 --> 00:23:13.400 140 is a bidirectional regulator,
NOTE Confidence: 0.807308
00:23:13.400 --> 00:23:16.094 so this is the regulator which
NOTE Confidence: 0.807308
00:23:16.094 --> 00:23:19.338 induces lag three and other Co
NOTE Confidence: 0.807308
00:23:19.338 --> 00:23:22.046 inhibitory molecules while inhibiting.
NOTE Confidence: 0.807308
00:23:22.050 --> 00:23:25.698 Going the opposite direction for ticket.
NOTE Confidence: 0.807308
00:23:25.700 --> 00:23:27.954 And then we looked at the relationship
NOTE Confidence: 0.807308
00:23:27.954 --> 00:23:30.049 between late faith covid for lag free,
NOTE Confidence: 0.807308
00:23:30.050 --> 00:23:32.255 Tim three and PD one and found
NOTE Confidence: 0.807308
00:23:32.255 --> 00:23:34.442 that BSL three instaff 3A positive
NOTE Confidence: 0.807308
00:23:34.442 --> 00:23:36.776 regulated flag 3 and 10 three.
NOTE Confidence: 0.807308
00:23:36.780 --> 00:23:37.652 And finally,
NOTE Confidence: 0.807308
00:23:37.652 --> 00:23:40.268 looking directly in patients to the
NOTE Confidence: 0.807308
00:23:40.268 --> 00:23:42.958 SP140B cell three and stat three
NOTE Confidence: 0.807308
00:23:42.958 --> 00:23:45.138 while elevated in COVID-19 cells,
NOTE Confidence: 0.807308
00:23:45.140 --> 00:23:47.564 so we’re able to recapitulate what
NOTE Confidence: 0.807308
00:23:47.564 --> 00:23:50.696 we saw in terms of induction wisco
inhibitory molecules in vivo in terms of what we thought on Pedro. So in summary, interferon is a major driver of cone hitori receptor regulation and human T cells. The computational and biologic approaches identifies. Regulatory networks under interferon one. Responses in human T cells. There are modules of transcription factors that control interferon stimulated genes. Colon, hip to receptors and interferon which really highlights the novel noncanonical transcription factors.
beyond the conventional Jack stat

pathways that we previously knew about.

We then demonstrate the relevance of our in vitro T cell type one interferon responses by integrating single cell RNA. See data from COVID-19. Patients were strong T cell into fair. One response was observed and finally we identify SP 140 as a key regulator that differentiates Lag 3 digit expression during acute viral infection as well as Aaron Vivo systems. So let me just acknowledge the individuals. Again, this truly represents the work of Thomas Amita. Here, members of the laboratory
contributed various parts of this. My long, long term collaborator, PJ Kutru Shadow Bergen is off Marty and also wondering knowledge. The covered work led by audio Untermann with Tomo Jonas Scoop and enough Tally Kaminski. So I’ll stop there and take any questions. Thank you. David, thank you. What an incredible body of work and congratulations on sorting through what is clearly a very complex. Regulatory system, let me ask,
00:25:37.420 --> 00:25:40.796 and this is sort of my concrete question,
NOTE Confidence: 0.8629724
00:25:40.800 --> 00:25:42.508 which is you know.
NOTE Confidence: 0.8629724
00:25:42.508 --> 00:25:44.216 Obviously you’re sorting through
NOTE Confidence: 0.8629724
00:25:44.216 --> 00:25:46.299 what’s driving expression of Tim.
NOTE Confidence: 0.8629724
00:25:46.300 --> 00:25:48.988 Three lag, three TIGIT an realizing
NOTE Confidence: 0.8629724
00:25:48.988 --> 00:25:51.246 that almost the Holy Grail
NOTE Confidence: 0.8629724
00:25:51.246 --> 00:25:53.906 today is what’s the next PD one?
NOTE Confidence: 0.8629724
00:25:53.910 --> 00:25:55.674 So does this work?
NOTE Confidence: 0.8629724
00:25:55.674 --> 00:25:57.879 Help us understand the relative
NOTE Confidence: 0.8629724
00:25:57.879 --> 00:26:00.453 merits of these targets and in
NOTE Confidence: 0.8629724
00:26:00.453 --> 00:26:02.503 the future of immuno oncology
NOTE Confidence: 0.8629724
00:26:02.586 --> 00:26:04.908 or give us some insight there.
NOTE Confidence: 0.83932835
00:26:06.100 --> 00:26:08.164 Great question. I think the short
NOTE Confidence: 0.83932835
00:26:08.164 --> 00:26:10.399 answer is probably not at one level.
NOTE Confidence: 0.83932835
00:26:10.400 --> 00:26:11.776 It gives us insight,
NOTE Confidence: 0.83932835
00:26:11.776 --> 00:26:14.410 so I guess one could ask what
what induces type one interferons in different tissues and. And how are tumors so presumably in tumors are secreting type one interferons. We know they are and that that may be influencing the local team environment. But the reason why I say no is my suspicion is that each organ has his own set of regulatory module for controlling LG cells work. We just completed an extensive analysis paper published in Science Immunology doing a single cell RNA seek. In T cells from normal spinal fluid is normal yell graduate students and
see that over 50% of the T cells.

In this DSL or PD, one positive high expression digit in three with spontaneous production of gamma interferon.

So I think each organ and that’s why I showed the Ms GBM data.

I think looking at what is expressed in tumors compared to autoimmune disease, which goes the opposite direction may give us insight as to what is the next Holy Grail coding inventory molecule.

I think that would be perhaps the best way of addressing it.

And this is more mechanistic, and it was surprising because it’s
a Vijay kept saying well Style 27.

Can’t you find it kept saying?

Well we keep looking and kept saying

what you’re doing the experiment

wrong and I didn’t show them

picture of Donald but you know,

we just couldn’t get it to work

and then we explore different

and then we explore different

like going hit or molecules.

And then it’s very simple observation

and actually predicted based on

all the viral immunology work.

Yeah, thank you, Ann Habermann has a

question which is how long does the

T cell response to interferon persist


and why would this be a desirable response during a viral infection?

Well, I think in terms of covid there cleared two phases. The initial phase of the high interferon response. We thought the intermediate phase and then with time disappears. If one can generate so there really are these biphasic interferon response? Is this what nature does to try to clear viruses and we suspect that one reason why patients do badly and we’re positive that the loss of TIGIT. Which is induced by interference. We have persistent high interference
signature leads to a loss of the mean regulation. We actually wrote a grant that supplemental grant. Hypothesising that Tim three PD one go up and teacher will go down in covid patients. I don’t like hypothesis driven science. It seemed like a long shot and were shocked to see that was going on. So so in terms of why be desire response because indifference help clear viruses. But then I think it becomes a less desirable response with time. And we suspect that will raise the
issue that loss of digit which is really quite remarkable in these individuals.
May late relate to the hyper mean response that we see in patients.

Well, David, thank you for a really a terrific talk and thank you for sharing that the work in progress. It’s really impressive.

Let me now turn to our next speaker, Doctor Hairy Cougar, who as you all know is a professor of medicine and along with Marcus Bosenberg leads or yell Sporen skin cancer which were so pleased, got renewed about a year ago and continues to be extremely productive.
Harriet’s work in the Cancer Center has been really Sort of the triple threat. Obviously she is a highly Respected and highly sought after physician, but at the same time leader in research and immunology in Melanoma and also a leader of our education program and not many people can do all that and do it so well. Harriet’s work I think has really been instrumental in understanding the biology of Melanoma. How do we leverage Immunobiology towards novel therapies?
And Anne frankly I suspect willingness to hear about it today, but her work on metastases very insightful, but Harriet thank you for taking the time and sharing your work with us. Thank you Charlie and thanks for that wonderful introduction. So it’s always humbling to talk after David Heffler, but that was the assignment I received, so I will do my best here. So I’m going to be talking to you about one of the sport projects which focuses
NOTE Confidence: 0.8806305
00:30:59.808 --> 00:31:03.188 on Co stimulating the the innate immune
NOTE Confidence: 0.8806305
00:31:03.188 --> 00:31:05.773 adaptive immunity to treat Melanoma.
NOTE Confidence: 0.8806305
00:31:08.330 --> 00:31:10.938 So just a few fast facts about Melanoma,
NOTE Confidence: 0.8806305
00:31:13.440 --> 00:31:15.354 so it’s a disease of the relatively young
NOTE Confidence: 0.8806305
00:31:16.630 --> 00:31:18.538 most patients present between age 45 and 55.
NOTE Confidence: 0.8806305
00:31:18.540 --> 00:31:20.298 The incidence has been going up
NOTE Confidence: 0.8806305
00:31:15.354 --> 00:31:16.630 actually for decades already,
NOTE Confidence: 0.8806305
00:31:20.298 --> 00:31:22.370 in 2003 there were around 54,000
NOTE Confidence: 0.8806305
00:31:22.370 --> 00:31:24.400 and just a decade and a half
NOTE Confidence: 0.8806305
00:31:24.400 --> 00:31:26.827 later it was already up to 87,000.
NOTE Confidence: 0.8806305
00:31:18.540 --> 00:31:20.298 It’s now the fifth most common malignancy
NOTE Confidence: 0.8806305
00:31:28.979 --> 00:31:30.300 among men and the seventh among women,
NOTE Confidence: 0.8806305
00:31:31.300 --> 00:31:33.124 but Fortunately most of our patients
NOTE Confidence: 0.8806305
00:31:33.124 --> 00:31:34.810 present with stage one disease,
NOTE Confidence: 0.8806305
00:31:34.810 --> 00:31:36.874 so stage one refers to diseases
NOTE Confidence: 0.8806305
00:31:36.874 --> 00:31:38.779 confined to the skin and is.
NOTE Confidence: 0.8806305
00:31:38.780 --> 00:31:41.388 Then stage two is confined to the skin
NOTE Confidence: 0.8806305
00:31:41.388 --> 00:31:43.609 and thicker stage three is disease.
NOTE Confidence: 0.8806305
00:31:43.610 --> 00:31:45.563 It’s spread to the lymph nodes and
NOTE Confidence: 0.8806305
00:31:45.563 --> 00:31:47.749 stage four is distant dissemination.
NOTE Confidence: 0.8806305
00:31:47.750 --> 00:31:49.820 And that’s essentially what kills patients.
NOTE Confidence: 0.8806305
00:31:49.820 --> 00:31:52.809 So we’re really going to be talking
NOTE Confidence: 0.8806305
00:31:52.809 --> 00:31:55.329 about stage four disease today.
NOTE Confidence: 0.8806305
00:31:55.330 --> 00:31:57.074 So for mortality, Interestingly,
NOTE Confidence: 0.8806305
00:31:57.074 --> 00:31:59.690 it was going up as well.
NOTE Confidence: 0.8806305
00:31:59.690 --> 00:32:02.306 So for 2000 three 7600 deaths,
NOTE Confidence: 0.8806305
00:32:02.310 --> 00:32:04.106 2017 ninety 700 deaths.
NOTE Confidence: 0.8806305
00:32:04.106 --> 00:32:07.540 But if you start tracking later on 2019,
NOTE Confidence: 0.8806305
00:32:07.540 --> 00:32:10.340 the death rate started to go down
for the very first time 7230 deaths, and the projected number for
this year is 6850. And this is because of our improved meta static.
Approved therapies for metastatic disease, particularly immunotherapy.
And that’s what I’m going to be talking about today.
So we’ve known for years that some Melanoma patients are cured by old-fashioned therapy.
If you do a medister tech, to me, this is an old series published in 2011.
You can see that eight or ten
00:32:43.073 --> 00:32:44.799 years at approximately 5 or 7%
NOTE Confidence: 0.8806305
00:32:44.800 --> 00:32:47.050 of patients are still alive.
NOTE Confidence: 0.8806305
00:32:47.050 --> 00:32:48.150 Chemotherapy you actually see
NOTE Confidence: 0.8806305
00:32:48.150 --> 00:32:49.800 a similar kind of a figure,
NOTE Confidence: 0.8806305
00:32:49.800 --> 00:32:51.175 and we don’t think chemotherapy
NOTE Confidence: 0.8806305
00:32:51.175 --> 00:32:52.000 really prolongs survival.
NOTE Confidence: 0.8806305
00:32:52.000 --> 00:32:53.710 Maybe it's just Natural History
NOTE Confidence: 0.8806305
00:32:53.710 --> 00:32:55.724 of disease that some people live
NOTE Confidence: 0.8806305
00:32:55.724 --> 00:32:56.696 for a long time.
NOTE Confidence: 0.8806305
00:32:56.700 --> 00:32:58.956 Now over here on the right you see
NOTE Confidence: 0.8806305
00:32:58.956 --> 00:33:01.578 the the five year survival data from
NOTE Confidence: 0.8806305
00:33:01.578 --> 00:33:04.101 our flagship phase three study of
NOTE Confidence: 0.8806305
00:33:04.101 --> 00:33:06.456 epilim abalon versus nivolumab alone
NOTE Confidence: 0.8806305
00:33:06.456 --> 00:33:08.727 versus the combination thereof at
NOTE Confidence: 0.8806305
00:33:08.727 --> 00:33:11.828 where at five years you see 26%
NOTE Confidence: 0.8806305
00:33:11.828 --> 00:33:14.156 patients are alive with EPI alone
44% with anti PD one alone and 52% or maybe even higher than that. With the combination of the two drugs. So what we’re really trying to do in the Melanoma field, especially the drug development field, is to raise the tennis tail at the end of the curve. So this is a figure that I borrowed from one in Microsoft students, Irina, who I’ll mention as we go along. just showing that targeted therapy and chemotherapy. You’re very low down here with people in Malibu starting.
00:33:42.465 --> 00:33:43.887 to push up. We’re pushing up
NOTE Confidence: 0.83978784
00:33:43.887 --> 00:33:45.501 further with Anti PD one even
NOTE Confidence: 0.83978784
00:33:45.501 --> 00:33:46.870 further with the combination.
NOTE Confidence: 0.83978784
00:33:46.870 --> 00:33:49.030 But really, what we need to do is to
NOTE Confidence: 0.83978784
00:33:49.030 --> 00:33:51.250 get new drugs and drug combinations,
NOTE Confidence: 0.83978784
00:33:51.250 --> 00:33:53.259 so hopefully in the next five years
NOTE Confidence: 0.83978784
00:33:53.259 --> 00:33:55.778 will have a five year survival of 80%.
NOTE Confidence: 0.83978784
00:33:55.780 --> 00:33:57.680 And eventually we’ll reach 100%,
NOTE Confidence: 0.83978784
00:33:57.680 --> 00:34:01.656 and until then we still have employment.
NOTE Confidence: 0.83978784
00:34:01.660 --> 00:34:04.635 So what are the limitations
NOTE Confidence: 0.83978784
00:34:03.785 --> 00:34:04.635 of immunotherapy’s,
NOTE Confidence: 0.83978784
00:34:04.640 --> 00:34:07.196 the Society of Immunotherapy or City?
NOTE Confidence: 0.83978784
00:34:07.200 --> 00:34:10.574 Which is the big society that Mario
NOTE Confidence: 0.83978784
00:34:10.574 --> 00:34:12.999 presides recently formed a
NOTE Confidence: 0.83978784
00:34:12.999 --> 00:34:15.687 task force to define to provide
NOTE Confidence: 0.83978784
00:34:15.687 --> 00:34:17.919 some clinical definitions of.
Limitations so firstly, not all patients respond upfront. We call that primary resistance. Then there’s some patients that will respond and subsequently progress. So we call that secondary resistance or required resistance. The third problem that we have is that we sometimes give combinations. So for example, when we give a pill and an urban Nevada map, we give the two together for four cycles and then we continue with Nevada map monotherapy. So if somebody has a nice response in
the beginning and then 18 months later
when they’re on monotherapy maintenance,
they then progress.
Is that resistance to the combination or
is that resistance to the monotherapy and
all of these things need to be defined?
And then how do we define regrowth
after patient stops therapy?
So we normally treat for a
limited period of time being at
one years one year or two years.
However long we treat for specific disease,
if a patient is in off therapy
and then has regrowth,
and then they’re actually
resistant to the original code?
Because in theory the tumor should have been gone. Or are they just dependent on it and we need to continue so the task force is starting to define all of these categories and to come up with specific definitions that can be used for clinical track for drug development so that all trials are designed the same way. We’ve started on that, but we’re chipping away at all of these questions, and I think many valuable faculty are actually participating in
this endeavour with concurrent with the clinical definitions, we really need to work on the science. So really, what I’m going to talk about mostly today is translation going back and forth. Why do patients develop resistance? Or many potential mechanisms of resistance have been described, and I think. You know half of the cancer immunology world is now working on one or other of these. So some of these tumors are just desert rumors, lack of tumor infiltrating
lymphocytes within the tumors you can have, in effect of priming of your T cells.

We know that defective antigen presentation, such as bile acid,

such as bile acid,

beta,

two microglobulin in the tumor

cells will cause resistance.

Sometimes T cells get exhausted as David just mentioned.

Sometimes T cells get exhausted

as David just mentioned.

Of course lack of PDL one in the tumor

or in the tumor microenvironment

suggests that we don’t live PD

one. Inhibition isn’t going
to do very much over there.

And then the other costimulatory
or Co inhibitory molecules that David just mentioned, particularly teachers and Lag 3 might also be present, and maybe it’s just not sufficient in all cases to inhibit PD one or PDL 1. And finally there there are many other immune inhibitory cells that we need to focus on in the tumor microenvironment, and sometimes those might just be overpowering the role of the T cells. So when we started putting together the renewal of the spore, one of the projects that we
worked on is specifically looking at the innate immune system.

So Sucic, when she was here, provided all of the preliminary data which I’ll be reviewing very quickly and some sewers left, Marcus has become a key collaborator, and actually it’s now become a whole village in the whole party because all of the investigators and trainees listed over here on the right are quite involved in this project, and I’ll mention some of their contribuciones as we go along.

So Sue started off looking at...
Marcus is young 1.7 models, so I'm sure everybody knows that this is a cell line that was generated from the gym model. It's byref mutant and P tenancy. This is a null and when you take this young 1.7 and you treated with anti PD one you see over here there's absolutely no tumor regression. If you irradiate the cells and generated the second. This tortoise airline called Yammer 1.7. You get some sensitivity to anti PD one, but ultimately with time these tumors to grow out as well.
00:38:21.880 --> 00:38:24.112 So the first question next to asked was
00:38:24.112 --> 00:38:26.705 what was actually in these in these tumors.
00:38:26.710 --> 00:38:29.730 So all of this work was done by Kurt Perry,
00:38:29.730 --> 00:38:31.536 who's over here on the right.
00:38:31.540 --> 00:38:33.868 We can see his picture and he's actually
00:38:33.868 --> 00:38:36.378 one of the new fellows that match to.
00:38:36.380 --> 00:38:38.372 Our program will be very thrilled
00:38:38.372 --> 00:38:41.163 to have him as part of our
00:38:41.163 --> 00:38:42.549 medical oncology fellowship.
00:38:42.550 --> 00:38:46.491 So first question that they asked
00:38:46.491 --> 00:38:48.816 was what was the infiltrating
00:38:48.820 --> 00:38:50.084 tumor content in these mass?
00:38:50.084 --> 00:38:52.506 And it turns out that the predominant
00:38:52.506 --> 00:38:55.272 cell type was actually terms or
tumor associated macrophages.

The next question that they asked was what kind of macrophages are these? Are there more inflammatory or inhibitory?

Classic definition of M1 and M2 and over here on the right you see a contour plot where on the X axis you’ve got F 480 and the Y axis you’ve got like 6 E. It turns out that there at least three populations, and they’re probably more than that, and just in a nutshell, the terms that have highlights 6, three like 6 E and low EF 480, or those that are more inflammatory.
00:39:27.834 --> 00:39:30.080 in the ones on the right over here
00:39:30.080 --> 00:39:31.724 are those that are presumed to
00:39:31.779 --> 00:39:32.820 be more inhibitory.
00:39:35.970 --> 00:39:38.500 So at that point they said, OK, we’ve got.
00:39:38.500 --> 00:39:39.620 We’ve got these terms.
00:39:39.620 --> 00:39:41.587 We need to try to modulate them,
00:39:41.590 --> 00:39:43.246 and there are many, many mechanisms
00:39:43.246 --> 00:39:44.680 out there for modulating terms.
00:39:44.680 --> 00:39:46.661 But the ones that they chose to
00:39:46.661 --> 00:39:48.332 work on with CD, 40, agonism,
00:39:48.332 --> 00:39:49.737 and CSF, one R inhibition,
00:39:49.740 --> 00:39:51.786 and in the beginning they used
00:39:51.786 --> 00:39:53.150 a small molecule inhibitor.
00:39:53.150 --> 00:39:55.316 So if you take these memory
00:39:55.316 --> 00:39:57.410 cells and implant them in mice,
and you treat either with control vehicle or.
The CD 40 agonist.
You'll see some decrease in the size of the tumors with the CD 40 agonist if you give the CSF receptor inhibitor you get a similar amount of tumor reduction. If you give the two together, you get synergism. As you can see by the red line over here. So to look back into the similar contour plots, what is the content of these different tumors within the mice treated in the graph over here on the left you can see that when you give doublet therapy,
00:40:29.040 --> 00:40:31.231 the CD 40 agonist in the CSF
00:40:31.231 --> 00:40:32.170 one receptor inhibitory,
00:40:32.170 --> 00:40:34.319 the main difference is that you get
00:40:34.319 --> 00:40:36.463 an increase in this little group over
00:40:36.463 --> 00:40:39.060 here on the right in the upper corner,
00:40:39.060 --> 00:40:41.924 which are like 60 high and in 480 low and are
00:40:41.924 --> 00:40:44.378 presumed to be more inflammatory macrophages,
00:40:44.380 --> 00:40:45.319 and that’s essentially
00:40:45.319 --> 00:40:46.884 verified on the bar graph.
00:40:46.890 --> 00:40:48.560 Over here on the left.
00:40:48.560 --> 00:40:49.430 On the right,
00:40:49.430 --> 00:40:51.460 at the bottom over here you can
00:40:51.525 --> 00:40:53.789 see this to the changes in the in
00:40:53.789 --> 00:40:55.780 the immune infiltrating content,
00:40:55.780 --> 00:40:57.730 and I think what’s most interesting
over here is that when you give

NOTE Confidence: 0.83383965

the CD 40 agonist along with

NOTE Confidence: 0.83383965

the CSF one receptor inhibitor,

NOTE Confidence: 0.83383965

you do get an increase of

NOTE Confidence: 0.83383965

infiltration of T cells.

NOTE Confidence: 0.83383965

So possibly we might be able to make
desert those desert tumors more

NOTE Confidence: 0.83383965

inflamed by using a regimen such as this.

NOTE Confidence: 0.83383965

And in addition you get more

NOTE Confidence: 0.83383965

PD one high T cells.

NOTE Confidence: 0.8104826

So Catherine Miller Jensen on the main
campus is developed a technology for

NOTE Confidence: 0.8104826

single cell site eccentric creation,

NOTE Confidence: 0.8104826

and she looked at what the difference of

NOTE Confidence: 0.8104826

was between these different treatment

NOTE Confidence: 0.8104826

arms and what you can see here on
the principle component analysis.

On the left is that if you only treat with assistive one receptor inhibitor versus the city for The Agonist inhibitor alone, versus the combination, you get quite a different pattern of cytokine secretion on the right. Oh, I’m sorry in the middle over here, you’ve got a heat map which we essentially depicts the differences, and some of them are highlighted over here on the right for cytokines and chemo kinds. Pretty much as as one would expect when you give the combination therapy, you get an increase in TNF Alpha.
I'll BIL 6 etc and the same for the panel of the side of kinds of the chemo kinds at the bottom. So essentially the doublet therapy over here is inducing quite vast changes in the animals. What does it do to the T cells? What else is important over here? When you give the doublet therapy, that when you give the doublet therapy, you can actually abrogate the effect if you give anti TNF Alpha or anti interferon gamma, again highlighting the the importance of the T cells in this process as well. So with that at the time we concluded
that CSF one receptor inhibitors in city
for The Agonist treatment can induce
an inflammatory term population in
the two in the tumor microenvironment.
It also induces a functional T cell response.
And this is dependent on TNF Alpha
and interferon gamma,
and these were the preliminary data
that we had to start our project.
So when we received funding,
we by then Curtis Perry had gone
off for residency.
So Bill Dembski came in to help
us and you’ll see a whole cast of
trainees along the way over here.
So Bill did a heroic job over here with bringing us closer to the clinic. So we decided at that point not to use a series of 1 receptor inhibitor, but rather to move towards and Antibody because of precision in the clinical arena, it would be very difficult to take a patient who progressed on a PD one and not to continue the PD one inhibitor with the next regiment. That’s essentially how most regimens are now being developed for Melanoma and renal cell as well.
So the question is what can we add onto a PD?
One inhibitor to get us there so these are large groups of mice treated either with control vehicle, either one of the three drugs alone so anti PD one.
Any doublet of the from among those three and the triplet, and you can see by the Brown line over here that by far the triplet therapy was superior on the
right you see the spider plots for the size of these tumors, which in the beginning
they’ll grow and then shrink.

Who’s MD PhD student who is in Marcus’s lab at the time or selection Marcus is lab did similar experiments on aranka model because we wanted to go into the clinic in kidney cancer as well. Again, showing their triple therapy was superior to double therapy. Not quite as pretty as in the Melanoma models, but that’s then that’s consistent with what we see in the clinic, whereby renal cell patients respond less well to these therapies then Melanoma patients. So because it’s a sport project,
00:44:44.650 --> 00:44:46.410 you have to have a clinical Pi and
NOTE Confidence: 0.8090304
00:44:46.410 --> 00:44:48.040 a basic science Pi and everything
NOTE Confidence: 0.8090304
00:44:48.040 --> 00:44:50.014 has to have a clinical trial so
NOTE Confidence: 0.8090304
00:44:50.014 --> 00:44:51.316 to go back to the bedside.
NOTE Confidence: 0.8090304
00:44:51.320 --> 00:44:53.534 What are we going to do with these data?
NOTE Confidence: 0.8090304
00:44:55.262 --> 00:44:57.381 So we formed collaborations with Bristol
NOTE Confidence: 0.8090304
00:44:57.381 --> 00:44:59.447 and a company called a passage
NOTE Confidence: 0.8090304
00:44:59.447 --> 00:45:02.590 Myers Squibb and we
NOTE Confidence: 0.8090304
00:45:02.590 --> 00:45:04.550 were able to get them to work together.
NOTE Confidence: 0.8090304
00:45:04.550 --> 00:45:06.388 Now could be oralism AB which is the
NOTE Confidence: 0.8090304
00:45:06.390 --> 00:45:08.366 CSF one receptor antibody and the
NOTE Confidence: 0.8090304
00:45:08.366 --> 00:45:10.329 volume Abbott being given to hundreds
NOTE Confidence: 0.8090304
the activity in Melanoma was modest, but there was a little bit of activity at that point. We knew that a CD 40 agonist can have significant activity in Melanoma based on studies by the group at Penn already years ago. But we didn’t know very much about the other combinations, so at the time sterilize, brought in a Phase 1 two study of APX. In other words, the CD 40 agonist plus nivo in Melanoma and lung cancer started at around that time and we rolled a
good number of patients there and actually saw phenomenal responses. So this is an example of a patient treated by doctors know who had a mucosal Melanoma, which tends to be very resistant to implement map in the volume. Evan the patient indeed had progressed on there. So we put the patient on the CD and remains of therapy couple of years later we have four of these patients and others and implement Melbourne Nivolumab.
We don’t actually see this, so maybe this is the answer to Charlie’s question is what’s the next anti PD? Why? So we’re very excited about this molecule and with that Sarah Weiss. This picture over here and I wrote a Phase one slash 1B or phase two for the combination of the triplet. We partnered with the yellow Spore in lung cancer and we were able to get support both from the Cancer Center Bristol Myers and Apixaban. So the phase one trial design is depicted on this picture over here.
anxious because nobody had ever given two macrophage modulating agents together and we were worried that we were going to get like diffuse macro activate macrophage activating syndrome and kill patients. So we had to go very very gingerly. We will also working with two pharmaceutical companies, each with its own opinion so it could be oralism AB which was being developed by Bristol Myers Squibb. dead already did it already defined the recommended phase two dose and we had to stick with the dose that
they gave us which was for me.

Ramza, kilogram.

We escalated the CD 40 agonist very gently,

so cohort one only had the doublet therapy

at a tenth of the recommended phase.

Two dose for the CD 40 agonist within

escalated by a half a log into cohort

three in Cohort 5 and concurrently

added the nevala map on with the goal

of ultimately reaching cohort six,

which would be 4 doses at the

record for Cabrera.

Lismer,

the pic surgeon drug and nivolumab at the.

Same recommended phase.

two dose of each one of these individually.
Once we get to the Cohort 6 or to the recommended phase two regimen, the plan is to go into the Phase 1B component, which is essentially three phase two studies, each one with its Simon phase. At this trial has lots of embedded correlates, both blood based and tumor based with pretreatment biopsies mandatory on treatment, biopsies etc. So to update you on what’s going on
with the Phase one trial which is an integral part of the sport project. We have completed the Phase 126 patients in total have been enrolled sarahs busy preparing the publication for this and that should be going out over the coming weeks. Overall it was reasonably well tolerated. It certainly wasn’t candy, though we saw a lot of periorbital edema as well as diffuse edema elevations in CPK AST and a Lt, but those didn’t appear to be particularly clinically significant. Fevers Insider Kind release, but a lot of fatigue.
I think that was the biggest problem. The other big problem that we saw was skipped. While there was some activity in some of the patients, it was mostly stable disease in progression of disease and not quiet what we’ve seen in the mice. The trial has preceded to the Phase 1B component in Melanoma and lung cancer. Both are in the first stage, but we’ve completed the phase one. I’m going to show you some examples of correlative studies that we’ve done and this is still a bit.
00:49:19.756 --> 00:49:21.300 of a work in progress,
NOTE Confidence: 0.8301139
00:49:21.300 --> 00:49:23.652 so we looked at cytokine panels before
NOTE Confidence: 0.8301139
00:49:23.652 --> 00:49:25.808 and on treatments at 24 hours later,
NOTE Confidence: 0.8301139
00:49:25.810 --> 00:49:28.295 and you can see nice increasing interferon
NOTE Confidence: 0.8301139
00:49:28.295 --> 00:49:30.638 gamma as well as in in TNF Alpha.
NOTE Confidence: 0.8301139
00:49:30.640 --> 00:49:32.894 The different cohorts are listed over here,
NOTE Confidence: 0.8301139
00:49:32.900 --> 00:49:35.330 but Code 5 and six are when we hit
NOTE Confidence: 0.8301139
00:49:35.330 --> 00:49:37.397 them at the recommended phase,
NOTE Confidence: 0.8301139
00:49:37.400 --> 00:49:39.338 two dose of deep excision drugs,
NOTE Confidence: 0.8301139
00:49:39.340 --> 00:49:43.570 so that’s where you see most of the activity.
NOTE Confidence: 0.8269034
00:49:43.570 --> 00:49:45.316 There are other changes in circulating
NOTE Confidence: 0.8269034
00:49:45.316 --> 00:49:47.060 cytokines and I could spend an
NOTE Confidence: 0.8269034
00:49:47.060 --> 00:49:48.375 hour just talking about this,
NOTE Confidence: 0.8269034
00:49:48.380 --> 00:49:50.151 but I selected a few just just
NOTE Confidence: 0.8269034
00:49:50.151 --> 00:49:52.060 to show you what we’re seeing,
NOTE Confidence: 0.8269034
00:49:52.060 --> 00:49:53.758 so we’ve got the CL 2,
NOTE Confidence: 0.8269034
00:49:53.760 --> 00:49:55.608 which is a side kind that’s primarily
NOTE Confidence: 0.8269034
00:49:55.608 --> 00:49:57.440 secreted by dendritic cells and macrophages.
NOTE Confidence: 0.8269034
00:49:57.440 --> 00:49:59.696 Very high levels of the higher dose levels,
NOTE Confidence: 0.8269034
00:49:59.700 --> 00:50:00.894 same with. P.
NOTE Confidence: 0.8269034
00:50:00.894 --> 00:50:02.884 10 and then the macrophage
NOTE Confidence: 0.8269034
00:50:02.884 --> 00:50:04.220 colony stimulating factor,
NOTE Confidence: 0.8269034
00:50:04.220 --> 00:50:06.705 also highest levels in Cohort
NOTE Confidence: 0.8269034
00:50:06.705 --> 00:50:08.693 6 but clear increases.
NOTE Confidence: 0.8269034
00:50:08.700 --> 00:50:09.573 Across the board,
NOTE Confidence: 0.8269034
00:50:09.573 --> 00:50:11.610 we do have the pretreatment and on
NOTE Confidence: 0.8269034
00:50:11.674 --> 00:50:13.299 treatment specimens show me jessel
NOTE Confidence: 0.8269034
00:50:13.299 --> 00:50:15.544 who supposed dark in my lab is
NOTE Confidence: 0.8269034
00:50:15.544 --> 00:50:17.224 busy analyzing these what you see
NOTE Confidence: 0.8269034
00:50:17.224 --> 00:50:18.950 over here is the basic analysis,
NOTE Confidence: 0.8269034
00:50:18.950 --> 00:50:21.449 so these are just this is just a
NOTE Confidence: 0.8269034

88
munificent staining a CD4 and CD8
before treatment and on treatments
on the left is pre and on the right
is post and you can see an increase
in the infiltration of the CD 8
cells which are colored in green.
There's also an increase of
the CD Force which are in red.
the CD 68 also actually.
CD 68 also actually.
In some patients we actually
see the opposite,
so over here you can see that the
C8 cells pretreatment were much
more dense than post treatment.
Although you do see some post treatment, I don’t know how well this projects. There’s an increase in the CD 68 though. Just to highlight one of the challenges that we have with doing this. Pre Anon treatments studies in that it may not come from this so the pretreatment was a containers tissue metastasis on the back and the post treatment in this particular patient came from the Gallbladder, so it’s possible that the tumor micro environment in the different organs is playing a part over here.
But because we didn’t see much activity in the Phase one trial, we’re going back to the bench to try to determine what can we do to improve our trial.

So Irina clickbait ever, who was the postdoc working?

I’m sorry there’s the doctoral student in Marcus’s lab, partnered with Deanna, who’s working in my lab to ask the question of whether we’re actually just giving too much CSF one receptor antibody. So more isn’t always better, particularly when we’re trying to polarize macrophages and not
NOTE Confidence: 0.8269034
00:51:49.997 --> 00:51:51.920 necessarily knock them off completely.
NOTE Confidence: 0.8269034
00:51:51.920 --> 00:51:54.539 So when we do these experiments in the mice,
NOTE Confidence: 0.8269034
00:51:54.540 --> 00:51:55.995 we were seeing much better
NOTE Confidence: 0.8269034
00:51:55.995 --> 00:51:57.159 activity than the humans,
NOTE Confidence: 0.8269034
00:51:57.160 --> 00:51:58.610 and the question is why?
NOTE Confidence: 0.8269034
00:51:58.610 --> 00:52:00.914 So the dose is selected for the Marin
NOTE Confidence: 0.8269034
00:52:00.914 --> 00:52:02.528 experiments with somewhat random we go
NOTE Confidence: 0.8269034
00:52:02.528 --> 00:52:05.020 based on what is done by other researchers,
NOTE Confidence: 0.8269034
00:52:05.020 --> 00:52:07.124 what’s done by format and the amount that
NOTE Confidence: 0.8269034
00:52:07.124 --> 00:52:09.376 we were giving them was 200MG kilogram.
NOTE Confidence: 0.8269034
00:52:09.380 --> 00:52:10.840 So we asked the question.
NOTE Confidence: 0.8269034
00:52:10.840 --> 00:52:11.130 Well,
NOTE Confidence: 0.8269034
00:52:11.130 --> 00:52:13.450 what happens if we give them more CSF?
NOTE Confidence: 0.8269034
00:52:13.450 --> 00:52:14.905 One receptor antibody and keep
NOTE Confidence: 0.8269034
00:52:14.905 --> 00:52:16.360 the other two drug steady?
NOTE Confidence: 0.8269034
And as you can see in this figure over here, if you give more CSF, one receptor antibody basically doubling the dose. The mice actually do less well die sooner or sacrificed sooner, and as you can see here on the left, the tumor volume is actually bigger when you give the higher dose of the CSF one receptor antibody. So we're still debating what to do about that as we go into the clinic. Meanwhile, because it's a small project, we still need to have an ongoing clinical trial, and the question was,
well, is the CSF one receptor
the optimal second target,
in addition to CD 40 agonist
and PD one inhibitors?
So it’s possible,
that CTA for is a better target because
CTA for new mission is is really
key for dendritic cell activation.
So Kelly Alina,
who’s one of our wonderful
surgeons in the Melanoma group
and also surgeon scientists,
is doing work in the lab.
It, primarily Marcus is lab where she
00:53:15.802 --> 00:53:18.020 is taking a very aggressive model
NOTE Confidence: 0.8289687
00:53:18.020 --> 00:53:20.080 marine model whereby she injects
NOTE Confidence: 0.8289687
00:53:20.080 --> 00:53:22.470 these cells into the left ventricle.
NOTE Confidence: 0.8289687
00:53:22.470 --> 00:53:24.305 So they developed vast mistake
NOTE Confidence: 0.8289687
00:53:24.305 --> 00:53:25.406 metastases all over,
NOTE Confidence: 0.8289687
00:53:25.410 --> 00:53:26.974 including in the brain.
NOTE Confidence: 0.8289687
00:53:26.974 --> 00:53:29.320 And this model is particularly resistant
NOTE Confidence: 0.8289687
00:53:29.384 --> 00:53:31.280 to anti PD one in Antici TLA 4.
NOTE Confidence: 0.8289687
00:53:31.280 --> 00:53:35.318 So the question is whether the addition
NOTE Confidence: 0.8289687
00:53:33.100 --> 00:53:35.318 of the CD 40 agonist adds something.
NOTE Confidence: 0.8289687
00:53:35.320 --> 00:53:37.018 And as you can see over
NOTE Confidence: 0.8289687
00:53:37.018 --> 00:53:38.500 here with the red bar,
NOTE Confidence: 0.8289687
00:53:38.500 --> 00:53:40.565 the addition of the CD 40 agonist
NOTE Confidence: 0.8289687
00:53:40.565 --> 00:53:42.574 does appear to improve the survival
NOTE Confidence: 0.8289687
00:53:42.574 --> 00:53:44.329 of these nice that typically
NOTE Confidence: 0.8289687
00:53:44.329 --> 00:53:46.329 will be dead within 20 days.
This is some subq injection data over here on the left, which we don’t have time to go through, but with those data we again approached the passage and we said, well, maybe we should do a different trial now in parallel, so it’s a phase one study of the CD 40 agonist in combination with epilim urban, and this is our second trial which Kelly and Sarah worked with me to write. So just to highlight some of the challenges of a study like this, we know that a polymer mabona volume
We’re talking about these immune-related adverse events all the time. And we also know that sometimes these events occur late, so you can have a patient who is treated comes off therapy, and six months later develops a horrendous toxicity. So how long? How do we design a study like that? How long can we follow the patients? For how long do we go from one cohort to the other? So it took a lot of negotiation back and forth with the FDA,
but we put a lot of thought into this really slow trial design where we actually have only two dose levels, so dose level one is a. Third of the recommended phase. Two dose of the seat of the CD 40 agonist which is the drug that we're adding, and we give people a map in the volume AB. We only treat three patients. Monitor them for 28 days and all of these six patients. They need to be monitored for six weeks so this is going to take
us a long time to get through.

But what we’re hoping is that we’ll have a regimen that may not be more toxic,

but that will be significantly more effective.

Then the PD one and see TLA for that.

We have right now to finally bring that tail of the curve up to 80%.

We have started.

We’ve enrolled three Melanoma patients or have completed their 28 day DLT

period and they did OK with there,

but they have not all completed their nine week observation.

Before Christmas, we going to enroll. Two more patients have consented and
we’re looking for the six patient, but they all have to be monitored for 9 weeks before we can proceed. So I’m going to conclude there that Co targeting the innate and adaptive immune system with the CSF one receptor inhibitor or antibody plus CD 40 agonist results in better anti tumor activity than either alone. It also increases the CD 8 tumor content in animals if we treat mice bearing PD one resistant tumors with all these drugs in combination with anti PD one.
The findings were confirmed in a renal cell carcinoma model where we are in the clinic already testing this. We're having some difficulty with. With insufficient activities, so we're back in the lab right now trying to modify the doses in the regimen before we go back again into the clinic, and this kind of back and forth between the lab in the clinic is something that can only be done at a place like this. We are also at the same time evaluating the combination with the CTL A4 inhibitor and hopefully this will be as exciting, more exciting and just to
say the final conclusion, that is that it really takes a village to do a project like this. So all of the folks have been involved acknowledged on this slide. The scientific collaborators at Yale, colleagues in other labs have helped a lot through this process. Members of my lab members of the Collaborating lab, clinical collaborators, pharmaceutical collaborators, patients and their family, and then finally the funding. So I did mention the sporting skin cancer
which is funded the core project.

But the K12 is funded a couple of the investigators here, Kelly Alina and Sarah Weiss, and Cancer Center has supported it, and some of our folks of which have received career development awards as well related to this.

So with that I’ll stop. I’m happy to take any questions. Thank you for listening.

Hurry, thank you.

What a great example of translating science into the clinic and folks can certainly submit questions online. So let me I have a question watching
'cause I you sort of anticipated my question by adding the CTA four antagonist. But to what extent do you think that triplet might have had greater benefit if they weren't previously exposed to a PD? One antibody? And that's really good question. So the masks were not exposed to PD one antibody, whereas the humans would. And it's possible that you know, we've we've just used that app and developed it yet, and you're of mechanism of resistance, so we haven't done that.
experiment in the mouse.

But that’s actually a really good next step to do. It’s a great thought.

We should expose the mice to PD one inhibitors and then add on the other ones instead of giving all three up front. And this may be impossible, but is there any consideration of combining all four agents in previously?

I mean that is a CSF one R CD40 anti CD L4, GTA 4 and PD one and I realized that’s a smorgasbord of agents, but is that a conceivable approach? We could, we just got it.
We can get through the 1st 3 first, so the CTA for CD for D and P1. So far we’re doing OK with toxicity. But we are only on the 1st dose level. It’s it’s very intimidating to do all of this sure, and then the other question is in what line do you do it? Mostly because of memory. We decided to go in first line.
with her previous settling for, you can get additive toxicity over there. But that’s a really good idea to do that in the mouse. Thank you. Yeah, well, I know where I know we’re just we’re out of. We’re a little past the hour and I want to be sensitive to everyone’s time. So Harriet and David. Thank you both for really exceptional talks. Congratulations on all your work and everyone in attendance. Thank you for joining us and enjoy your day. Thanks. Bye bye.